HUMAN LYMPHOCYTE ANTIGENS

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PREFACE

This thesis embodies much of my work done over the past 25 years. The impetus for these studies was the need to provide the best tissue typing available for organ transplantation and to overcome the problems of defining HLA antigens in different ethnic groups. These goals were achieved by extensive international collaboration and participation in the International Histocompatibility Workshops.

The discovery that the HLA antigens are associated with many diseases led to an epidemic of investigations in which over 500 diseases have been studied. In retrospect, it is not surprising that auto-immune diseases such as diabetes and rheumatoid arthritis showed such marked associations with HLA antigens. The studies in Part II of this thesis were aimed at finding out if the HLA associations reported in Caucasian populations were also present in the Black and Indian populations.

These research interests led to my being invited by the National Science Council of the Republic of China in Taiwan to be a Visiting Professor at the National Taiwan University in Taipei for the 1989 academic year. I investigated the association between HLA and naso-pharyngeal carcinoma in Chinese during that year.

I wish to express my appreciation to Dr Peter Brain who inspired the early investigations and continued to encourage and support my research. I am grateful to all my co-authors and the many colleagues, clinicians and laboratory staff who have contributed to the various research programmes.

Studies of the relationship of the HLA system to cancer, diabetes, arthritis and other diseases have been supported in part by grants from the National Cancer Association and the Medical Research Council of South Africa.

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BIBLIOGRAPHY

1. Brain P and Hammond MG. **Leucocyte antigens in three race groups.** *Med Proc* **14**: 589, 1968

2. Hammond MG and Brain P. **Leucocyte groups in baboons tested with human antisera.** *S Afr Med J* **44**: 380, 1970

3. Hammond MG and Brain P. **Reactions of HLA antisera in three populations.** *Vox Sang* 20: 492, 1971

4. Brain P and Hammond MG. **Frequency of HLA antigens in South African Bantu, Indians and Caucasians.** In: Dausset J, Colombani J (eds) *Histocompatibility Testing 1972* p433. Munksgaard, Copenhagen. 1972

5. Hammond MG, Appadoo B and Brain P. **HLA antigens and antibodies in South African Bantu.** *Transplantation* 14: 159, 1972

6. Downing HJ, Brain P, Hammond MG, Vos GH and Webb GR. **Leucocyte antigens of baboons.** *Transplant Proc* 4: 33, 1972

7. Vos GH, Hammond MG, Vos D, Grobbelaar BG, Auslander HP, and Marescotti G. **An evaluation of humoral antibody responses in patients with carcinoma of the cervix.** *J Obs Gyn* 79: 1040, 1972

8. Hammond MG, Appadoo B and Brain P. **HLA antigens and antibodies in South African Indians.** *Tissue Antigens* 2: 389, 1972

9. Brain P and Hammond MG. **Association between histocompatibility type and the ability to make Rh antibodies.** *Eur J Imm* 4, 223. 1974

10 Hammond MG, Appadoo B and Brain P. **Subdivision of HLA 5 and comparative studies of the HLA polymorphism in South African Indians.** *Tissue Antigens* 4, 42. 1974

11 Vos GH, Hammond MG and Marescotti G. **Changeable lymphocytotoxic antibody activity in patients with cervical carcinoma.** *Vox Sang* 28: 285, 1975

12 Hammond MG, Appadoo B and Brain P. **HLA antigens in Bantu and Indians.** In: Kissmeyer-Nielsen F (ed) *Histocompatibility Testing 1975* p173 Munksgaard, Copenhagen. 1975

13 Hammond MG, Appadoo B and Brain P. **HLA and cancer in South African Negroes.** *Tissue Antigens* 9: 1, 1977

14 Hammond MG, Appadoo B and Brain P. **HLA antigens in South African Negroes and Indians.** *Tissue Antigens* 10: 230, 1977

15 Hammond MG, Appadoo B and Brain P. **HLA in non-Caucasian populations.** In: Bodmer W F et al.(eds) *Histocompatibility Testing 1977* p407. Munksgaard, Copenhagen. 1977

16 Schreuder I, Bos A and Hammond MG. HLA-Bw40 is heterogeneous In: Bodmer W F et al.(eds) *Histocompatibility Testing 1977* p408. Munksgaard, Copenhagen. 1977

17 Biegel A, Botha MC, Bouysou C, Briggs B, Hasty B, Herbert S, Pollack M, Wolf E, Duquesnoy R and Hammond MG. **Joint Report. The "Black" antigens : Aw36, Aw34, Aw43 and Bw42.** In: Bodmer W F et al.(eds) *Histocompatibility Testing 1977* p164. Munksgaard, Copenhagen. 1977

18 Amos DB, Engelfriet P, Hammond MG, Mazzilli C, Payne R, Richiardi P and Ting A. **Joint Report. B5, Bw51, Bw52, Bw53, Bw35.** In: Bodmer W F et al.(eds) *Histocompatibility Testing 1977* p166. Munksgaard, Copenhagen. 1977

19 Downing HJ, Criticos A, Burgess LE and Hammond MG. **An antigen resembling HLA 7 on the leucocytes of vervet monkeys.** *J Med Primat* 7: 174, 1978

20 Hammond MG, Appadoo B and Brain P. **HLA and cancer in South African Indians.** *Tissue Antigens* 14: 296, 1979

21 Hammond MG. **Further splits of HLA B5.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p758. UCLA Tissue Typing Laboratory, Los Angeles. 1980

22 Hammond MG and Appadoo B. **Confirmation of ST-1 in Asian Indians.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p844. UCLA Tissue Typing Laboratory, Los Angeles. 1980

23 Hammond MG. **Heterogeneity of HLA B40.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p782. UCLA Tissue Typing Laboratory, Los Angeles. 1980

24 Hammond MG and Asmal AC. **HLA and insulin dependent diabetes in South African Indians.** *Tissue Antigens* 15: 244, 1980

25 Hammond MG and Lamm L. **A/C crossover in a South African Indian family.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p794. UCLA Tissue Typing Laboratory, Los Angeles. 1980

26 Hammond MG. **Antigen Report. HLA Bw53.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p429. UCLA Tissue Typing Laboratory, Los Angeles. 1980

27 Svejgaard A, Platz P, Ryder LP, Bertrams J, Bodmer JG, Brautbar C, Acton RT, Bartova A, Bashir H, Ceppelini R, de Mouzon A, Guttman RD, Hammond MG, Hansen JA, Juji T, Kastelan A, Kreisler JM, Moller E, Mayr WR, Raffoux C, Rubinstein P, Saito S, Sucia-Foca N, Tiilikainen A, Tsuji K and Arnaiz-Villena A. **Joint Report: Insulin-dependent Diabetes** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p638. UCLA Tissue Typing Laboratory, Los Angeles. 1980

28 Stastny P, Barger BD, Batchelor JR, Bertrams J, Betuel H, Botha MC, Braun WE, Carpenter CB, Darke C, Dawkins RL, Engelfriet CP, Fauchet R, Festenstein H, Gazit E, Gorodezky C, Hammond MG, Hansem JA, Juji T, Mayr WR, Mayer S, McConnachie PR, Oh JH, Perez-Rojans GE, Petranyi GG, Radvany R, Rodey GE, Sasazuki T, Sucia-Foca N, Walford R and Winchester RJ. **Joint Report: Rheumatoid Arthritis.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p681. UCLA Tissue Typing Laboratory, Los Angeles. 1980

29 Hammond MG and Moshal MG. **HLA and duodenal ulcer in South African Indians.** *Tissue Antigens* 15, 508. 1980

30 Hammond MG and Asmal AC. **HLA and insulin dependent diabetes in South African Negroes.** *Diabetologia* 19, 101. 1980

31 Hammond MG and Angorn B. **HLA and cancer of the oesophagus - in South African Negroes.** *Tissue Antigens* 16, 254. 1980

32 Coovadia HM, Wesley A, Hammond MG and Kiepiela P. **Measles, Histocompatibility leukocyte antigen polymorphism, and natural selection in humans.** *J. Inf. Dis.* 144, 142. 1981

33 Hammond MG. **HLA and cancer of the oesophagus.** In: Pfeiffer CJ (ed) *Cancer of the Oesophagus* Vol 1, Chapter 11. C R C Press. Boca Raton, Florida. 1982

34 Haffajee IE, Hammond MG and Moosa A. **HLA antigens in black South African children with rheumatic heart disease.** *Ann. Trop. Paed.* 2,17. 1982

35 Hammond MG. **Antigen Report. HLA Bw35.** In: Simons MJ, Tait BD (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p112. Immunopublishing, Toorak, Australia, 1983

36 Hammond MG. **Antigen Report. HLA Bw53.** In: Simons MJ, Tait BD (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p65. Immunopublishing, Toorak, Australia, 1983

37 Hammond MG. **Antigen Report. HLA Cw4.** In: Simons MJ, Tait BD (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p138. Immunopublishing, Toorak, Australia, 1983

38 Hammond MG. **Subdivision of HLA B15 in Indians.** In: Simons MJ, Tait BD (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p413. Immunopublishing, Toorak, Australia, 1983

39 Hammond MG. **Anomalous reactions with Bw4 sera in Indian families.** In: Simons MJ, Tait Bd (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p411. Immunopublishing, Toorak, Australia, 1983

40 Bashir H, Juji T, Moffitt P, Amos DB, Kostyu D, Chen RB, Chiewsilp P, Fong R, Hammond MG, Hirota M, Sasazuki T, Vaidya M, Mehra N, Woodfield G, Ye YG, Kirk R, Dawkins RL and Elliott RG. **Diabetes Mellitus** In: Simons MJ, Tait Bd (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p332. Immunopublishing, Toorak, Australia, 1983

41 Christiansen FT, Komori K, Dawkins RL, Mehra M, Bashir H, Sekiguchi S, Saito H, Mehra M, Hammond MG, Chandanayingyong D, and Chan SH. **Rheumatoid Arthritis** In: Simons MJ, Tait Bd (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p348. Immunopublishing, Toorak, Australia, 1983

42 Hammond MG, Betuel H and Gebuhrer L. **Antigen Report. HLA A29.** In: Albert ED et al. (eds) *Histocompatibility Testing 1984* p126. Springer-Verlag, Berlin 1984

43 Campbell EM, du Toit ED, Hammond MG and Oudshoorn M. **Antigen Report. Aw43.** In: Albert ED et al. (eds) *Histocompatibility Testing 1984* p132. Springer-Verlag, Berlin 1984

44 Taylor C, Ting A, Hammond MG, de Waal L, de Lange GG and Engelfriet P. **Antigen Report. Bw53.** In: Albert Ed et al. (eds) *Histocompatibility Testing 1984* p161. Springer-Verlag, Berlin 1984

45 Chandanayingyong D, Cambon-Thomsen A and Hammond MG. **Bw62 and other B15 variants - Bw6 associated.** In: Albert ED et al. (eds) *Histocompatibility Testing 1984* p169. Springer-Verlag, Berlin 1984

46 Cambon-Thomsen A, Chandanayingyong D, Thomsen M and Hammond MG. **Bw63 and other Bw4 associated variants.** In: Albert ED et al. (eds) *Histocompatibility Testing 1984* p171. Springer-Verlag, Berlin 1984

47 Hammond MG. **Definition of Bw53 in South African Indians.** *Ninth International Histocompatibility Workshop Newsletter VI* p6 1984

48 Appadoo B and Hammond MG. **Splits of DR4 in South African Indians.** *Ninth International Histocompatibility Workshop Newsletter VI* p15 1984

49 Hammond MG. **The HLA A10 and Aw19 complex in South African Indians and Negroes.** *Ninth International Histocompatibility Workshop Newsletter VII* p6 1984

50 Hammond MG. **HLA B15 complex in South African Indians.** *Ninth International Histocompatibility Workshop Newsletter VIII* p4 1984

51 Hammond MG. **Short Bw41.** *Ninth International Histocompatibility Workshop Newsletter VIII* p9 1984

52 Omar MAK, Hammond MG and Asmal AC. **HLA A, B, C and DR antigens in young South African Blacks with Type I (insulin-dependent) diabetes mellitus.** *Diabetologia* 26, 20-23. 1984

53 Omar MAK, Hammond MG, Rajput MC and Asmal AC. **HLA A, B, C and DR antigens in young South African Indians with insulin-dependent diabetes mellitus.** *S. Afr. Med. J.* 66, 765-767. 1984

54 Omar MAK, Hammond MG, Seedat MA and Asmal AC. **HLA antigens and non-insulin dependent diabetes mellitus in young South African Indians.** *S. Afr. Med. J.* 67, 130. 1985

55 Adhikari M, Coovadia HM and Hammond MG. **Associations between HLA antigens and nephrotic syndrome in African and Indian children in South Africa.** *Nephron* 41, 289. 1985

56 Norman RJ, Reddi K, Richards A, Hammond MG and Joubert SM. **Male transmission of the gene for isolated gonadtropin releasing hormone deficiency.** *Fert. and Steril* 43, 225. 1985

57 Omar MAK and Hammond MG. **The HLA system and diabetes mellitus.** Editorial *S. Afr. Med. J.* 68, 333. 1985

58 Mauff G, Hitzeroth H, Hammond MG and Kleeberg HH. **Immunogenetic aspects of TB and leprosy.** *S. Afr. J. Science* 82, 392 1986

59 Naidoo C, Jialal I, Hammond MG, Omar MAK and Joubert SM. **HLA and NIDDM in the Young.** *Diabetes Care* 9, 436. 1986

60 Hammond MG. **Antigen Report HLA A3.** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p27 Hokkaido University Press, Sapporo 1986

61 Hammond MG. **Antigen Report HLA A11.** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p29 Hokkaido University Press, Sapporo 1986

62 Mehra NK, Taneja V, Kailash S, Chaudhuri TK, Vaidya MC, Balakrishnan K, Contractor N, Seth GS, Hammond MG, Undevia JV and Khan R. **North Indians - Ethnic Study** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p271. Hokkaido University Press, Sapporo 1986

63 Hammond MG and Appadoo B. **HLA antigens in African Blacks.** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p316 Hokkaido University Press, Sapporo 1986

64 Naito S, Kong FH, Hawkins BR, Mehra NK, Serjeantson SW and Hammond MG. **Joint Report: HLA and Disease: SLE.** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p364 Hokkaido University Press, Sapporo 1986

65 Hawkins BR, Chan SH, Charoenwongse P, Guo SS, Hammond MG, Pei J, Sun YP, Tian D, Ye GY and Yi YN. **Thyroid disease Joint Report.** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p369 Hokkaido University Press, Sapporo 1986

66 Sewdarsen M, Hammond MG, Vythilingum S and Appadoo B. **Histocompatibility Antigens in Indian patients with myocardial infarction.** *Tissue Antigens* 29,21. 1987

67 Maharaj B, Hammond MG, Appadoo B, Leary WP and Pudifin DJ. **HLA A, B, C and DR antigens in black patients with rheumatic heart disease.** *Circulation* 76, 259. 1987

68 Omar MAK, Hammond MG, Motala AA and Seedat MA. **HLA Class I and II antigens in South African Indians with NIDDM.** *Diabetes* 37, 796. 1988

69 Johnson N, Moodley J and Hammond MG. **Human leucocyte antigen status in African women with eclampsia.** *Brit J Obs Gyn* 95, 877. 1988

70 Albert ED, Chandanayingyong D, Thompson JS, Zhao T, Hammond MG, Naito S and Sierp GM. **Antigen Society #9 Report (Bw46 and the subgroups of B15)** In: Dupont B (ed).*Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p153-195.

71 Sekiguchi S, Neumeyer N, Kashiwagi N, Tsuji K, Kobayashi K, Konoeda Y, Ohkubo M, Atoh M, Tokunaga K, Yagita A, Inoko H, Fong R, Mervart H, Paik Y, Reekers P, Hammond MG, du Toit ED and Call E. **Antigen Society #12 Report (Bw54, Bw55, Bw56 and Bw42)** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p199-201.

72 Arnaz-Villena A, Belvedere M, Decary F, Fotino M, Heise E, Hogan V, Martinetti M, Muller C, Richiardi P, Vicario JL, Barbanti M, Bruyere J, Caruso C, Conighi C, Gelsthorpe K, Hammond MG, Lopez-Larrea C, Mervart H, Peruccio D, Regueiro JR and Schreuder I. **Antigen Society #15 Report (Bw4 and Bw6)** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p214-216.

73 Conighi C, Contu L, Grappa MT, du Toit ED, Hammond MG, Lulli P, Mayr WR, Menicucci A, Mervart H and Pupura M. **Antigen Society #18 Report (Cw4 and Cw6)** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p222-235.

74 Stastny P, Layrisse Z, Singal DP, Svejgaard A, van den Berg-Loonen E, Dohi K, Caraballo L, Chiewsilp P, Colombe B, Fauchet R, Haas E, Hammond MG, Jakobsen BK, Knight S, Lee J, Mervart H, Schreuder GMT and Sullivan K. **Antigen Society #24 Report (DRw11, DRw12, DRw8)** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p249-251.

75 Cambon-Thomsen A, Calot M, Sommer E, Ohayon E, Goeken N, Kaplan C, Mattiuz PL, Menicucci A, Cross R, Tait B, Buc M, Jeannet M, Irle C, Mayer S, Tongio MM, Contu L, Purpura M, Nikaen A, Mervart H, Sullivan K, Schweizer R, Hansen JA, du Toit ED and Hammond MG. **Antigen Society #26 Report (DR3, DR7, DQw2): Part 1** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p255-258.

76 Gazit E, Fauchet R, Jones M, van Leeuwen A, Longo A, Mahoney R, Navarrete C, Richiardi P, Tongio MM, Altshuler R, Bouhallier O, Balboni S, Belvedere M, Cappelacci S, Crepaldi T, Ferrara GB, Hammond MG, Lulli P, Martinetti M, Savi M, D'Amaro J, Yunis EJ and van Rood JJ. **Antigen Society #31 Report Part 2: Antigen Society #31 Report.** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p281-282.

77 Paulsen G, Markussen G, Acton RT, Tiercy JM, Hammond MG and Fauchet R. **RFLP Standardization Report for DR Beta/Hind III**: In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p598-600.

78 Paulsen G, Markussen G, Barger BO, Fauchet R, Hammond MG and Tiercy JM. **RFLP Standardization Report for DP Beta/HindIII** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p662-663.

79 Mody GM, Hammond MG and Naidoo PD. **HLA associations with rheumatoid arthritis in African blacks.** *J Rheum* 16:10 1326 1989

80 Omar MAK, Hammond MG, Desai RK, Motala AA, Abboo N and Seedat MA. **HLA Class I and II antigens in South African blacks with Graves' disease.** *Clin Imm Immunopath* 54;98 1990

81 Maharaj B and Hammond MG. **HLA-A, B, DR and DQ Antigens in Black patients with idiopathic dilated cardiomyopathy.** *Amer J Cardiology* 65; 1402. 1990

82 Johnson N, Moodley J and Hammond MG. **HLA Status of the Fetus Born to African Women with Eclampsia.** *Clin and Exper Hyper in Pregnancy* B9 (3): 311. 1990

83 Hammond MG, Hsu M-M, Ko J-Y, Hsieh R-P, Yang C-S. **Preliminary results of HLA Class I and Class II antigens in Chinese with nasopharyngeal carcinoma.** In: Ablashi DV et al (eds) *Epstein-Barr Virus and Human Diseases*, p407. Humana Press, Clifton, New Jersey. 1991.

84 O'Farrell N and Hammond MG. **HLA antigens in Donovanosis (Granuloma Inguinale).** *Genitourin Med*; 67:400-402 1991

85 Bhigjee AI, Hammond MG, Bill PLA and Windsor IM. **HLA Profile and HTLV-I Associated Myelopathy (HAM/TSP) in Natal, South Africa.** *J Neurology, Neurosurgery and Psychiatry*; 55:329-330 1992

86 Hammond MG, Tokunaga K, Fotino M, Grunnet N, Graugaard B and Vives J. **Antigen Society #112 Report.** In: Tsuji K (ed) *HLA 1991* Oxford University Press, Oxford (in press)

87 Hammond MG, Marcelli A and Poirier JC. **Complement polymorphism in African Blacks.** In: Tsuji K (ed) *HLA 1991*. Oxford University Press, Oxford (in press)

88 Hammond MG, du Toit ED, Sachs JA, Kaplan C and Mbayo K. **HLA in Southern African Black Populations.** In: Tsuji K (ed) *HLA 1991* Oxford University Press, Oxford (in press)

89 Bodmer JG, Tonks S, Oza AM, Mikata A, Takenouchi T, Lister TA and collaborating centres. **11th International Histocompatibility Workshop Hodgkin's Disease Study.** In: Tsuji K (ed) *HLA 1991* Oxford University Press, Oxford (in press)

90 Hammond MG. **HLA and mate selection.** (submitted to *Human Immunology*)

91 Hammond MG. **Paternity calculations in court.** (submitted to *Forensic Science International*).

92 van Wyk CW, Hammond MG. **HLA associations in oral submucous fibrosis caused by chewing betel nuts** (in preparation)

I am a member of the international Transplantation Society; a founder member of the South African Transplantation Society and of the South African Immunology Society. I am also a member of the South African Society of Human Genetics.

Part I

THE DEFINITION OF HLA ANTIGENS

Introduction

Human lymphocyte antigens (HLA) occur on lymphocytes and all other nucleated cells. They were catapulted into importance when their function as histocompatibility antigens made them an essential part of transplantation programmes.

The definition of the antigens, in all their complexities of 'splits' and cross-reactions was facilitated by International Histocompatibility Workshops. The study of HLA antigens in different race groups emphasised the complexities of this system and revealed the difficulties in defining the antigens in different races.

The HLA system is one of the most complex antigenic systems known in man. There are at least 15 loci determining histocompatibility antigens and they are sufficiently close to exhibit linkage, ie they segregate together. The products of the A, B and C loci are glycoprotein components of the plasma membrane of nucleated cells and are referred to as Class I antigens while the Class II genes control the expression of DR, DQ and DP antigens which have a restricted distribution, notably on B lymphocytes. The number of clearly defined antigens has increased dramatically as a result of a series of International Histocompatibility Workshops and the fact that nearly all the genes have now been sequenced. There are now 82 Class I antigens and 33 Class II antigens that can be serologically defined. However, 153 Class II alleles have been defined by DNA sequencing and this has proved a useful tool in establishing defined serological reactions for use in tissue typing for transplantation.

The frequency of these antigens varies in different races and antigens which are rare or of low frequency in Caucasians are often more common in other races. Linkage disequilibrium is the tendency for some alleles at different loci to occur together more often than would be expected from the frequencies of the individual alleles concerned. In different populations, linkage disequilibrium produces different haplotypes; these haplotypes are frequently characteristic of population groups.

The first HLA antigen was described by Dausset in 1958 and was called 'Mac'. In 1959 van Rood et al. described leucocyte antigens 2 and 3. Rapid progress followed the observation that leucocyte antibodies are present in the sera of about 10% of parous women.

Tremendous progress was made as a result of a series of International Histocompatibility Workshops. In 1964, the first International Histocompatibility Workshop was held to compare various tissue typing methods. The second Workshop in 1965 showed that different laboratories, using different techniques, could detect the same specificities. The third Workshop studied families and showed the inheritance patterns. The locus was named HLA. A standardised technique - the microlymphocytotoxicity test of Terasaki - was introduced for the fourth Workshop in 1970. The use of micro quantities of serum made it possible for many laboratories to participate by sending small amounts of sera through the mail.

Anthropology was the focus of the fifth Workshop in 1972 and our early work on the distribution of HLA antigens in three race groups led to an invitation from the organiser of the Workshop, Jean Dausset, to present our results. Only 29 laboratories in the world participated in testing 49 different ethnic populations. I have since participated in all the International Histocompatibility Workshops and our studies have been accepted in the series "Histocompatibility Testing" which is published after each Workshop. The C locus was clearly identified during the sixth Workshop (1975) and the seventh Workshop concentrated on the definition of the DR antigens by typing B lymphocytes in 1977. The eighth Workshop (1980) was able to define 78 specificities and in 1984 the ninth Workshop explored the DQ and DP loci. Molecular biology was introduced at the Tenth Workshop (1987). By this time 157 laboratories were involved with the serological aspects but we were one of only 80 laboratories world-wide that performed Southern blots on DNA extracted from lymphocytes in one of the earliest attempts to define the genes responsible for the Class II determinants. The Eleventh International Histocompatibility Workshop introduced a refinement of the earlier methods of studying the DNA of HLA genes by using the polymerase chain reaction (PCR) to amplify specific alleles and detecting slight variations with sequence specific oligonucleotide probes (SSO's) by means of "dot-blots".

In addition to the International Histocompatibilty Workshops, I participated in the Asia-Oceania Histocompatibility Workshops which are organised on a regional basis and involve most of the HLA laboratories bordering the Pacific ocean. I participated in the Second and Third Asia-Oceania Histocompatibility Workshops and I am now a Councillor for this series of Histocompatibility Workshops.

The forty nine papers dealing with the definition of HLA antigens in the different races from 1968 to the present form Part I of this thesis.

SEROLOGY OF HLA

p10 Brain P and Hammond MG. **Leucocyte antigens in three race groups.** *Med Proc* 14: 589, 1968

p14 Hammond MG and Brain P. **Reactions of HLA antisera in three populations.** *Vox Sang* 20: 492, 1971

p22 Hammond MG, Appadoo B and Brain P. **HLA antigens and antibodies in South African Bantu.** *Transplantation* 14: 159, 1972

p29 Hammond MG, Appadoo B and Brain P. **HLA antigens and antibodies in South African Indians.** *Tissue Antigens* 2: 389, 1972

p37 Hammond MG, Appadoo B and Brain P. **Subdivision of HLA 5 and comparative studies of the HLA polymorphism in South African Indians.** *Tissue Antigens* 4, 42. 1974

p45 Hammond MG, Appadoo B and Brain P. **HLA antigens in South African Negroes and Indians.** *Tissue Antigens* 10: 230, 1977

p46 Hammond MG and Brain P. **Leucocyte groups in baboons tested with human antisera.** *S Afr Med J* 44: 380, 1970

p49 Downing HJ, Brain P, Hammond MG, Vos GH and Webb GR. **Leucocyte antigens of baboons.** *Transplant Proc* 4: 33, 1972

p53 Downing HJ, Criticos A, Burgess LE and Hammond MG. **An antigen resembling HLA 7 on the leucocytes of vervet monkeys.** *J Med Primat* 7: 174, 1978

HISTOCOMPATIBILITY TESTING 1972

p62 Brain P and Hammond MG. **Frequency of HLA antigens in South African Bantu, Indians and Caucasians.** In: Dausset J, Colombani J (eds) *Histocompatibility Testing 1972* p433. Munksgaard, Copenhagen. 1972

HISTOCOMPATIBILITY TESTING 1975

p70 Hammond MG, Appadoo B and Brain P. **HLA antigens in Bantu and Indians.** In: Kissmeyer-Nielsen F (ed) *Histocompatibility Testing 1975* p173 Munksgaard, Copenhagen. 1975

HISTOCOMPATIBILITY TESTING 1977

p77 Hammond MG, Appadoo B and Brain P. **HLA in non-Caucasian populations.** In: Bodmer W F et al.(eds) *Histocompatibility Testing 1977* p407. Munksgaard, Copenhagen. 1977

p78 Schreuder I, Bos A and Hammond MG. HLA-Bw40 is heterogeneous In: Bodmer W F et al.(eds) *Histocompatibility Testing 1977* p408. Munksgaard, Copenhagen. 1977

p80 Biegel A, Botha MC, Bouysou C, Briggs B, Hasty B, Herbert S, Pollack M, Wolf E, Duquesnoy R and Hammond MG. **Joint Report. The "Black" antigens : Aw36, Aw34, Aw43 and Bw42.** In: Bodmer W F et al.(eds) *Histocompatibility Testing 1977* p164. Munksgaard, Copenhagen. 1977

p82 Amos DB, Engelfriet P, Hammond MG, Mazzilli C, Payne R, Richiardi P and Ting A. **Joint Report. B5, Bw51, Bw52, Bw53, Bw35.** In: Bodmer W F et al.(eds) *Histocompatibility Testing 1977* p166. Munksgaard, Copenhagen. 1977

HISTOCOMPATIBILITY TESTING 1980

p88 Hammond MG. **Further splits of HLA B5.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p758. UCLA Tissue Typing Laboratory, Los Angeles. 1980

p90 Hammond MG and Appadoo B. **Confirmation of ST-1 in Asian Indians.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p844. UCLA Tissue Typing Laboratory, Los Angeles. 1980

p91 Hammond MG. **Heterogeneity of HLA B40.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p782. UCLA Tissue Typing Laboratory, Los Angeles. 1980

p93 Hammond MG and Lamm L. **A/C crossover in a South African Indian family.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p794. UCLA Tissue Typing Laboratory, Los Angeles. 1980

p94 Hammond MG. **Antigen Report. HLA Bw53.** In: Terasaki PI (ed) *Histocompatibility Testing 1980* p429. UCLA Tissue Typing Laboratory, Los Angeles. 1980

SECOND ASIA-OCEANIA HISTOCOMPATIBILITY WORKSHOP

p99 Hammond MG. **Antigen Report. HLA Bw35.** In: Simons MJ, Tait BD (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p112. 1983

p102 Hammond MG. **Antigen Report. HLA Bw53.** In: Simons MJ, Tait BD (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p65. 1983

p103 Hammond MG. **Antigen Report. HLA Cw4.** In: Simons MJ, Tait BD (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p138. 1983

p104 Hammond MG. **Subdivision of HLA B15 in Indians.** In: Simons MJ, Tait BD (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p413. 1983

p107 Hammond MG. **Anomalous reactions with Bw4 sera in Indian families.** In: Simons MJ, Tait Bd (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p411. 1983

HISTOCOMPATIBILITY TESTING 1984

p110 Hammond MG, Betuel H and Gebuhrer L. **Antigen Report. HLA A29.** In: Albert ED et al. (eds) *Histocompatibility Testing 1984* p126. Springer-Verlag, Berlin 1984

p111 Campbell EM, du Toit ED, Hammond MG and Oudshoorn M. **Antigen Report. Aw43.** In: Albert ED et al. (eds) *Histocompatibility Testing 1984* p132. Springer-Verlag, Berlin 1984

p113 Taylor C, Ting A, Hammond MG, de Waal L, de Lange GG and Engelfriet P. **Antigen Report. Bw53.** In: Albert Ed et al. (eds) *Histocompatibility Testing 1984* p161. Springer-Verlag, Berlin 1984

p114 Chandanayingyong D, Cambon-Thomsen A and Hammond MG. **Bw62 and other B15 variants - Bw6 associated.** In: Albert ED et al. (eds) *Histocompatibility Testing 1984* p169. Springer-Verlag, Berlin 1984

p116 Cambon-Thomsen A, Chandanayingyong D, Thomsen M and Hammond MG. **Bw63 and other Bw4 associated variants.** In: Albert ED et al. (eds) *Histocompatibility Testing 1984* p171. Springer-Verlag, Berlin 1984

p118 Hammond MG. **Definition of Bw53 in South African Indians.** *Ninth International Histocompatibility Workshop Newsletter VI* p6 1984

p119 Appadoo B and Hammond MG. **Splits of DR4 in South African Indians.** *Ninth International Histocompatibility Workshop Newsletter VI* p15 1984

p121 Hammond MG. **The HLA A10 and Aw19 complex in South African Indians and Negroes.** *Ninth International Histocompatibility Workshop Newsletter VII* p6 1984

p123 Hammond MG. **HLA B15 complex in South African Indians.** *Ninth International Histocompatibility Workshop Newsletter VIII* p4 1984

p126 Hammond MG. **Short Bw41.** *Ninth International Histocompatibility Workshop Newsletter VIII* p9 1984

THIRD ASIA-OCEANIA HISTOCOMPATIBILITY WORKSHOP

p128 Hammond MG. **Antigen Report HLA A3.** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p27 Hokkaido University Press, Sapporo 1986

p130 Hammond MG. **Antigen Report HLA A11.** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p29 Hokkaido University Press, Sapporo 1986

p132 Mehra NK, Taneja V, Kailash S, Chaudhuri TK, Vaidya MC, Balakrishnan K, Contractor N, Seth GS, Hammond MG, Undevia JV and Khan R. **North Indians - Ethnic Study** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p271. Hokkaido University Press, Sapporo 1986

p138 Hammond MG and Appadoo B. **HLA antigens in African Blacks.** In: Aizawa M (ed) *HLA in Asia-Oceania 1986* p316 Hokkaido University Press, Sapporo 1986

HISTOCOMPATIBILITY TESTING 1987

p143 Albert ED, Chandanayingyong D, Thompson JS, Zhao T, Hammond MG, Naito S and Sierp GM. **Antigen Society #9 Report (Bw46 and the subgroups of B15)** In: Dupont B (ed).*Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p153-195.

p186 Sekiguchi S, Neumeyer N, Kashiwagi N, Tsuji K, Kobayashi K, Konoeda Y, Ohkubo M, Atoh M, Tokunaga K, Yagita A, Inoko H, Fong R, Mervart H, Paik Y, Reekers P, Hammond MG, du Toit ED and Call E. **Antigen Society #12 Report (Bw54, Bw55, Bw56 and Bw42)** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p199-201.

p190 Arnaz-Villena A, Belvedere M, Decary F, Fotino M, Heise E, Hogan V, Martinetti M, Muller C, Richiardi P, Vicario JL, Barbanti M, Bruyere J, Caruso C, Conighi C, Gelsthorpe K, Hammond MG, Lopez-Larrea C, Mervart H, Peruccio D, Regueiro JR and Schreuder I. **Antigen Society #15 Report (Bw4 and Bw6)** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p214-216.

p194 Conighi C, Contu L, Grappa MT, du Toit ED, Hammond MG, Lulli P, Mayr WR, Menicucci A, Mervart H and Pupura M. **Antigen Society #18 Report (Cw4 and Cw6)** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p222-235.

p208 Stastny P, Layrisse Z, Singal DP, Svejgaard A, van den Berg-Loonen E, Dohi K, Caraballo L, Chiewsilp P, Colombe B, Fauchet R, Haas E, Hammond MG, Jakobsen BK, Knight S, Lee J, Mervart H, Schreuder GMT and Sullivan K. **Antigen Society #24 Report (DRw11, DRw12, DRw8)** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p249-251.

p211 Cambon-Thomsen A, Calot M, Sommer E, Ohayon E, Goeken N, Kaplan C, Mattiuz PL, Menicucci A, Cross R, Tait B, Buc M, Jeannet M, Irle C, Mayer S, Tongio MM, Contu L, Purpura M, Nikaen A, Mervart H, Sullivan K, Schweizer R, Hansen JA, du Toit ED and Hammond MG. **Antigen Society #26 Report (DR3, DR7, DQw2): Part 1** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p255-258.

p215 Gazit E, Fauchet R, Jones M, van Leeuwen A, Longo A, Mahoney R, Navarrete C, Richiardi P, Tongio MM, Altshuler R, Bouhallier O, Balboni S, Belvedere M, Cappelacci S, Crepaldi T, Ferrara GB, Hammond MG, Lulli P, Martinetti M, Savi M, D'Amaro J, Yunis EJ and van Rood JJ. **Antigen Society #31 Report Part 2: Antigen Society #31 Report.** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p281-282.

HISTOCOMPATIBILITY TESTING 1991

p219 Hammond MG, Tokunaga K, Fotino M, Grunnet N, Graugaard B and Vives J. **Antigen Society #112 Report.** In: Tsuji K (ed) *HLA 1991* Oxford University Press, Oxford (in press)

p223 Hammond MG, du Toit ED, Sachs JA, Kaplan C and Mbayo K. **HLA in Southern African Black Populations.** In: Tsuji K (ed) *HLA 1991* Oxford University Press, Oxford (in press)

p226 Hammond MG, Marcelli A and Poirier JC. **Complement polymorphism in African Blacks.** In: Tsuji K (ed) *HLA 1991* Oxford University Press, Oxford (in press)

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LEUCOCYTE ANTIGENS IN THREE RACE GROUPS

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The antigens of leucocytes are important in transplantation, and there is already some evidence[1-3] that their incidence, like that of the red cell antigens, varies from one race to another. In this country tissue typing may have to be done on donors and recipients of at least 3 different races. It is therefore necessary to know something of the distribution of these antigens in the main population groups. This paper reports a preliminary study in which a number of antisera were characterized and tested against the white cells of 3 groups of donors, viz. White (European), Bantu and Indian.

MATERIAL AND METHODS

The EDTA agglutination test of van Rood *et al.*[4] was used with certain modifications. Red cells were sedimented with 3% gelatin in normal saline. Only one drop of antiserum was used for each test; the quantities of the other reagents were correspondingly reduced. All tests were read by the same worker. No sera were absorbed.

Sera from pregnant women were screened daily against the white cells of 4 blood donors, and larger samples obtained from some of the women found to have antibodies. From these samples 39 of the most avid sera were selected. Nothing was known in advance of their specificity; 19 were from Coloured (mixed Bantu-White), 14 from White, 4 from Indian and 2 from Bantu donors. Reference sera obtained through the collaborative programme of the Transplantation Immunology Branch, National Institutes of Health, Bethesda, Md., were run in parallel with these antisera.

White cell donors (40 each of Whites, Bantu and Indians) were healthy adult staff members or blood donors. These race groups are relatively pure in the sense that there has been little intermarriage between the groups. Bantu were of the Zulu tribe. The Indians were inhabitants of Natal whose forefathers (mostly Hindi, Tamil and Telegu speakers) came from India about 60 years ago.

The White series was begun first, and 3 sera (84, 86 and 88) were introduced too late to be included in it. With this exception all the sera were tested against the white cells of all the donors. From the laboratory protocols the results for each race group were assembled in a large matrix, coding any positive result as 1, negative as 0. The rows of these 3 matrices (each representing the reactions of one serum with the cells of 40 donors) were transferred to punch cards and the reactions within the race group of each serum compared with those of every other, using a comparator that has been described elsewhere.[5] The output of this comparator was fed to a Diehl Combitron calculator programmed to compute χ^2 for 2 × 2 tables. Yates' correction was not applied.

A further 32 White donors were studied, but in order to make the results exactly comparable in the 3 populations these results were not considered when calculating χ^2. The frequencies with which the White sera react, however, are calculated from the larger sample.

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RESULTS

Fig. 1 shows the associations of the sera in each population group. Each serum is represented by a circle of diameter proportional to the frequency with which it reacts. Positive associations between sera with χ^2 of 6.6 or more are represented by solid lines. Those with a χ^2 of 10 or more have thicker lines. Dotted lines represent negative associations with a χ^2 of at least 3.0.

The sera fall into 5 well-defined groups, corresponding to the antigenic complexes 7d, 6b-7c, 4a, 4b and an unidentified group defined by the 2 sera 27 and 29. The 4a and 4b groups, against which no reference sera were included in the run, were identified afterwards by 2 sera of known specificity. The anti-8a reference serum (Pinquette) is not consistently associated with any group; neither is the serum T0/01/II of Ceppellini, which recognizes his antigen To1.

DISCUSSION

The criteria of association must be explained. Two sera with identical reactions (as long as these are neither all positive nor all negative) will be positively associated by a χ^2 equal to the number of individuals in the panel, here 40. The maximum value of χ^2 thus depends on the size of the panel, and any quoted value of χ^2 means little unless this is stated. A comparatively high level of χ^2 can be adopted as the criterion of positive association. But where 2 reliable antisera are recognizing the products of a pair of antithetical alleles, many individuals will type positive with both sera because

they are heterozygotes. This greatly reduces the maximum value of χ^2 for the kind of negative association of the greatest interest, that between alleles. As Dausset[1] has shown, it is therefore only reasonable to adopt a much lower standard of χ^2 for negative associations.

No 2 sera gave identical results in all race groups, but 49 and 88 were identical in the Bantu. In the Indian group they are associated by a χ^2 of 15.3.

The same well-marked groups of tightly associated sera appear in all 3 populations, and each of these groups of sera identifies a complex of antigenic factors that are frequently inherited in association.[6] The important entities, at this stage of knowledge, are the antigenic complexes; they must not be regarded as simple antigens. An antiserum described as anti-4a, for example, recognizes a certain arbitrary though common combination of antigens within the 4a group. It is of no greater or less value in tissue typing than another antiserum in the same group that recognizes a slightly different combination of antigens. Hence it is essential to use batteries of related antisera against each of the complexes when undertaking tissue typing for transplantation. They will not all give the same results.

TABLE 1: MEAN FREQUENCIES OF ANTIGENIC COMPLEXES IN THREE RACE GROUPS

(FIGURES IN BRACKETS SHOW NUMBER OF SERA USED TO IDENTIFY THE COMPLEX)

Complex	*% Frequency*		
	White	*Indian*	*Bantu*
4a	54(6)	78(5)	68(7)
4b	84(3)	82(5)	93(5)
8a	52(1)	60(1)	70(1)
6b	47(1)	55(1)	52(1)
7c	32(4)	24(5)	36(5)
7d	28(8)	30(10)	42(9)
To1	72(1)	78(1)	70(1)
Unknown (sera 27, 29)	21(2)	22(2)	26(2)

Many of the sera appear in the same tightly associated groups in all 3 populations. Such, e.g. are 12, 72, 25, 64, 66 and Willett in the anti-7d group; 9. 10, 14 and 76 in anti-6b-7c; 44, 7, and 32 in anti-4a; and 23, 46 and 49 in anti-4b. From such sera a general-purpose tissue-typing kit could be assembled for use with any of the 3 race groups. But to do this would exclude many sera; examples are 51, 48 and 52 which are tightly associated with the 7d group in the Indians but not in the Whites. Dausset[1] has also observed that some of the sera identifying a complex in one population do not identify it in another. It is better, therefore, to have a special set of sera for use with each race group. Such sera should be chosen because they have several strong positive associations within the group and few or none, except negative ones, outside it. Using such sets of sera we can calculate a mean frequency of each antigenic complex in each race group, as Table 1 shows.

There are some interesting differences between the race groups:

Serum 73 is a member of the anti-4a group in Whites and Bantu, but among the Indians it is associated with 54, a member of the anti-4b group: 54 in its turn is a respectable member of anti-4b in Indians and Bantu, but in the Whites it has a much lower frequency and no strong associations with this group;

48 is not strongly associated with the anti-7d group in Whites; in both the other populations it is;

51 is in the anti-7d group in the Indians, but in anti-6b-7c in the Bantu;

84 is connected with the anti-4a group in the Bantu; in the Indian its only strong associations are negative ones with the new group 27/29, whose specificity is unknown.

The complex of anti-7d sera is larger and shows more numerous and stronger associations between its members in the Indians than in Whites.

The 4a and 4b complexes, by our criteria of association, are negatively associated in the Whites, less strongly so in the Indians, and not at all in the Bantu. In the Bantu there are many strong negative associations between 6b-7c and 4a. These are weaker in the Whites and absent in the Indians.

The sera of the anti-7c group are almost entirely contained in the reference serum anti-6b (Rens) when tested against the Indian and White panels (i.e. they seldom give positive results when Rens is negative), but there are many exceptions to this in the Bantu. Fig. 1 shows several other differences between the race groups. Because the panels are small, such associations (or the lack of them) should be treated with some reserve.

Differences in the frequencies of leucocyte antigens may be of interest to anthropologists. Dausset,[1] in a study of a small sample of Negroes from the West African state of Mali, found lower frequencies of 8a, 4a, 4b, 7d and 6b than in the French population. New York Negroes[2] also had lower frequencies of 8a, 4a, 7c and 7d; they were not tested for 4b. The findings in the Bantu are quite different. Every antigen we could test for, except To1, had a higher frequency in the Bantu than in the White group. The Indian group appears to have a higher incidence of 4a, and a lower one of 4b, than either of the other populations. Figures such as these are, of course, to some degree arbitrary, as they depend on the choice

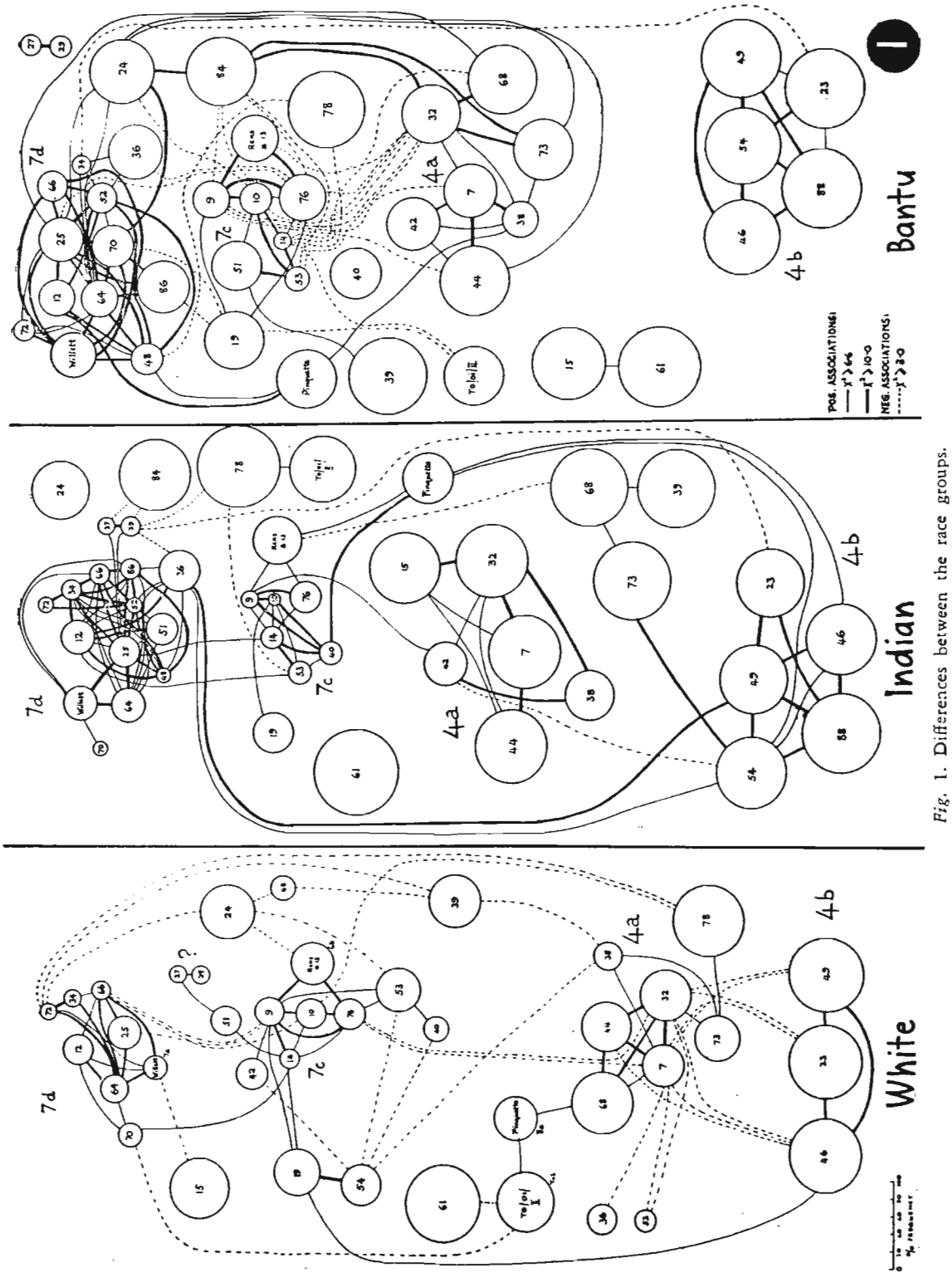

Fig. 1. Differences between the race groups.

of sera used to obtain them. Until more work has been done they should, therefore, be regarded as provisional.

It is not clear why we failed to find a good anti-8a serum. Such sera are common. They are avid and would thus not be excluded by our criteria of selection. These criteria may well have excluded sera like anti-7a and anti-7b, which are said[7] to give weak and unreliable results. No allowance has been made in this study for false negative reactions caused by the ANAP (agglutination negative absorption positive) phenomenon, but our choice of avid sera probably makes this unimportant.[7]

Several of the sera that have not been classified probably detect known specificities; 61 may be anti-5b. The group detected by the sera 27 and 29 is a well-defined one, but does not appear to correspond to any of van Rood's specificities.

The knowledge gained from this study will help us to undertake tissue typing in 3 race groups with more confidence, but it is still incomplete. Rubinstein *et al.*[3] have pointed out that new antigenic specificities may be found as new populations are examined and that to detect some of these it may be necessary to use antisera derived from the population groups concerned. We had relatively few Bantu and Indian sera in this study, and not surprisingly did not detect convincingly any new groups confined to one population. If experience with red cell antigens is any guide, we may expect to find such specificities in the future.

SUMMARY

Thirty-nine leucoagglutinating sera from pregnant women were tested against the white cells of 3 panels of 40 donors each, from the White, Indian and Bantu race groups.

Many of the sera could be classified into groups detecting the 7d, 6b-7c, 4a and 4b antigenic complexes, together with another complex of unknown specificity.

The sera identifying each of these complexes differed in number and in their interrelationships from one population group to another.

The frequency of the 7d, 6b-7c, 4a, 4b and 8a antigenic complexes was higher in the Bantu than in the Whites. The Bantu thus differ notably from West African and American Negroes, who have been found by other workers to have lower frequencies than Whites for most of these complexes.

Groups of sera chosen to identify the antigenic complexes in each race group were assembled for use in tissue typing.

We are grateful to Dr. Kayhoe and Dr. Ohanesian of the NIH Transplantation Immunology Branch for valuable reference sera, and to Mrs. G. C. Buckle, Miss B. Hall and Mrs. A. D. Skinner for help in the laboratory.

REFERENCES

1. Dausset, J., Ivanyi, P. and Ivanyi, D. (1965): *Histocompatibility Testing*, 1965, p. 51. Copenhagen: Munksgaard.
2. Dausset, J., Ivanyi, P., Colombani, J., Feingold, N. and Legrand, L. (1967): *Histocompatibility Testing*, 1967, p. 189. Copenhagen: Munksgaard.
3. Rubinstein, P., Costa, R., van Leeuwen, A. and van Rood, J. J. (1967): *Histocompatibility Testing*, 1967, p. 251. Copenhagen: Munksgaard.
4. van Rood, J. J., van Leeuwen, A., Schippers, A. M. J., Pearce, R., van Blankenstein, M. and Volkers, W. (1967): *Histocompatibility Testing*, 1967, p. 203. Copenhagen: Munksgaard.
5. Brain, P. (1968): Transplantation. In the press.
6. Amos, D. B. (1967): Transplantation, **5**, 1015.
7. van Rood, J. J., van Leeuwen, A., Schippers, A. M. J., Vooys, W. H., Frederiks, E., Balner, H. and Eernisse, J. G. (1965): *Histocompatibility Testing*, 1965, p. 37. Copenhagen: Munksgaard.

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Reactions of HL-A Antisera in Three Populations

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Abstract. Cytotoxic antisera against HL-A antigens were tested in panels of Caucasian, Bantu and Indian donors. A serum that gives reliable results in Caucasians may prove quite unreliable when used in Bantu or Indians, since it may possess extra antibodies against antigens that are very rare in Caucasians but common in the other groups. Every serum that is to be used in a population different from that in which it was standardized must therefore be re-standardized in the new group before use. Results obtained in previous studies by EDTA agglutination cannot be compared with those obtained by cytotoxicity. The NIH serum WILLETT (anti-HL-A 8) gives identical results by agglutination and by cytotoxicity when tested in Caucasians; but in the Bantu it reacts with 49% by agglutination and only 8% by cytotoxicity. The Bantu evidently possess several unidentified HL-A antigens.

It is now well established [2–4, 7, 8] that the frequency of HL-A antigens differs considerably from one population group to another. In an earlier study, [2], using leukoagglutination, we found – as DAUSSET [3] had previously observed – that a serum giving reliable results in one population group would not necessarily do so in another. The hospital population in this part of South Africa consists of 3 important groups, Bantu, Caucasians and Indians, and it soon became clear to us that if we were to perform reliable tissue typing we must have sera that have been tested and found effective in all 3 groups. Since adopting the microcytotoxicity test we have screened some 20,000 sera of parous women in our own laboratory. We present in this paper some of the results obtained with selected sera of our own, and with sera from the National Institutes of Health and other sources.

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Materials and Methods

Lymphocytes were isolated by the method of BOYUM [1], using a Ficoll-Isopaque mixture, and a final suspension was made to contain between 4,000 and 6,000 cells/cu mm. The cytotoxicity test was performed in 60-well Microtest tissue culture plates (Falcon Plastics) using the two-stage method recommended by the National Institutes of Health plus trypan blue, as follows: antisera were dispensed in 1 µl amounts under paraffin, and 1 µl of the cell suspension added. After 30 min at room temperature 5 µl of fresh unabsorbed rabbit serum was added and the plates left to stand for 60 min. Five microliter of a fresh preparation of trypan blue, made daily by diluting a 1% aqueous stock solution with an equal volume of 1.7% saline, was then added. After 15 min at room temperature (20° C) the excess dye was flicked off the plates and they were examined with an inverted microscope and 20 x objective; phase contrast was not used. We have not found the results in plastic trays to be unreproducible, as suggested by DICK [5], as long as the two-stage procedure is used.

The serum donors were parous women of all race groups, but up to the time of this study there were relatively few Bantu among them. There were, however, a large number of coloured (mixed European and Bantu origin) donors, as many coloured women attended an antenatal clinic situated near our laboratory. Sera were screened daily against the lymphocytes of 6 blood donors. Screened but uncharacterized sera were obtained also from the South African Institute for Medical Research, Johannesburg. Reference sera were obtained from the National Institutes of Health bank and through them (in ready prepared trays) from Dr. P. I. TERASAKI; also from the National Tissue Typing Reference Laboratory, Bristol (Dr. G. H. TOVEY), from commercial sources (identified by the prefix C), and one (394 CH) from the Massachusetts General Hospital (Dr. PAUL S. RUSSELL). Positively reacting sera from the screening tests were put up, together with reference sera, against panels of donors who were all in the first instance Caucasian. An IBM 1130 computer was used to compare the reactions of every serum with those of every other and to print out X^2 [2]. Selected sera were later tested in the same way against panels from the other 2 race groups.

Donors of lymphocytes were healthy unrelated adult blood donors and staff members of either sex. The Caucasian population of South Africa is of Western European origin. Bantu were almost all of the Zulu tribe. Indians are the descendants of immigrants who arrived about a century ago, principally from the Madras Presidency. The 3 groups are quite distinct in appearance and none of the individuals used by us appeared to be of mixed origin. The group of mixed origin which appears among the serum donors was not included among the lymphocyte donors.

The groups of antisera identifying the antigenic complexes were characterized in previous unpublished studies. Those groups used in this study that do not include a reference serum obtained from elsewhere contained (among others) sera characterized as follows against those supplied in trays by the National Institutes of Health (tray NIH 202), using a panel of 30 Caucasian donors:

Anti-HL-A10: Serum V 104, X^2 19.3 with both sera 2527.0 and 1617.1.
Anti-HL-A5: Serum S 21: X^2 23.1 with both 951.0 and 2532.

Table I. Percent frequency of reaction of sera in 3 population groups

Anti-	Serum	Origin[1]	% frequency		
			Caucasian	Bantu	Indian
HL-A1	324	I	49	0	38
	C1		49	3	43
	89	C	46	20	38
HL-A2	291	C	33	20	40
	C2		33	20	40
	317	C	36	25	40
HL-A3	394 CH		33	8	20
	C3		33	8	33
	STORM		31	8	35
	125	C	46	8	38
HL-A9	275	Co	23	23	10
	300	I	26	18	18
	C9		33	28	20
	JONES 05		36	25	18
	42	Co	33	50	38
HL-A10	V104		8	30	15
HL-A5	310	C	13	5	35
	S21		13	5	40
HL-A7	101	I	21	25	15
	247	Co	23	28	15
	130	C	26	35	18
HL-A8	GT29		33	13	8
	311	C	33	15	10
	284	C	46	15	8
	S71		44	63	23
	C8		41	45	20
HL-A12	137	C	28	18	20
	271	C	28	20	25
	GT61		23	18	18
	328	C	23	18	10
	320	I	28	38	23
Te10(BB)	V8		21	15	25
Te10+HL-A7	253	C	33	28	33
Te17(SL)	35	C	18	53	43
	S90		21	53	28
	24	Co	28	60	30
	131	B	21	45	33
Te50(4c)	204	Co	31	15	43
	301	Co	31	10	45

[1] Origins of sera: C = Caucasian. Co = coloured. B = Bantu. I = Indian.

Anti-HL-A7: Serum 247, X^2 25.5, 17.9, 17.9, and 25.5 respectively with Te 473.2, 4070, 3186.0 and 1953.0.
Anti-HL-A 12: Serum 271: X^2 19.8 with each of 719.1 and 975.1.
Anti-Te 17: Serum 35, X^2 17.4 with each of Te 3346.4 and Te 479.5.
Anti-Te 10: Serum V 8: X^2 18.5 with 2717.0, 18,9 with 2659.
Anti-Te 50: Serum 204, X^2 25.5 with each of Te 889.1 and Te 10.21; 15.1 with 2526.0.

Results

Table I shows the frequencies of reaction of each serum in each population, together with the population group (where known) of the serum donor.

Table II. Alleles detected in individuals of 3 population groups, Caucasian, Bantu and Indian

First (LA) sub-locus				Second (Four) sub-locus contd.			
Alleles	Number of individuals			Alleles	Number of individuals		
	C	B	I		C	B	I
HL–A1	7	0	7	Te10(BB)	3	3	2
2	3	5	4	Te17(SL)	1	10	7
3	3	3	2	HL–A5,7	0	1	1
9	5	6	6	5,8	1	0	0
10	2	5	3	5,12	0	0	2
1,2	4	0	6	5,Te10	0	0	3
1,3	4	0	2	5,Te17	0	0	4
1,9	3	0	0	7,8	1	1	1
2,3	4	0	3	7,12	3	2	1
2,9	2	0	1	7,Te10	1	2	1
2,10	0	3	2	7,Te17	1	4	0
9,10	1	3	0	8,12	1	0	0
Blank	1	15	4	8,Te10	2	0	0
Totals	39	40	40	8,Te17	0	2	0
Second (Four) sub-locus				12,Te10	0	0	3
HL–A5	3	0	5	12, Te17	3	2	0
7	2	2	2	Te10, Te17	2	1	0
8	8	1	2	Blank	4	8	5
12	3	1	1	Totals	39	40	40

Table II shows the alleles detected in the individuals of the 3 population groups (39 Caucasians, 40 Bantu, 40 Indians) at each of the sub-loci LA and Four.

Table III shows the reactions of one serum (Willett) tested by both EDTA agglutination [2] and by cytotoxicity in three population groups.

Table III. Antiserum Willett: % frequency of reaction in 3 population groups

Method	Caucasian	Bantu	Indian
Cytotoxicity (NIH)	31	8	13
Agglutination (EDTA)	31	49	40

Discussion

It is of interest to compare these results with those obtained by agglutination in our earlier study [2]. There we concluded that the Bantu were quite different from the West African and American Negroes, since they showed higher frequencies of most of the common antigens than did Caucasians. Negroes had been found, by cytotoxicity, to have lower frequencies. It is now clear that although our findings were correct our conclusions were not. Although the numbers tested are small it is probably safe to say that by cytotoxicity the Bantu show lower frequencies than Caucasians for HL-A 1, 2, 3, 8, Te 50. and perhaps HL-A 9, 5 and 12. Bantu frequencies are higher for Te 17 and HL-A 7. Using agglutination the findings are very different, the frequencies for HL-A 2 and 8 being higher in the Bantu than in the Caucasians. The NIH anti-HL-A 8 reference serum Willett, which works by both agglutination and cytotoxicity, is of great interest, It was used in earlier studies by us but not in this one since supplies were exhausted. The 1969 edition of the NIH catalogue states that its activity as an agglutinin corresponds exactly to its cytotoxic activity. In table III we see that this is perfectly true as long as

testing is confined to Caucasians. (The panels on which Willett was tested were not the same as in the present study.) But in the Bantu the frequency of reactions by cytotoxicity is 8 % and by agglutination 49 %, and there is also a lesser but still marked difference in Indians. Results obtained in a population by agglutination are internally perfectly consistent, but they cannot be compared with those obtained by cytotoxicity. It is obvious that the serum Willett is not identifying the same antigens by agglutination and by cytotoxicity. In Caucasians it appears to be doing so because the 2 antigens have the same frequency and are associated. In the Bantu and Indians they are quite distinct. The moral of this is that a serum that behaves perfectly in the population group against which it was originally characterised may perform quite differently in another. Consider the commercial cytotoxic serum C 8. When used in Caucasians, against whom it must have been originally standardised, this is an excellent serum. In the Bantu, however, it reacts with a frequency of 45 %, whereas the frequency of a true anti-HL-A 8 is 15 % or less. Results with the Bristol reference serum GT 29 and our serum 311 are similar in all 3 race groups; C 8 gives similar results in Caucasians (χ^2>23) but quite dissimilar in the Bantu (X^2 1.3). This commercial serum tested in the Bantu includes GT 29, but it is reacting also against another antigen that is evidently common in the Bantu and very rare in Caucasians; too rare, that is, to have been observed in the doubtless very extensive tests the serum received before being released for sale. We are not criticising this serum; in Caucasians it is almost perfect. The point we are making is that any serum that has been standardised in one population group must be re-standardised before it is used in another. We suspect that there may be no such thing as a monospecific serum; to misquote WIENER, the number of antibodies that can be detected is limited only by the ingenuity of the experimenter. Other sera that behave differently in different races include S 71, which resembles the commercial serum in its reactions but is not strongly associated with it in the Bantu and must therefore contain a different second antibody; 89, which might have been regarded as an acceptable anti-HL-A 1 had it not been tested in the Bantu; and 320. Some sera, however, are encouragingly uniform from one population to the next. Of the anti-HL-A 2 sera, the commercial product C 2 and our 291 are absolutely identical in all the 3 groups, and 317 is identical with them in Indians. All 4 anti-HL-A 3 sera (C 3, 394 CH, 125 and Storm) are identical in the Bantu.

We were gratified that table II shows no individual with more than 2 alleles at 1 sub-locus. We have not reported our findings for HL-A 11 and Te 19, since the sera are inadequately characterised; if included, they would abolish the only blank at the first sub-locus in the Caucasians and 3 of the 4 in the Indians. There would still be 14 individuals blank for the first sub-locus among the Bantu; while among those in whom only 1 allele was detected, some may be heterozygotes for an unknown antigen rather than homozygotes for a known one. The behaviour of some of the sera makes it clear that unknown antigens must be common in the Bantu. As RUBINSTEIN *et al.* [7] have observed, such antigens are likely to be found by using sera derived from the population groups concerned; we have already begun a study of sera from Bantu women in the hope of finding some of them.

Our frequencies for the antigens in the 3 race groups must be regarded as tentative because of the size of the panels and the uncertain reliability of the sera detecting some of the more obscure factors. The anthropological significance of these findings deserves more work on larger panels, and another paper.

Acknowledgments

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References

1. BOYUM, A.: Separation of leucocytes from blood and bone marrow. Scand. J. clin. lab. Invest. *21:* suppl. 97, p. 7 (1968).
2. BRAIN, P. and HAMMOND, M.: Leucocyte antigens in three race groups. Med. Proc. *14:* 589 (1968).
3. DAUSSET, J.; IVANYI, P., and IVANYI, D.: Tissue alloantigens in humans – identification of a complex system (Hu-1). Histocompatibility testing, p. 51 (Munksgaard, Copenhagen 1965).
4. DAUSSET, J.; IVANYI, P.; COLOMBANI, J.; FEINGOLD, N., and LEGRAND, L.: The Hu-1 system. Histocompatibility testing, p. 189 (Munksgaard, Copenhagen 1967).
5. DICK, H. M.: *cit.* KISSMEYER-NIELSEN and THORSBY Human transplantation antigens. Transpl. Rev. *4:* 1 (1970).
6. KISSMEYER-NIELSEN, F. and KJERBYE, K. E.: Lymphocytotoxic microtechnique: purification of lymphocytes by flotation. Histocompatibility testing, p. 381 (Munksgaard, Copenhagen 1969).

7. RUBINSTEIN, P.; COSTA, R.; VAN LEEUWEN, A., and VAN ROOD, J. J.: The leukocyte antigens of Mapuche Indians, ibid., p. 251 (Munksgaard, Copenhagen 1969).
8. SINGAL, D. P., MICKEY, M. R., and TERASAKI, P. I.: Serotyping for homotransplantation XXIX. Two new HL-A antigens. Transplantation *8:* 235 (1969).

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HL-A ANTIGENS AND ANTIBODIES IN SOUTH AFRICAN BANTU[1]

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SUMMARY

We previously found that nearly 35% of the Bantu had no antigens at the first locus detectable with the antisera available to us. The sera of 1,004 Bantu women were therefore screened and those containing antibodies were tested against 50 unrelated Bantu donors in parallel with known antisera, using the 2-stage microlymphocytotoxic test. Antisera for Te 63 and Te 66 were obtained from the National Institutes of Health (NIH). These two specificities almost completely filled the gap previously found at the first locus. Two hundred two of the 1,004 Bantu sera contained HL-A antibodies, but only one had the specificity anti-Te 63. One hundred twenty selected sera were then used to test a further 100 Bantu and 100 Caucasians. We tested for 10 antigens at the first locus, HL-A1,2,3,9,10,11, W28, W19, Te 63, and Te 66; and at the second locus we tested for 12 antigens, HL-A5,7,8,12,13, W5, W22, W15, W17, W10, and W27. HL-A1 has a very low frequency in the Bantu (5%) and no Bantu were found with HL-A11, while HL-A3 had a lower frequency (12%) than in Caucasians. W28 (19%), HL-A9 (17%), HL-A10 (23%), Te 63 (13%), and Te 66 (31%) all had higher frequencies in Bantu than in Caucasians. At the second locus, the frequency of HL-A7 was only 11% but W22 was found in 34% of the Bantu (5% in Caucasians). Thirty-five anti-HL-A12 sera could be divided into two groups, one reacting as a short anti-HL-A12.

There are significant differences in the frequencies of HL-A antigens in various races (*1–3, 7, 8, 11–13*). We have reported (*5, 9*) the antigen frequencies in small samples from the three large population groups of Durban: Caucasian, Indian, and Bantu.

This study is the result of our finding (*9*) that nearly 35% of the Bantu had no antigens at the first locus detectable with the antisera available to us. The corresponding percentages for Caucasians and Indians were 2.5 and 10%, respectively. Evidently, the Bantu possess, at relatively high frequencies, antigens that are unknown or rare in Caucasians. Antibodies against such antigens, therefore, might be expected to occur in Bantu women, and the original aim of this study was to find them.

MATERIALS AND METHODS

Lymphocytes were isolated by the method of Boyum (*4*), using a Ficoll-Hypaque mixture, and the cytotoxicity test was performed in Falcon microtest trays using the 2-stage procedure recommended by the NIH (*6*) as follows: 1 μl of antiserum and 1 μl of cell suspension were added to each well under paraffin. After 30 min at room temperature, 5 μl of unabsorbed rabbit complement was added and, after a further 60 min at room temperature, 5 μl of freshly prepared 0.6% trypan blue in saline was added. After 15 min at room temperature, excess dye was flicked off and the wells were examined with an inverted microscope.

Blood samples were collected from 1,004 Bantu women of the Zulu tribe attending antenatal and postnatal clinics. The number of pregnancies was not recorded, but more than one-half were multiparas. The serum was separated and stored at −30 C.

Their sera were screened daily against the lymphocytes of normal adult Bantu blood donors or staff members. A serum was not regarded as negative until it had given no positive reactions with 40 different Bantu donors. Posi-

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TABLE 1. Numbers and sources of antisera used to identify HL-A antigens

Antigens	Antisera Nos. from			Total
	Natal Institute of Immunology	NIH serum bank	NIH tray N621	
HL-A1	9	1	3	13
HL-A2	14	1	3	18
HL-A3	3	3	3	9
HL-A9	2	3	2	7
HL-A10	4	1	4	9
HL-A11	1	1	3	5
W28	4	3	2	9
W19	1	2	2	5
Te 63	1	0	2	3
Te 66	0	1	2	3
HL-A5	6	2	3	11
W5	3	1	3	7
HL-A7	9	2	4	15
W22	3	1	1	5
HL-A8	4	4	3	11
W14	3	1	3	7
HL-A12	11	0	3	14
HL-A13	4	2	2	8
W15	3	0	2	5
W17	5	1	3	9
W10	3	1	3	7
W27	1	1	0	2
Total	94	32	56	182

TABLE 3. Percentage of frequency of HL-A antigens in three race groups

Antigen	150 Bantu	147 Indians	100 Caucasians
HL-A1	5	27	27
HL-A2	20	31	51
W28	19	12	6
HL-A3	12	15	35
HL-A11	0	25	13
HL-A10	23	7	8
HL-A9	17	16	13
W19	17	10	14
Te 63	13	1	5
Te 66	31	2	5
Blank	3	3	0
HL-A5	4	37	12
W5	9	34	22
HL-A7	11	13	26
W22	34	3	5
W27	3	1	12
HL-A8	13	5	21
W14	7	1	6
HL-A12	22	10	28
HL-A13	5	7	7
W15	16	17	15
W17	29	26	9
W10	5	34	16
Blank	3	1	2

TABLE 2. Specificity of antibodies detected in sera of 1,004 parous Bantu women

Specificity	No.	Specificity	No.
HL-A1	0	HL-A5	0
HL-A2	3	W5	1
Associated with HL-A2	4	Associated with W5	6
Associated with HL-A2 + W28	1	HL-A7	3
W28	1	HL-A7 + W22	12
Associated with W28	1	Associated with HL-A7 + W22	17
<W28	1	Associated with HL-A7 + W27	1
HL-A2 + W28 + W17	1	HL-A8	1
HL-A3	1	Associated with HL-A8	3
HL-A11	0	W14	0
HL-A9	2	HL-A12	3
Associated with HL-A9	2	Associated with HL-A12	10
HL-A9 + 8	1	<HL-A12	5
Associated with HL-A10	3	HL-A13	2
Associated with HL-A10 + W28	2	Associated with HL-A13	1
W19	1	HL-A13 + W17	1
Associated with W19	2	Associated with W15	2
Te 63 + HL-A13	1	W17	4
Te 66	0	Associated with W17	8
		Associated with W10	1
Multispecific	32	W27	1
		Associated with W27	2
Unrelated	59		
	Total 202		

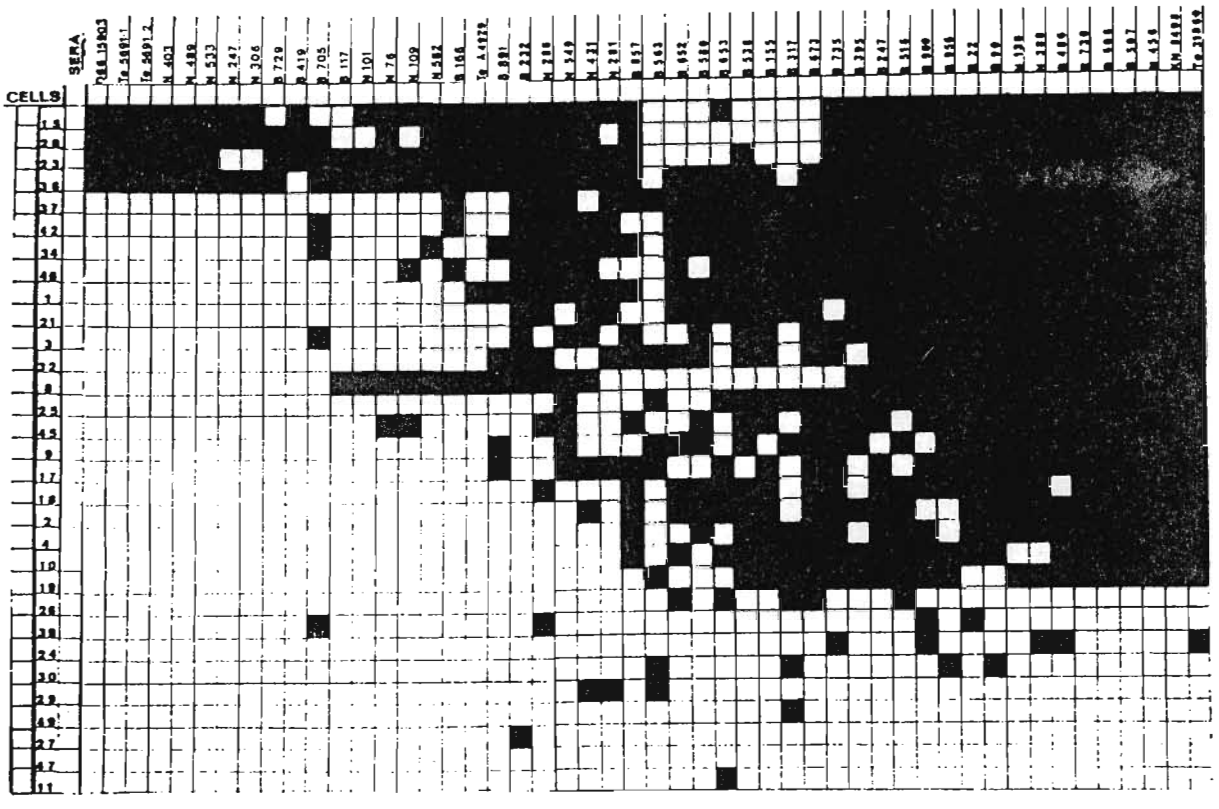

FIGURE 1. Reaction pattern of anti-HL-A7 and W22 sera with 50 Bantu cell donors.

tively reacting sera were kept for further study.

These sera were tested, together with a large number of others, against the lymphocytes of 50 unrelated normal Bantu. Of the other sera, some had been characterised by us in the past, some came from the NIH serum bank, and others were obtained already dispensed on tissue typing trays (No. N621) from the NIH. In addition to sera of our own that were regarded as monospecific or of known specificity, we included 96 that had previously given obscure results. In all, 480 sera were tested against this panel; 120 selected sera were then used to test two further panels of 100 Bantu and 100 Caucasians. The results were analysed using an IBM 1130 computer (*5*).

We used large numbers of sera because we have previously found that a serum which appears to be monospecific in one population may not be so at all in another (*7*). This is usually because it contains extra antibodies against antigens very rare in the first population and relatively common in the second. As a rule the antisera with the lowest frequency within a particular group are usually defining the antigen correctly. The numbers used and the origins of these sera are shown in Table 1.

RESULTS

Table 2 shows the specificities of the 202 out of the 1,004 Bantu sera that were found to have lymphocytotoxic antibodies.

Table 3 shows the frequencies of the HL-A antigens in Bantu and Caucasians. For the second sample of Bantu, no more preloaded trays were available and the results for Te 63 and Te 66 should therefore be treated with reserve, as Te 66 was detected with only a single serum and Te 63 was detected with only two sera, both of which are mixtures. For comparison, frequencies obtained on a panel of 147 Indians are included in this table.

Figure 1 shows the reaction patterns of sera containing antibodies to HL-A7 and W22 in the first 50 Bantu. Table 4 gives the 2 × 2 tables of these sera. Figure 2 and Table 5 illustrate the reactions of antisera against 150 Bantu. Figure 3 and Table 6 show the reaction pattern and 2 × 2 tables of sera with antibodies recognising HL-A12 or part of it.

DISCUSSION

Antisera for Te 63 and Te 66 which were available for this study almost completely filled

TABLE 4. 2 × 2 comparisons of sera illustrated in Figure 1

Leading serum	Antisera	%++	++	− −	+ −	− +
K-N 8498	Te 31860	100	22	27	0	1
	N456	100	22	28	0	0
	B507	82	22	28	0	0
	B606	95	22	28	0	0
	B730	100	22	28	0	0
	B486	100	21	27	1	1
	N388	82	21	27	1	1
	N139	81	21	28	1	0
	B22	95	21	27	1	1
	B90	68	21	27	1	1
	B956	100	20	27	2	1
	B900	95	20	26	2	2
	B516	81	20	27	2	1
	B247	71	21	28	1	0
	B395	89	18	28	4	0
	B735	76	20	27	2	1
	B673	95	18	27	4	1
	B317	57	11	25	11	3
	B155	88	17	28	5	0
	B538	83	18	28	4	0
	B653	53	13	26	9	2
	B580	69	13	28	9	0
	B652	54	12	27	10	1
	B563	50	6	26	16	2
	B857	73	15	28	7	0
	N281	27	10	27	12	1
	N431	64	13	27	9	1
	N549	73	15	28	7	0
	N286	80	14	27	8	1
	B232	57	13	27	9	1
	B891	70	10	28	12	0
	Te A4929	83	6	28	16	0
	B166	63	8	28	14	0
	N582	67	6	28	16	0
	N109	67	6	28	16	0
	N76	50	6	28	16	0
	N101	50	4	28	18	0
	B117	33	3	28	19	0
	B705	71	6	27	16	1
	B419	33	3	28	19	0
	B729	33	3	28	19	0
	N306	0	3	28	19	0
	N247	0	3	28	19	0
	N533	75	4	28	18	0
	N489	100	4	28	18	0
	N403	50	4	28	18	0
	Te 5691.1	50	4	28	18	0
	Te 5691.2	75	4	28	18	0
	D 66-15903	75	4	28	18	0

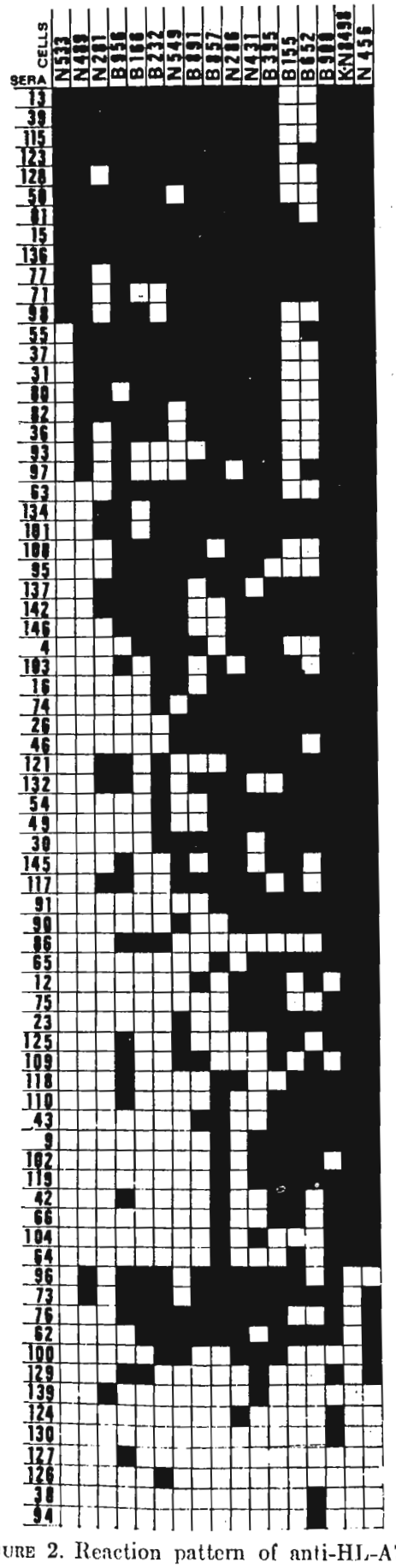

FIGURE 2. Reaction pattern of anti-HL-A7 and W22 sera with 150 Bantu cell donors.

TABLE 5. 2 × 2 comparisons of sera illustrated in Figure 2

Leading serum	Antisera	%4+	+ +	− −	+ −	− +
K-N 8498	N456	92	60	85	0	5
	B900	100	57	83	3	7
	B652	66	32	86	28	4
	B155	97	36	87	24	3
	B395	93	53	85	7	5
	N431	83	47	84	13	6
	N286	55	45	84	15	6
	B857	73	48	86	12	4
	B891	79	34	86	26	4
	N549	76	35	87	25	3
	B232	73	34	84	26	6
	B166	77	25	85	35	5
	B956	88	38	85	22	5
	N281	50	20	89	40	1
	N489	65	20	88	40	2
	N533	79	12	90	48	0

the gap previously found in the Bantu at the first locus. No cell donor was found with more than two antigens and in only three donors were none detected at the first locus.

It is interesting that we did not find antibodies to these specificities among our 202 positive sera, except for one in a mixture. Presumably the antigenicity of these factors is low. Several good antisera were found. Antisera listed in Table 2 as specific did not have more than one discrepancy with the reference serum for that specificity. Those associated with a specificity had two or more discrepancies. Some of the 32 multispecific antisera included common specificities, e.g., HL-A2, but generally reacted with three antigens or more. The 59 antisera that showed no relation to any other recognised specificity generally had a very low frequency of reaction (10%) and few of the reactions gave 100% kill. They were not restricted to those cells with only one detectable allele at either locus. One possible explanation is that they are recognising products of *HL-B* locus (*14*).

In Caucasians, HL-A1 and HL-A8 have similar frequencies and a high degree of association and consequently antisera containing anti-HL-A1 and anti-HL-A8 may be difficult to identify. Indians, however, have a very low frequency of HL-A8 (5%) while 27% possess HL-A1, whereas in the Bantu the reverse is found. Only 5% were HL-A1-positive while 13% are positive for HL-A8. These differences can be put to good use by using a selected panel from each race group for characterising antisera.

Figure 3 shows the reaction pattern of sera associated with or included in HL-A12 in 150 Bantu. It appears that HL-A12 is a heterogenous antigen which can be subdivided into two parts. The 2 × 2 tables of these reactions are listed in Table 6. Next to each serum is shown the percentage of reactions that gave 100% killing of lymphocytes. The (− +) reactions of sera N137 and B374 are with cells that are positive for W19. Svejgaard et al. (*15, 16*) have described an antigen, EL*, which is defined by an antiserum that reacts with HL-A12 cells and with cells that are EL*-positive. However, the differences in the Bantu presented here are within HL-A12.

The most interesting difference was the high frequency of W22 and the large number of sera that contained antibodies to HL-A7 and W22 (Table 2). Figure 1 illustrates the reaction patterns obtained with the sera in the first panel of 50 Bantu. Table 4 lists the 2 × 2 tables for these sera and the percentage of 4+ reactions for each serum. Six sera, including Engelfriet's serum D66-15903 (obtained from the NIH) and Te 5691.1 gave identical results and all have been previously characterised as anti-HL-A7. They reacted with only 4 out of 50 donors. Four

TABLE 6. 2 × 2 comparisons for sera shown in Figure 3

Antigen	Antisera	%4+	+ +	− −	+ −	− +
HL-A12	N399	81	31	118	0	1
	B12	100	30	118	1	1
	B872	100	25	117	6	2
	B894	100	30	119	1	0
	B855	97	31	119	0	0
	B836	97	30	119	1	0
	B364	67	27	119	4	0
	B389	75	28	119	3	0
	N513	65	26	119	5	0
	B315	96	27	118	4	1
	B898	44	5	117	26	2
	N328	50	3	118	28	1
	PE102	25	8	119	23	0
	B374	89	22	114	9	5
	N137	58	23	115	8	3
	N546	100	22	119	9	0
	B225	74	22	118	9	1
	B228	75	21	116	10	3
	N366	37	19	119	12	0
	B849	31	15	118	16	1
	N320	50	10	119	21	0

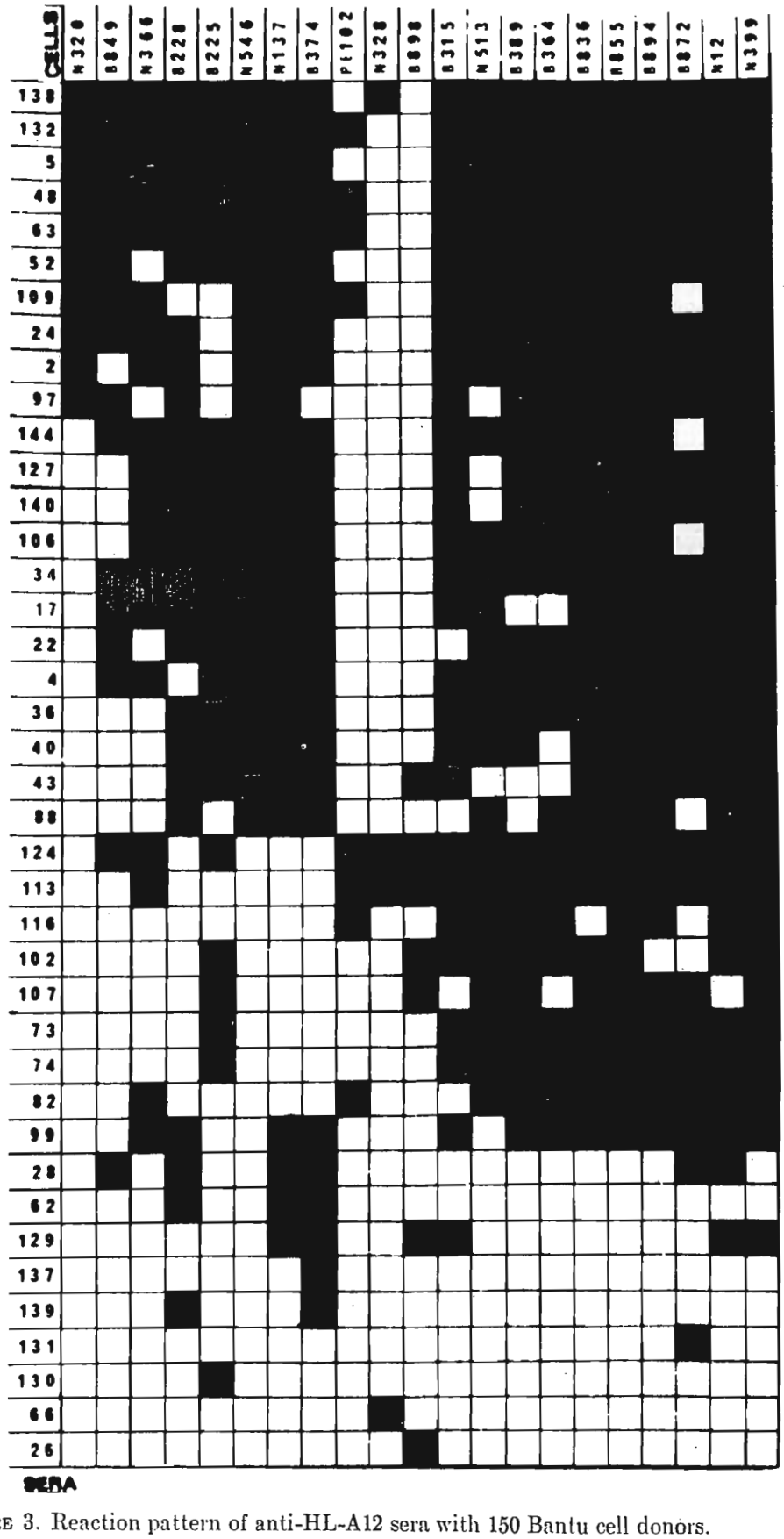

FIGURE 3. Reaction pattern of anti-HL-A12 sera with 150 Bantu cell donors.

antisera gave identical reactions to K-N 8498 (from Kissmeyer-Nielsen) which is anti-HL-A7 + W22 and were positive with 22 out of 50 donors. Another 11 sera including Te 3186.0 (HL-A7 + W22) all had a coefficient of correlation ($r = \sqrt{\chi^2/N}$) greater than 0.87 with K-N 8498. Eight sera appear to identify W22. The negative reactions of these eight sera are not attributable to weakly reacting sera because at least 50% of the reactions give total killing of lymphocytes (Table 4). Another six sera (B232, N286, N549, N431, N281, B857) react with HL-A7 and part of W22.

We then tested a further 100 Bantu using two anti-HL-A7 sera and two anti-HL-A7 + W22 sera and 12 other sera that showed associations with this complex. Figure 2 shows the overall pattern of reactions of these sera with cells from 150 Bantu. Two sera, B155 and B652, appear to recognise W22 only. Three cell donors appear to possess both HL-A7 and W22.

Kissmeyer-Nielsen (personal communication) has found that the antigen AA (W22) can be subdivided into two categories which he calls AA* and AA-AJ. This study of the Bantu has shown that there are even more parts to this complex. The identification of these subgroups may only be possible in a race group such as the Bantu where the frequency of this antigen is so much greater than in Caucasians.

REFERENCES

1. Albert, E. D.; Mickey, M. R.; McNicholas, A. C.; Terasaki, P. I. 1970. p. 221. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.
2. Amos, D. B.; Cabrera, G.; Bias, W. B.; Macqueen, J. M.; Lancaster, S. L.; Ward, F. E. 1970. p. 259. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.
3. Bodmer, J. G.; Bodmer, W. F. 1970. Amer. J. Human Genet. *22:* 396.
4. Boyum, A. 1968. Scand. J. Clin. Lab. Invest. *21:* 7.
5. Brain, P.; Hammond, M. G. 1968. Med Proc. *14:* 589.
6. Brand, D. L.; Ray, J. G.; Hare, D. B.; Kayhoe, D. E.; McClelland, J. D. 1970. p. 357. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.
7. Dausset, J.; Ivanyi, P.; Colombani, J.; Feingold, N.; Legrand, L. 1967. p. 189. *In* E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi (eds.). *Histocompatibility testing 1967*. Munksgaard, Copenhagen.
8. Dausset, J.; Ivanyi, P.; Ivanyi, D. 1965. p. 51. *In* H. Balner; F. J. Cleton; J. G. Eernisse (eds.). *Histocompatibility testing 1965*. Munksgaard, Copenhagen.
9. Hammond, M. G.; Brain, P. 1971. Vox Sang. *20:* 492.
10. Robillard, P.; Potworowski, E. F. 1970. Transplantation *9:* 137.
11. Rubinstein, P.; Costa, R.; van Leeuwen, A.; van Rood, J. J. 1967. p. 251. *In* E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi (eds.). *Histocompatibility testing 1967*. Munksgaard, Copenhagen.
12. Singal, D. P.; Mickey, M. R.; Terasaki, P. I. 1969. Transplantation *8:* 235.
13. Singal, D. P.; Mickey, M. R.; Terasaki, P. I. 1969. Transplantation *8:* 829.
14. Singal, D. P.; Sengar, D. P. S.; Terasaki, P. I. 1970. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.
15. Svejgaard, A.; Kissmeyer-Nielsen, F. 1970. Vox Sang. *18:* 12.
16. Svejgaard, A.; Kissmeyer-Nielsen, F.; Thorsby, E. 1970. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.

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HL-A Antigens and Antibodies in South African Indians

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The Indian population of South Africa has been found to have a higher frequency of the antigens HL–A5 and W5 than do either Caucasians or Bantu. Some antisera that appeared to be good anti-HL–A5 or anti-W5 in South African Caucasians gave anomalous results when tested in Indians. The sera of 1,000 Indian women were tested for lymphocytotoxic antibodies and those sera found to contain antibodies were tested in parallel with known antisera against the cells of 150 Indians.

We tested for 10 antigens at the first locus, HL–A1, 2, 3, 9, 10, 11, W28, W19, Te63 (= W19-1) and Te66 (= W19-4) and at the second locus for 12 antigens, HL–A5, 7, 8, 12, 13, W5, W14, W15, W17, W22, W27 and W10.

The frequency of HL–A1 is 22 %, which agrees with the Caucasian origin of the Indian population. There apparently are subdivisions of HL–A5 and W5, and one serum was found to be a "short" W10. HL–A11 has a relatively high frequency in Indians and may also be subdivided.

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The frequency of HL–A antigens varies in the different race groups, and antigens which are rare in one race group may be more common in other race groups. We have previously reported an intensive search in South African Bantu for new antigens (Hammond et al. 1972). This paper describes a similar search in the Indian population for antisera that would resolve the anomalous results we obtained with antisera for HL–A5 and W5. The Indian population has a relatively high frequency of these antigens and we were more likely to find antibodies to these specificities in such a population.

There are three distinct racial groups in Durban: Caucasian, Bantu and Indian. The Caucasian population is of Western European origin. The Indian population is concentrated in the province of Natal and accounts for approximately 13 % of its total population. In Durban (population 650,000) the proportion of Indians is much higher (34 %). The Indians are descendants of immigrants who arrived about a century ago, principally from the Madras

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Presidency. They speak mostly Hindi, Tamil and Telegu. There has been little admixture with other groups.

Materials and Methods

Lymphocytes were isolated by the method of Böyum (1968) using a Ficoll-Hypaque mixture, and the cytotoxicity test was performed in Falcon microtest trays using the two-stage procedure recommended by the National Institutes of Health.

Blood samples were collected from 1,000 Indian women attending ante-natal and post-natal clinics. The serum was separated and stored at — 30° C. All the sera were screened against a panel of 12 selected donors, but as this panel would not contain antigens that were "new", the sera were also screened against the cells of 40 random Indian blood donors.

A serum was not regarded as negative until it had given no positive reactions with 40 donors. The positively reacting sera were tested in parallel with previously characterised sera against the lymphocytes of 99 randomly selected but unrelated Indians. Altogether 318 sera were tested and the results analysed by computer. A total of 120 selected sera were then tested against the cells of a further 51 Indians.

Results

Table 1 shows the number and specificity of the antibodies detected. Table 2 shows the 2 × 2 comparisons of the sera illustrated in Fig. 1. Table 3 shows inclusions in HL-A2. Table 4 and Fig. 2 show the relationship between sera reacting with the HL-A5-W5 complex. Table 5 and Fig. 3 illustrate a subdivision of W10. Tables 6 and 7 give the phenotypic and gene frequencies of HL-A antigens in the Indian population with those of Bantu and Caucasians for comparison. Table 8 gives the calculated haplotype frequencies in the three races, using the method described by Mattiuz et al. 1970.

Discussion

Two recent studies by Ting et al. (1971) and Singal et al. (1971) of HL-A fre-

Table 1

Number and specificity of antibodies detected in 1000 Indian women

Specificity	No.	Specificity	No.
HL-A1	2	Associated with HL-A5 + W5	12
HL-A2	4	HL-A7	3
Associated with HL-A2	2	HL-A7 + W22	6
W28	1	HL-A7 + W10	1
HL-A2 + W28	3	HL-A8	1
HL-A3	1	W14	–
Associated with HL-A3	1	HL-A12	2
Associated with HL-A11	3	HL-A13	1
HL-A9	2	Associated with HL-A13	3
HL-A10	2	Associated with W15	6
Associated with W19	1	W17	3
Te63 (= W19-1)	–	Associated with W10	4
Te66 (= W19-4)	–	W27	–
Multispecific	69	Unknown	57

Total 190

quencies in Asian Indians show similar antigen frequencies except that we have found a much higher incidence of HL–A5 and HL–A11. These differences may be attributable to sectarian differences. Milner & Calitz (1968) and Milner (1970) have shown differences in the strength of the B antigen in various Indian religious sects.

Of the 190 antisera from Indian women, 126 gave reactions that could not be identified (Table 1). Of these sera, 57 had a frequency of less than 10 % and showed no correlation with any other known antisera, nor were their reactions included in those of any known sera. It does not seem possible that they are all recognising specific HL–A antigens or combinations of rare antigens, and one explanation is that some of them may be recognising non-HL–A antigens such as those described as HL–B antigens by Singal et al. (1970), although these authors found HL–B antibodies primarily as extra antibodies in HL–A antisera.

The Indian population has a higher frequency of HL–A11 than do either Caucasians or Bantu, but the reactions of the sera we used differed significantly among themselves. Serum N597 contains antibodies to HL–A3 and HL–A11, and when it was characterised in Caucasians there were no positive reactions outside these two specificities. In the Indians this serum has a frequency of 42 %, of which 32 % appeared to be HL–A11. HL–A3 was identified with two monospecific sera. Three antisera obtained from Indian women gave a reaction pattern which was included in HL–A11, but they had no significant correlation with each other. This is shown in Fig. 1 and Table 2.

Two sera gave reactions which were included in HL–A2 but the possibility exists that these are reacting with only some cells from homozygous subjects, although there were no weak reactions with these sera. The 2 × 2 comparisons are shown in Table 3.

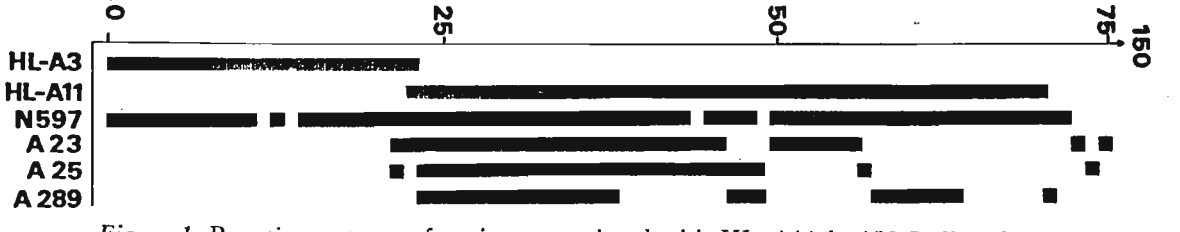

Figure 1. Reaction pattern of antisera associated with HL–A11 in 150 Indian donors.

Table 2

2 × 2 comparisons of sera illustrated in Figure 1, tested in 150 Indian donors

%4+	Serum	Serum	++	+−	−+	−−
97	N597	anti-HL–A3 + 11	66	2	4	78
97	N597	anti-HL–A11	46	22	2	80
82	A23	anti-HL–A11	31	3	17	99
66	A25	anti-HL–A11	27	2	21	100
73	A289	anti-HL–A11	25	1	23	101
82	A23	A25	24	10	5	111
82	A23	A289	15	19	11	105
66	A25	A289	18	11	8	113

Table 3

2 × 2 comparisons of sera included in HL–A2 tested in 150 Indian donors

Serum	Serum	%4+	++	+−	−+	−−
anti-HL–A2	A689	100	34	12	0	104
	A1	89	35	11	3	101
A689	A1	89	26	8	12	104

Table 4

2 × 2 comparisons of sera reacting with the HL–A5, W5 complex (as in Figure 2) tested in 150 Indian donors

Leading serum	%4+	Antisera	%4+	++	+−	−+	−−
AGST	92	N442	97	63	1	3	83
		N310	93	58	6	1	85
		PE27	78	43	21	2	84
		S152	88	38	26	2	84
		V52	78	36	28	0	86
		A110	86	40	24	1	85
		A168	69	16	48	0	86
		A803	46	13	51	0	86
		A177	55	11	53	0	86
		N579	38	5	59	3	83
		N335	88	10	54	6	80
N429	89	N277	94	43	2	4	101
		A568	100	43	2	7	98
		A125	70	36	9	4	101
		A911	89	34	11	3	102
		N482	83	20	25	3	102
		A831	84	26	19	5	100
		A587	86	14	31	0	105
		N335	88	16	29	0	105

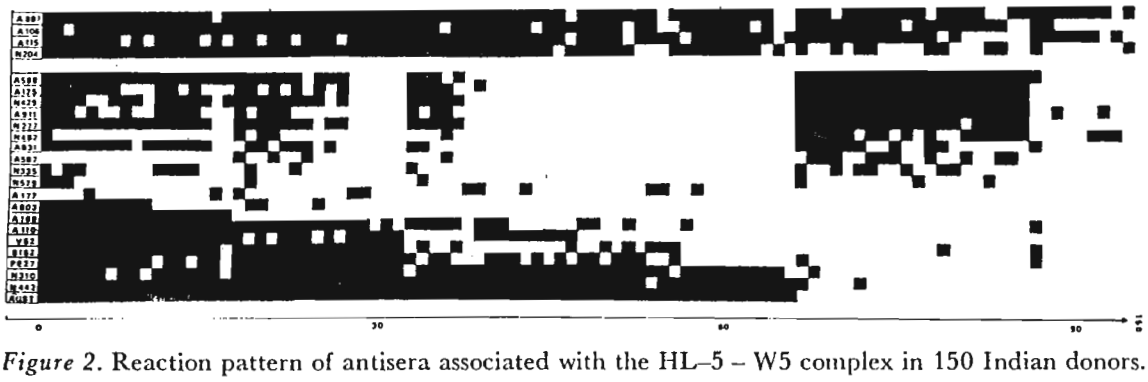

Figure 2. Reaction pattern of antisera associated with the HL–5 – W5 complex in 150 Indian donors.

The most interesting results, however, are those obtained with the antisera recognising HL–A5 and W5. These antigens are relatively frequent in the Indian population, so that differences in reaction patterns are conspicuous. Table 4 shows 2 ×

2 tables for several sera that show associations with this complex, which is illustrated in Fig. 2. It seems that there may be more factors involved than the three described in the 1970 Workshop data, i.e. HL–A5, W5 and W18. AGST, N442 and N310 are operationally monospecific anti-HL–A5 sera in Caucasians, and in the Indian series they agree very well. Four sera, V52, PE27, S152 and A110, have patterns that are included in HL–A5. The reactions of sera A168 and A803 are included in these four sera.

Across the middle of Fig. 2 is the reaction pattern of sera that are associated with W5. The block at the right centre represents cells that are positive for W5 and negative for HL–A5. Two sera from this group (N429 and N277) have previously been characterised as anti-W5. At the top are four sera which appear to react with both HL–A5 and W5. Unfortunately no sera for W18 were available and this specificity has been shown to be associated with W5 (Albert et al. 1971). It appears that there are other antigens present which cross-react with antisera against HL–A5, W5, and W18.

Table 5 shows the 2 × 2 comparisons of sera reacting with the W10 antigen, and these reaction are also shown in Fig. 3. The serum A488 appears to be a short W10 and this serum gives strong reactions with no weak positives. Serum A150 appears to be even shorter than A488.

The serological identification of HL–A antigens is not yet complete and if (as Dausset (1971) suggests) each specificity consists of several factors, then the HL–A system may have an almost individual-specific configuration. On the other hand, unrelated individuals have been found (Eijsvoogel et al. 1971) who are phenotypically identical in mixed lymphocyte cultures (MLC), which indicates that there is a restricted, though large, number of allelic variations.

The data presented here show that these antigenic factors can be more easily identified by testing different race groups

Table 5

2 × 2 comparisons of W10 antisera illustrated in Figure 3 tested in 150 Indian donors

Leading serum	%4+	Antisera	%4+	++	+−	−+	−−
A150	64	A488	94	10	1	10	129
		A530	96	10	1	36	103
		A561	90	10	1	30	109
		BAUER	94	10	1	21	118
		V8	95	10	1	29	110
		N253	100	8	3	22	117
A488	94	A530	96	19	1	27	103
		A561	90	18	2	22	108
		BAUER	94	17	3	14	116
		V8	95	19	1	20	110
		N253	100	16	4	14	116
BAUER	94	A530	96	30	1	16	103
		A561	90	24	7	16	103
		V8	95	30	1	9	110
		N253	100	23	8	7	112

in which these factors have a higher frequency. Our studies in the Bantu (Hammond et al. 1972) have shown this to be true for other antigens. The elucidation of all the factors governed by the HL–A locus may be possible only in this manner.

Figure 3. Reaction pattern of antisera associated with W10 in 150 Indian donors.

Table 6

Percentage frequency of antigens in Caucasians, Bantu and Indians

Antigen	Caucasian	Bantu	Indian
	N=446	N=150	N=150
HL–A1	30.3	4.7	22.0
HL–A2	48.2	20.7	30.1
W28	7.6	18.7	15.3
HL–A3	29.8	12.0	15.3
HL–A11	10.1	0.0	32.0
HL–A9	15.0	23.3	26.7
HL–A10	8.5	16.7	6.7
W19	9.9	18.0	3.3
Te63	4.3	13.3	0.7
Te66	3.4	30.0	0.7
Blank	0.4	3.3	0.7
HL–A5	10.1	2.7	42.0
W5	13.7	11.3	26.7
W15	12.6	15.3	16.0
HL–A7	23.5	10.7	14.0
W27	9.4	3.3	2.7
W22	3.4	35.3	4.7
HL–A8	22.0	12.0	6.0
W14	5.2	8.0	1.3
HL–A12	29.6	22.7	11.3
HL–A13	6.1	4.7	8.0
W10	15.5	5.3	19.3
W17	9.0	30.0	20.7
Blank	3.1	2.7	0.7

Table 7

HL–A gene frequencies in Caucasians, Bantu and Indians

Gene	Caucasian	Bantu	Indian
HL–A1	.165	.024	.117
HL–A2	.280	.109	.167
W28	.039	.098	.080
HL–A3	.162	.062	.080
HL–A11	.052	.000	.175
HL–A9	.078	.124	.144
HL–A10	.044	.087	.034
W19	.051	.095	.017
Te63	.022	.069	.003
Te66	.017	.163	.003
Blank	.002	.017	.003
HL–A5	.052	.013	.239
W5	.071	.058	.146
W15	.065	.080	.084
HL–A7	.126	.055	.073
W27	.048	.017	.013
W22	.017	.196	.024
HL–A8	.177	.062	.031
W14	.026	.041	.007
HL–A12	.161	.121	.058
HL–A13	.031	.024	.041
W10	.081	.027	.102
W17	.046	.163	.109
Blank	.016	.013	.003

Table 8

Haplotype frequencies for Caucasian, Bantu and Indian populations (number per 1000)

	HL–A5	W5	W15	HL–A7	W27	W22	HL–A8	W14	HL–A12	HL–A13	W10	W17	Blank	
HL–A1	0	2	8	14	7	0	88	4	2	0	3	20	1	C
	0	2	0	2	0	0	2	0	8	0	0	8	0	B
	24	26	21	10	0	5	0	0	0	2	2	47	0	I
HL–A2	15	9	28	36	18	5	0	4	80	10	31	6	6	C
	2	0	0	0	2	0	0	10	22	0	4	47	6	B
	43	18	17	19	1	4	6	3	25	0	22	16	0	I
W28	3	4	2	0	4	4	0	0	10	6	0	1	2	C
	2	5	19	9	6	6	0	7	2	5	5	32	0	B
	11	2	12	0	6	2	5	3	6	11	22	0	0	I
HL–A3	2	20	10	59	0	9	13	2	0	4	8	3	2	C
	3	4	0	0	0	0	18	16	3	0	2	25	0	B
	8	19	13	8	0	2	2	0	6	4	17	2	0	I
HL–A11	6	11	0	11	5	0	0	3	2	0	6	4	0	C
	0	0	0	0	0	0	0	0	0	0	0	0	0	B
	46	0	21	14	9	0	0	0	13	16	21	20	0	I
HL–A9	3	8	6	6	2	1	1	6	14	1	11	2	1	C
	0	0	17	8	0	37	7	10	15	1	0	22	0	B
	35	31	2	25	0	0	11	3	6	5	16	0	0	I
HL–A10	3	0	4	0	1	2	1	0	7	2	3	2	3	C
	6	2	19	6	2	9	2	0	16	0	5	25	0	B
	12	6	0	1	3	10	6	0	0	2	0	0	0	I
W19	0	0	8	3	7	0	3	3	17	2	11	0	0	C
	0	28	7	2	9	16	13	0	3	1	5	0	0	B
	4	1	17	2	0	3	0	0	3	0	6	2	0	I
Te63	1	3	1	0	0	2	0	0	16	1	1	0	0	C
	3	0	5	7	0	13	3	0	14	6	2	15	0	B
	0	3	0	0	0	0	0	0	0	0	0	0	0	I
Te66	3	0	0	0	0	0	2	1	2	3	3	0	0	C
	1	13	5	10	0	76	17	0	14	8	7	0	5	B
	0	0	0	0	0	0	0	0	0	0	0	0	0	I
Blank	2	1	0	0	0	0	0	0	0	0	1	0	0	C
	0	3	2	3	0	8	0	0	2	3	0	0	0	B
	3	0	0	0	0	0	0	0	0	0	3	0	0	I

C = Caucasian, B = Bantu, I = Indian.

References

Albert, E. D., Mickey, M. R. & Terasaki, P. I. (1971) Genetics of four new HL–A specificities in the Caucasian and Negro populations. *Transplant. Proc.* **3**, 95.

Böyum, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97.

Dausset, J. (1971) The genetics of transplantation antigens. *Transplant. Proc.* **3**, 8.

Eijsvoogel, V. P., Schellekens, P. T. A., Breur-Vriesendorp, B., Koning, L., Kock, C., van Leeuwen, A. & van Rood, J. J. (1971) Mixed lymphocyte cultures and HL–A. *Transplant. Proc.* **3**, 85.

Hammond, M. G., Appadoo, D. & Brain, P. (1972) HL–A antigens and antibodies in South African Bantu. *Transplantation* **14**, 159.

Mattiuz, P. L., Ihde, D., Piazza, A., Ceppelini, R. & Bodmer, W. F. (1970) New approaches to the population: Genetic and segregation analysis of the HL–A system. *Histocompatibility Testing 1970*. Munksgaard, Copenhagen.

Milner, L. V. (1970) The strength of the B antigen in South African Asiatics related to language group and religious sect. *Social Biology* **17**, (3) 213.

Milner, L. V. & Calitz, F. (1968) Quantitative studies of the erythrocytic B antigen in South African Caucasians, Bantu and Asiatic blood donors. *Transfusion* **8**, (5) 277.

Singal, D. P., Sengar, D. P. S. & Terasaki, P. I. (1970) Detection of non-HL–A antibodies. *Histocompatibility Testing 1970*, ed. Terasaki, P. I. Munksgaard, Copenhagen.

Singal, D. P., Mickey, M. R. & Terasaki, P. I. (1971) HL–A typing in Asian Indians. *Tissue Antigens* **1**, 286.

Ting, A., Wee, G. B., Simons, M. J. & Morris, P. J. (1971) The distribution of HL–A leucocyte antigens in Singapore Chinese, Malays and Indians. *Tissue Antigens* **1**, 258.

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Subdivision of HL-A5 and Comparative Studies of the HL-A Polymorphism in South African Indians

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The HL–A5 antigen has a higher frequency in the Indian population than in either the Caucasian or Bantu populations of South Africa. Ninety-five Asian Indians were tested by microcytotoxicity using 34 anti-HL–A5 and nine anti-W5 sera. The results confirm the heterogeneity of the HL–A5 antigen and show that it may be subdivided into at least four parts. The Indian population of South Africa is here subdivided into four groups. The HL–A antigen frequencies in each group are compared, and haplotype frequencies and gametic associations (delta values) have been calculated. The genetic distances (f) between these groups and between Indians, Caucasians and Bantu also are calculated. The results may indicate a differential selection with respect to the HL–A polymorphism.

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A previous investigation (Hammond et al. 1972b) revealed significant differences in the reaction patterns of different anti-HL–A5 sera when tested in the Indian population of Durban. Further work is described in this paper.

Materials and Methods

Subjects

The Indians of Natal are the descendants of immigrants who arrived about a century ago to work on the sugar plantations. They can be grouped firstly into Tamil and Telegu speakers from southern India, both Hindu by religion but subdivided here by language, and secondly into two groups from the north, northern Hindus from the eastern side of the continent and a group from the west who are Moslem by religion. Most of the latter would have been Hindus before conversion but are separated geographically from the Hindu group in the north-east.

Caucasians are of western European origin. The Bantu are Negroes, mostly of the

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Zulu tribe. The Coloured population is of mixed Caucasian and Bantu origin. The proportion of each race group in the greater Durban area is shown in Table 1.

Table 1
Population of Durban

Race	Number	%
Caucasian	269,635	24.4
Indian	348,483	31.5
Bantu	443,382	40.0
Coloured	45,376	4.1
Total	1,106,876	100.0

Although Indians are normally considered to be Caucasians, for the purposes of this paper the terms Caucasians, Indians and Bantu will refer to the populations as defined above.

Serology

A total of 250 antisera were used in a two-stage microlymphocytotoxicity test as recommended by the National Institutes of Health (Brand et al. 1970); of these, 34 were anti-HL–A5 and nine were anti-W5. Serum Lindford was kindly donated by Dr. M. Shapiro of the South African Blood Transfusion Service, Johannesburg, and several antisera were obtained from the N.I.H. serum bank. More than 120 of these sera have been characterised in parallel with N.I.H. tray N621 (Hammond et al. 1972a). Lymphocytes from 95 randomly selected Indians were isolated by the method of Böyum (1968) using a Ficoll-Hypaque density gradient.

Statistical Analysis

The distribution of the different groups in the 95 Indians tested was as follows:

Hindu	34	Tamil	37
Moslem	14	Telegu	8
Others	2		

The subdivision of HL–A5 emerged from the analysis of the reaction patterns of these 95 Indians. A total of 303 Indians was used for the population frequencies and 258 of these could be classified by language and religion into the four groups mentioned. There were 45 other Indians who could not be classified.

Haplotype frequencies and delta values were calculated according to Mattiuz et al. (1970), and the genetic distances (f) between populations were calculated according to Cavalli-Sforza & Bodmer (1971) using the formula

$$f = 4\ (1-\text{Cos}\,\theta)/K-1$$

$$\text{where Cos}\,\theta = \sum_{i=1}^{K} \sqrt{Pi1 \times Pi2},$$

K is the number of alleles, and Pi1, Pi2 are the respective allele frequencies in the two populations.

Results

Fig. 1 shows the reaction pattern obtained with 34 anti-HL–A5 sera, nine anti-W5 sera and serum Laskey (anti-W18). Also shown is the distribution of the other antigens at the second segregant series and the distribution of Indians from the North and South of India. Table 2 lists the 2 × 2 comparisons for these sera versus HL–A5 and W5, as illustrated in Fig. 1. Table 3 shows the distribution of subdivisions of HL–A5 amongst the four groups of Indians and the significance is calculated in Table 4.

Tables 5 and 6 show the HL–A antigen frequencies at the first and second segregant series in each of the Indian subgroups compared with the frequencies in Caucasians and Bantu. Table 7 shows the frequency of haplotype HL–A1, W17 in all the populations with the standard error and delta values.

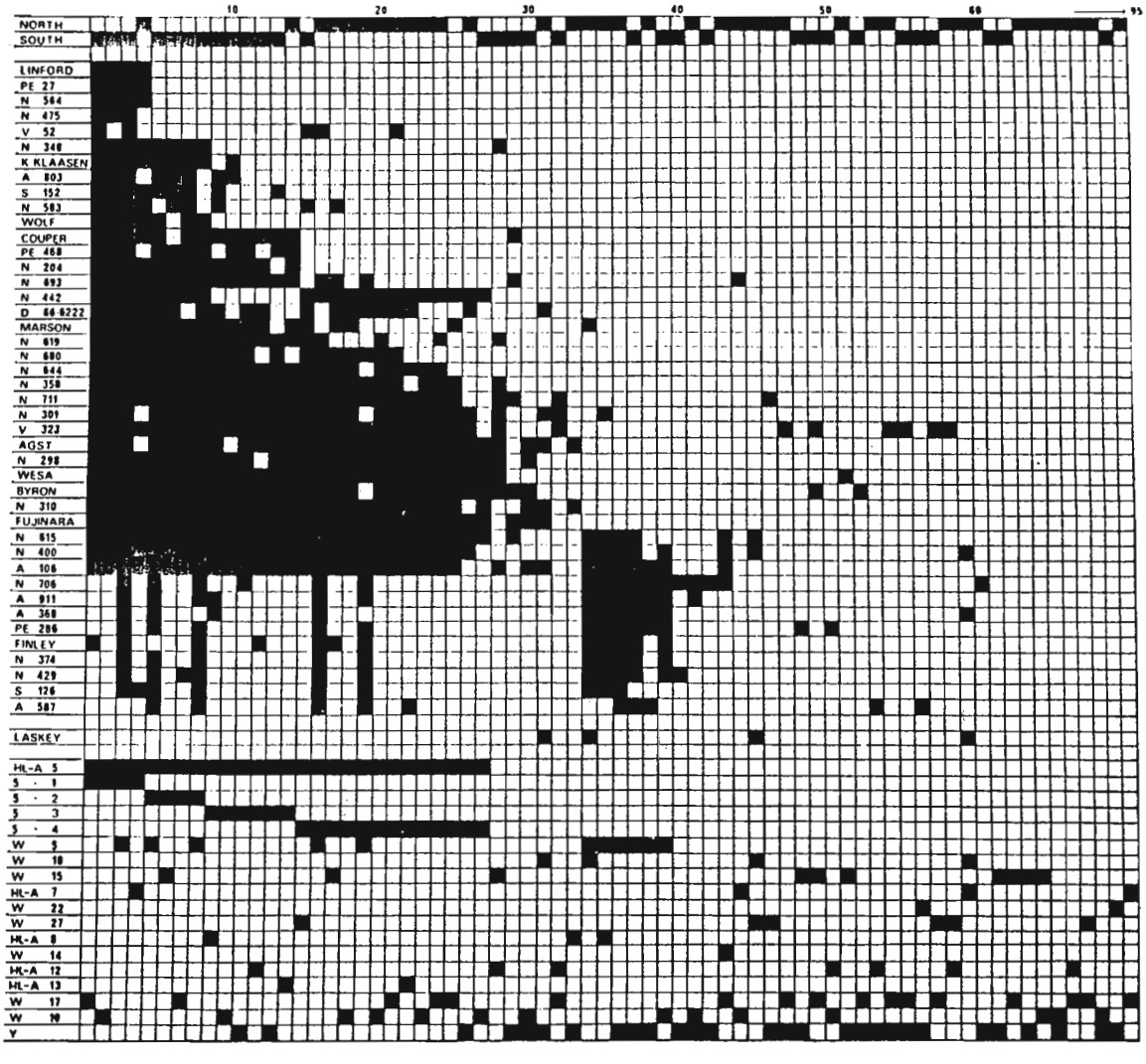

Figure 1. Reaction pattern of HL–A5 and W5 antisera in Indians.

Table 2

2×2 comparisons of antisera reacting with HL–A5 and W5 as defined in Fig. 1

	Antisera	Dilution	% 8+	++	+−	−+	−−	χ^2
HL–A5	LINFORD	2	100	4	23	0	68	10.5
	PE27	30	75	4	23	0	68	10.5
	N564	8	75	4	23	0	68	10.5
	N475	2	33	3	24	0	68	7.8
	V52	3	80	5	22	0	68	13.3
	N348	2	56	8	19	1	67	17.9
	K. KLAASEN	1	44	9	18	0	68	25.0
	A803	1	37	8	19	0	68	22.0
	S152	20	90	9	18	0	68	25.0
	N583	1	33	9	18	0	68	25.0
	WOLF	1	86	7	20	0	68	19.0
	COUPER	1	71	13	14	1	67	33.5
	PE468	1	36	11	16	0	68	31.3
	N204	4	92	13	14	0	68	37.9
	N693	2	79	17	10	2	66	43.5
	N442	5	52	21	6	0	68	67.9
	D66–6222	2	32	18	9	1	67	51.3
	MARSON	1	50	17	10	1	67	47.6
	N619	2	79	18	9	1	67	51.3
	N680	1	53	19	8	0	68	59.8
	N644	1	91	23	4	0	68	76.4
	N358	6	68	24	3	1	67	76.2
	N711	1	72	25	2	4	64	68.5
	N301	1	79	24	3	4	64	64.1
	V323	1	79	26	1	8	60	60.1
	AGST	100	93	25	2	4	64	68.5
	N298	1	96	26	1	2	66	81.0
	WESA	1	86	27	0	2	66	85.8
	BYRON	1	87	26	1	5	63	69.5
	N310	1	90	26	1	3	65	76.9
	FUJINAKA	1	100	27	0	3	65	81.7
	N615	1	74	27	0	7	61	67.7
	N400	1	71	26	1	8	60	60.1
	A106	1	71	25	2	10	58	50.4
W5	N615	1	74	9	2	25	59	11.5
	N400	1	71	10	1	24	60	16.4
	A106	1	71	11	0	24	60	21.3
	N706	2	100	11	0	6	78	57.1
	A911	1	92	11	0	2	82	78.5
	A368	1	45	9	2	2	82	59.9
	PE286	4	38	11	0	2	82	78.5
	FINLEY	1	50	9	2	3	81	54.0
	N374	1	80	10	1	0	84	85.3
	N429	1	75	10	1	2	82	69.1
	S126	30	44	8	3	1	83	58.0
	A587	1	40	7	4	3	81	37.3

Table 3

Distribution of sub-divisions of HL–A5

	HL–A5			
	5.1	5.2	5.3	5.4
Hindu	1	0	1	7
Moslem	0	0	0	3
Telegu	1	1	0	0
Tamil	2	3	5	2

Note: One cell donor (No. 25) of unknown origin had been omitted.

Table 4

Association between subgroups of HL–A5 and Indians from the North and South of India

	HL–A5 .1 + .2 + .3	HL–A5 .4	Total
North of India Moslem and Hindu	2	10	12
South of India Tamil and Telegu	12	2	14
	14	12	26

$\chi^2 = 12.4$ $P < 0.001$

Fisher's exact method $P = 0.00064$

Note: One cell donor (No. 25) of unknown origin has been omitted from the calculation.

Genetic distances (f) between the populations are shown in Tables 8 and 9. The calculated gene frequencies for Caucasians, Bantu and Indians are given in Table 10.

Table 5

HL–A antigen frequencies at the first locus in Indian sub-groups compared with the frequencies in Caucasians and Bantu

	Hindu 70	Moslem 38	Telegu 45	Tamil 105	North 108	South 150	Indian 303	Caucasian 704	Bantu 166
HL–A1	15.7	21.1	24.4	32.4	17.6	30.0	25.7	31.5	4.2
HL–A2	17.1	42.1	33.3	31.4	25.9	32.0	30.0	46.3	22.9
W28	17.1	15.8	6.7	10.5	16.7	9.3	13.2	7.8	19.3
HL–A3	21.4	10.5	17.8	17.1	17.6	17.3	17.8	30.0	12.1
HL–A11	31.4	10.5	26.7	28.6	24.1	28.0	26.7	11.4	0.0
HL–A9	30.0	36.8	28.9	25.7	32.4	26.7	28.7	16.5	23.5
HL–A10	2.9	10.5	11.1	8.6	5.6	9.3	7.9	9.0	16.3
W19–6	10.0	7.9	8.9	6.7	9.3	7.3	7.9	7.0	16.3
W29	0.0	0.0	2.2	1.0	0.0	1.3	0.7	3.6	12.7
W31	2.9	0.0	0.0	0.0	1.9	0.0	0.7	3.3	30.7
X	51.5	44.8	40.0	38.0	48.9	38.8	40.7	33.6	42.0

Table 6

HL–A antigen frequencies at the second locus in Indian sub-groups compared with the frequencies in Caucasians and Bantu

	Hindu 70	Moslem 38	Telegu 45	Tamil 105	North 108	South 150	Indian 303	Caucasian 704	Bantu 166
HL–A5	40.0	36.8	33.3	33.3	38.9	33.3	36.0	9.9	2.4
W5	17.2	13.2	28.9	23.8	15.7	25.3	21.8	13.6	10.8
HL–A7	7.1	7.9	17.8	13.3	7.4	14.7	12.2	23.3	12.7
W22	0.0	0.0	4.4	1.9	0.0	2.7	2.6	3.8	34.3
W27	8.6	0.0	0.0	1.9	5.6	1.3	3.3	7.8	3.0
HL–A8	5.7	5.3	13.3	4.8	5.6	7.3	5.9	23.7	11.5
W14	0.0	5.3	0.0	0.0	1.9	0.0	1.0	6.4	7.8
HL–A12	14.3	21.1	8.9	9.5	16.7	9.3	12.9	29.8	22.3
HL–A13	8.6	2.6	6.7	6.7	6.5	6.7	6.3	5.1	4.2
W10	27.1	29.0	33.3	23.8	27.8	26.7	25.4	13.4	4.8
W15	14.3	13.2	20.0	12.4	13.9	14.7	14.9	11.4	15.1
W17	18.6	23.7	20.0	22.9	20.4	22.0	21.1	7.8	30.7
Y	38.5	41.9	13.4	45.7	39.6	36.0	36.6	44.0	40.4

Table 7

Distribution of the HL–A1, W17 haplotype. All figures are $\times 10^3$

	Hindu	Moslem	Telegu	Tamil	North	South	Indians	Caucasian	Bantu
Frequency	25	49	66	86	33	80	61	19	7
s.e.	40.4	57.7	37.3	37.1	33.2	28.3	19.4	10.0	23.5
Delta	16.7	34.9	52.5	64.6	23.3	61.1	45.1	11.7	3.1

Table 8

Genetic distances (f) between the four Indian groups based on the HL–A gene frequencies at the first and second locus

	First locus	Second locus	Average
Hindu – Moslem	0.0963	0.0548	0.0755
Hindu – Telegu	0.0864	0.0383	0.0623
Hindu – Tamil	0.0818	0.0553	0.0686
Moslem – Telegu	0.0720	0.0418	0.0569
Moslem – Tamil	0.0699	0.0642	0.0670
Telegu – Tamil	0.0571	0.0340	0.0456

Table 9

Genetic distances between Caucasians and Indian subgroups

	First locus	Second locus	Average
Caucasian – Hindu	0.1015	0.0994	0.1004
Caucasian – Moslem	0.0803	0.1015	0.0909
Caucasian – Telegu	0.0689	0.0744	0.0716
Caucasian – Tamil	0.0668	0.1027	0.0847
Caucasian – North	0.0880	0.0941	0.0910
Caucasian – South	0.0675	0.0914	0.0794
Caucasian – Indian	0.0679	0.0865	0.0772
Caucasian – Bantu	0.1271	0.0951	0.1111
Indian – Bantu	0.1561	0.1072	0.1316

Table 10

HL–A gene frequencies

Gene	Caucasian	Bantu	Indian
HL–A1	0.1726	0.0213	0.1383
HL–A2	0.2672	0.1219	0.1635
W28	0.0399	0.1015	0.0683
HL–A3	0.1632	0.0622	0.0935
HL–A11	0.0585	N.O.	0.1440
HL–A9	0.0861	0.1253	0.1557
HL–A10	0.0458	0.0849	0.0404
W19.6	0.0354	0.0849	0.0404
W29	0.0179	0.0654	0.0033
W31	0.0165	0.1677	0.0033
'O'	0.0969	0.1649	0.1493
HL–A5	0.0510	0.0121	0.1998
W5	0.0707	0.0558	0.1156
HL–A7	0.1242	0.0654	0.0630
W22	0.0194	0.1897	0.0133
W27	0.0399	0.0152	0.0166
HL–A8	0.1266	0.0590	0.0302
W14	0.0325	0.0400	0.0050
HL–A12	0.1623	0.1185	0.0666
HL–A13	0.0259	0.0213	0.0319
W10	0.0692	0.0244	0.1364
W15	0.0585	0.0784	0.0772
W17	0.0399	0.1677	0.1119
W18	N.T.	N.T.	0.0213*
'O'	0.1799	0.1525	0.1062

* N = 95
N.O. = Not observed
N.T. = Not tested

Discussion

The reaction patterns illustrated in Fig. 1 show how the anti-HL–A5 sera may be subdivided into groups. We have called these groups 5.1, 5.2, 5.3 and 5.4, and together they make up HL–A5. Those cells which are 5.1 (the first four subjects) reacted positively with almost all the HL–A5 antisera. Those which are 5.2 reacted positively with most of the antisera except the first five sera, which reacted only with 5.1 cells. 5.3 cells reacted with fewer antisera and 5.4 with fewer still. There were no antisera that reacted specifically with 5.2, 5.3 or 5.4, although one serum (N442) reacted only with 5.1 + 5.2 + 5.4 and not with 5.3, and four sera (Linford, PE 27, N564 and N475) reacted only with 5.1.

There is no question of the "short" sera reacting only with cells homozygous for HL–A5 because only three of the 27 cell donors did not have another antigen present at the second locus. Two of these subjects are in group 5.3, with which serum N442 did not react.

There is, of course, the possibility that only the cell donors on the left of Fig. 1 possess the HL–A5 antigen and that the extra reactions are caused by an antigen (or antigens), common in Indians but extremely rare in other populations, cross reacting with anti-HL–A5 sera and commonly associated with HL–A5 in Indians. Fig. 1 and Table 4 also show that 5.4 seems to be closely associated with Indians from the north of India. It is known that these northern populations were subjected to successive waves of infiltration of Mongoloid races from the northeast. These waves did not penetrate to the south of India, which is occupied by Dravidian races who originated in Western Asia and settled in India in prehistoric times. This is confirmed by the frequency of the HL–A1 antigen in the sub-groups investigated here. It is lowest in the Hindu population which emigrated to South Africa from the northeast of India, slightly higher in Moslems from the northwest and is the same in the Caucasians as in the Tamil population which emigrated from the southern-most part of India. (The Telegu occupy provinces to the North of the Tamils.) The significance of the differences in antigen frequency was tested by calculating χ^2 values for each antigen between all possible pairs of Indian subgroups. The only value with $P < 0.01$ was that for HL–A2 between Hindu and Moslem ($\chi^2 = 7.99$). Considering the total number of comparisons and the small number of Moslems tested, this value is not significant.

If only one antigen was detected at the first segregant series the cells were classified as, e.g., HL–A3, X,HL–A7, W27. Thus 'X' represents an unknown, or more precisely, an undetermined antigen because of the possibility of homozygosity. Similarly a 'Y' is used at the second segregant series. The frequencies of 'X' and 'Y' are therefore, to some degree, a measure of heterozygosity, because of the low frequency of null genes ('0') at each locus.

The frequency of 'X' at the first segregant series ranges from 33.6 % in Caucasians to 51.5 % in the Hindu population. At the second segregant series similar frequencies are observed for 'Y' except for the Telegus where the frequency is only 13.4 %. The next lowest frequency of 'Y' is in Hindus (38.5 %) and the difference is significant ($\chi^2 = 6.97$; $P < 0.05$), perhaps even more so, considering that the genetic distance (f) between Telegu and Hindu at the second locus is only 0.0383 (Table 8). The difference in the frequency of 'Y' between Telegu and Tamil is highly significant ($\chi^2 = 14.3$; $P < 0.0005$). This implies that there may be a selective advantage to the heterozygote at the second segregant series in the Telegu populations. We have found (Brain & Hammond, submitted for publication) a correlation between heterozygosity at the first segregant series and the ability to make Rh antibodies. There may be a correlation between heterozygosity at the second segregant series and the ability to make antibodies to pathogens which are (or were) common in the Telegu provinces of India.

Haplotype frequencies and delta values were calculated from the phenotype data for each population. The only significant delta values were for the haplotype HL–A1, W 17 and these are shown in Table 7. Singal et al. (1971) found the frequency of this haplotype to be 24/1000 in a study of 80 Indians of whom more than half (47) were from Northern India. Its delta value was not significant and their figures

are almost the same as our figures in the Hindu population. Ting et al. (1971) found a significant delta value for HL–A1, W17 in a study of Indians in Singapore. In our Caucasian population the delta value is greater than the standard error of the haplotype frequency. The data collected during the Fifth Workshop (*Histocompatibility Testing 1972*) show an increasing frequency of the HL–A1, W17 haplotype with increasing distance east of Europe.

If Caucasians and Dravidians have prehistoric ancestors in common it may be interesting to speculate on the high frequency of the W17 antigen and the HL–A1, W17 haplotype in the Tamil and Telegu groups, compared with the frequency of HL–A8 and the HL–A1, HL–A8 haplotype in Caucasians. There is also a negative delta value (— 0.0096) for the HL–A1, HL–A8 haplotype in Indians from the south of India. In fact only one individual out of 150 possessed both HL–A1 and HL–A8. What selective pressures can there have been to favour the HL–A1, W17 haplotype (and act against HL–A1, HL–A8) in the Dravidian races? There also must have been different selective pressures at work in the Caucasian population that were advantageous to the HL–A1, HL–A8 haplotype and did not affect the HL–A1, W17 haplotype.

Table 8 shows the genetic distances (f) between the Indian subgroups. The lowest values are between Tamil and Telegu from the south of India. The genetic distances between Caucasians and the other populations are shown in Table 9.

The f-values between Caucasians and Bantu and between Indians and Bantu are lower at the second segregant series than at the first segregant series. But the f-values between Caucasians and the Indian sub-groups are greater at the second segregant series than at the first, with the exception of Hindus, who show the greatest disparity with Caucasians.

References

Bodmer, J. G. & Bodmer, W. F. (1970) Studies on African pygmies. IV. A comparative study of the HL–A polymorphism in the Babinga Pygmies and other African and Caucasian populations. *Amer. J. hum. Genet.* **22,** 396–411.

Böyum, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21,** Suppl. 97.

Brand, D. L., Ray, J. G., Hare, D. B., Kayhoe, D. E. & McClelland, J. D. (1970) Preliminary trials toward standardization of leukocyte typing. *Histocompatibility Testing 1970,* p. 357–367. Munksgaard, Copenhagen.

Cavalli-Sforza, L. L. & Bodmer, W. F. (eds.) (1971) *The Genetics of Human Populations.* Freeman, San Fransisco.

Dausset, J. & Colombani, J. (eds.) (1972) *Histocompatibility Testing 1972.* Munksgaard, Copenhagen.

Hammond, M. G., Appadoo, B. & Brain, P. (1972a) HL–A antigens and antibodies in South African Bantu. *Transplantation* **14,** 159–165.

Hammond, M. G., Appadoo, B. & Brain, P. (1972b) HL–A antigens and antibodies in South African Indians. *Tissue Antigens* **2,** 389–396.

Mattiuz, P. L., Ihde, D., Piazza, A., Ceppellini, R. & Bodmer W. F. (1970) New approaches to the population: Genetic and segregation analysis of the HL–A system. *Histocompatibility Testing 1970,* p. 193–205. Munksgaard, Copenhagen.

Singal, D. P., Mickey, M. R. & Terasaki, P. I. (1971) HL–A typing in Asian Indians. *Tissue Antigens* **1,** 286–289.

Ting, A., Wee, G. B., Simons, M. J. & Morris, P. J. (1971) The distribution of HL–A leukocyte antigens in Singapore Chinese, Malays and Indians. *Tissue Antigens* **1,** 258–264.

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HLA ANTIGENS IN SOUTH AFRICAN NEGROES AND INDIANS. M.G. Hammond, B. Appadoo and Peter Brain. Natal Institute of Immunology, Durban, South Africa.

The Seventh Workshop serum set was tested in Zulu Negroes and Indians (the descendants of 19th century immigrants from India) because of the high frequency in these races of antigens that have been 'split'. At the A locus Aw30 is very common in Zulus and the reaction pattern of Aw30 sera shows that this antigen is probably heterogeneous although no clearcut split could be defined. As in the Sixth Workshop there were no sera recognising Aw31 but Aw32 was clearly defined by sera W312 and W327. Aw33 was detected only in Indians and a possible split is defined by two workshop sera which reacted as 'short' Aw33 (W331 and W427). Only serum W427 did not react with HLA A10 cells.

Several splits can now be defined at the B locus. The Seventh Workshop sera confirm our earlier subdivision of HLA B5 (M.G. Hammond et al, Tissue Antigens (1974) 4, 42), although serum W336 appears to define B5.3 and not B5.2 while B5.1 is split into B5.1 and B5.2. HR is not as clear as in the Sixth Workshop and the relationship between HR and B5.4 needs clarification. These subdivisions are common in the Indian population but Zulus have only B5.1 and HR. Bw35 was fairly well defined except for two Indians whose cells reacted with only some of the Bw35 sera. Nearly all Bw5.1, 5.2, 5.3, 5.4 and HR cells were 4a positive. while Bw35 cells were 4b.
Bw42 was found only in Zulus and was clearly different from Bw22 although some sera reacted with cells that were also positive with Bw41.
It is clear that Bw40 can be split. Bw40.1 is defined by serum W457 but the other Bw40 sera showed a very complex reaction pattern which was confined to Indians. Further splits cannot be excluded. There were no helpful 4a or 4b associations. The question of a split of Bw17 defined with Bw15 sera is not clear at all. Only Indians were positive with the short Bw15 sera in the workshop set. Eight local sera and one workshop serum (W436) reacted as long Bw15 sera with five Zulus and four Indians. Four of these local sera were positive with a further four Indians and two Zulus. Eleven of these donors were also Bw17 and ten of them had another antigen present at the B locus. However, six local sera and six workshop sera defining Bw17 showed no differences between these 10 cells and 16 other Bw17 cells. All Bw17 cells were 4a while the short Bw15 cells were 4b.

The first five antigens at the C locus presented no problems. At the Sixth Workshop we reported that Cw2 was absent in Indians but we have since found that it is present at a low frequency. T7 was present in 42% of Indians and 35% of Zulus.

A preliminary analysis of the B cell antisera gave the following percentage frequencies for the D locus antigens. A split of Dw2 was present in Indians.

	Zulu	Indian
Dw2	8	26
< Dw2	0	10
Dw3	19	13
Dw5	14	8
LD107	11	21
W85-W86	11	10

Dw1, Dw4, Dw6 could not be defined.

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LEUCOCYTE GROUPS IN BABOONS TESTED WITH HUMAN ANTISERA*

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The South African baboon (*Papio papio*) is a useful animal for research in transplantation, and Murphy *et al.*[1] have already shown that its leucocytes will cross-react with human leuco-agglutinating sera. The human antisera used in Murphy's study, however, were not characterized by leucocyte group. We report here a study of 29 baboons tested with 62 leuco-agglutinating sera previously characterized in a human panel.

MATERIAL

Twenty-nine adult baboons, of which 13 were male, were examined. They had been collected in five widely separated parts of South Africa and, as far as is known, were unrelated.

The human antisera were obtained from parous women as previously described. They had been characterized by χ^2 and sometimes by factor analyses against the leucocytes of panels of Caucasian donors varying in size from 40 to 188 individuals. A study of some of the sera has been previously published.[2] A composite χ^2 map of all these results is seen in Fig. 1. The antigenic complexes which the sera were recognizing were identified in earlier studies by the use of reference sera obtained from the National Institutes of Health and elsewhere, and many of the sera have also been examined by another laboratory. Many antisera that fell into positions intermediate between the main complexes, and some of unknown specificity, were deliberately included in the survey.

*Date received: 16 October 1969

METHODS

The EDTA agglutination test of Van Rood *et al.*[3] was used with certain modifications. Red cells were sedimented with 3% gelatin in normal saline. Only one drop of antiserum was used for each test, and the quantities of the other reagents were correspondingly reduced. All tests were read by the same worker.

From the laboratory protocols the results were assembled in a 29 × 63 matrix, coding any positive reaction as 1, negative as 0. An IBM 1130 computer was programmed to perform the following analyses:

1. Compare the reactions of every serum with those of every other, and print out χ^2 and r. $\left(r = \sqrt{\frac{\chi^2}{29}}\right)$

2. Compare the pattern of reactions of each individual baboon with that of every other, and perform a χ^2 analysis as above.

RESULTS

Fig. 1 shows the χ^2 associations of the sera in the human and Fig. 2 in the baboon panel. Each serum is represented by a circle of diameter proportional to the frequency with which it reacts. The unbroken lines between circles represent positive associations with χ^2 such that $r \geqslant 0{\cdot}5$ (thick lines) or $\geqslant 0{\cdot}32$ (thinner lines). Negative associations with $r \leqslant 0{\cdot}20$ are shown by dotted lines.

Table I shows the percentage frequencies with which the sera react in humans and baboons and the antigenic clusters to which the sera correspond in the human panel.

TABLE I. SPECIFICITY OF ANTISERA, AND FREQUENCIES OF REACTION IN MAN AND IN BABOONS

Serum No.	*Antigenic complex identified in man*	*Frequency in man %*	*Frequency in baboons %*	*Serum No.*	*Antigenic complex identified in man*	*Frequency in man %*	*Frequency in baboons %*
7	4a	48	14	76	7c	37	31
9	7c	34	31	78	4a	59	79
10	7c	34	10	86	7d	45	10
12	7d	36	35	88	4b	88	86
14	7c	22	21	89	7d	29	66
15	4a+	71	41	99	4a+	78	59
19	7c+	53	10	101	7c—7d	29	24
23	4b	88	38	103	4a+	74	72
24	±8a	51	38	105	4a+	85	59
25	7d	42	7	106	4b	90	59
27	New	17	62	109	7c—7d	34	31
29	New	21	28	112	7d	22	38
32	4a	52	55	113	7c—7d	22	10
34	7d	25	41	114	?5b	89	90
38	4a	32	76	115	7d	51	17
39	?	65	55	116	4a+	75	24
40	7c+4a	22	14	121	7d	61	31
44	4a	64	10	124	?	38	17
46	4b	83	79	127	?	60	10
48	8a+7d	46	35	129	?	53	31
49	4b	75	100	132	4b	80	28
52	7d	28	31	135	4b	83	28
53	7c	43	59	136	?	48	31
54	<4b	48	38	137	4a	43	14
61	?	80	52	138	?5b	93	79
64	7d	35	31	142	7c	65	79
66	7d	32	17	143	7c	60	35
68	4a	73	41	144	?	45	48
70	7d	32	10	145	7c—7d	15	10
72	7d	29	24	146	?	88	52
73	7c—4a	58	66	152	4b	88	7

Mean of all frequencies in man = 52·7%
Mean of frequencies in baboons = 39·1%

TABLE II. ANTIGENIC CLUSTERS IN BABOONS

Cluster No.	*Sera Nos.*	*Antigenic complex identified by serum in man*
1	145	7c—d
	10	7c
	124	?
	113	7c—d
	19	7c
	40	7c+4a
2	72	7d
	44	4a
	115	7d
	127	?
	14	7c
3	54	<4b
	29	New
	64	7d
	52	7d
	12	7d
4	66	7d
	101	7c—d
	86	7d
	116	4a+
	70	7d
	76	7c
5	135	4b
	121	7d
	15	4a+
	144	?
	112	7d
6	109	7c—d
	32	4a
	48	7d—8a
	137	4a

group detected by agglutination tests with these sera differs from the HL-A 8 group detected by cytotoxic tests. Studies of baboon leucocyte groups using cytotoxic sera of human origin would be interesting, and we hope to undertake them.

SUMMARY

The leucocytes of 29 South African baboons (*Papio papio*) were tested with 62 EDTA agglutinating human antisera, mostly of known specificity against human leucocytes. All these sera reacted with the cells of some baboons. Before it can be assumed that baboons have the equivalent of human white cell groups, however, it must be shown that the same sera that identify an antigenic complex in man are associated to form a complex in baboons also. This was found to be so only with some of the anti-7c (HL-A7) sera. There was no evidence of the equivalent of a human 4a or 4b group in the baboons tested, but the χ^2 map showed several antigenic complexes that do not appear to be related to human groups and may represent groups peculiar to the baboon. It is concluded that the South African baboons tested have a leucocyte group that is related to the human 7c group, but no groups related to the human 4a or 4b groups.

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REFERENCES

1. Murphy, G. P., Brede, H. D., Weber, H. W., Retief, F. P., Van Zyl, J. J. W., Van Zyl, J. A. and De Klerk, J. N. (1969): S. Afr. Med. J., **43**, 689.
2. Brain, P. and Hammond, M. (1968): Med. Proc., **14**, 589.
3. Van Rood, J. J., Van Leeuwen, A., Schippers, A. M. J., Pearce, R., Van Blankenstein, M. and Volkers, W. (1967): *Histocompatibility Testing*, p. 203. Copenhagen: Munksgaard.
4. Dausset, J., Ivanyi, P. and Ivanyi, D. (1965): *Histocompatibility Testing*, p. 51. Copenhagen: Munksgaard.

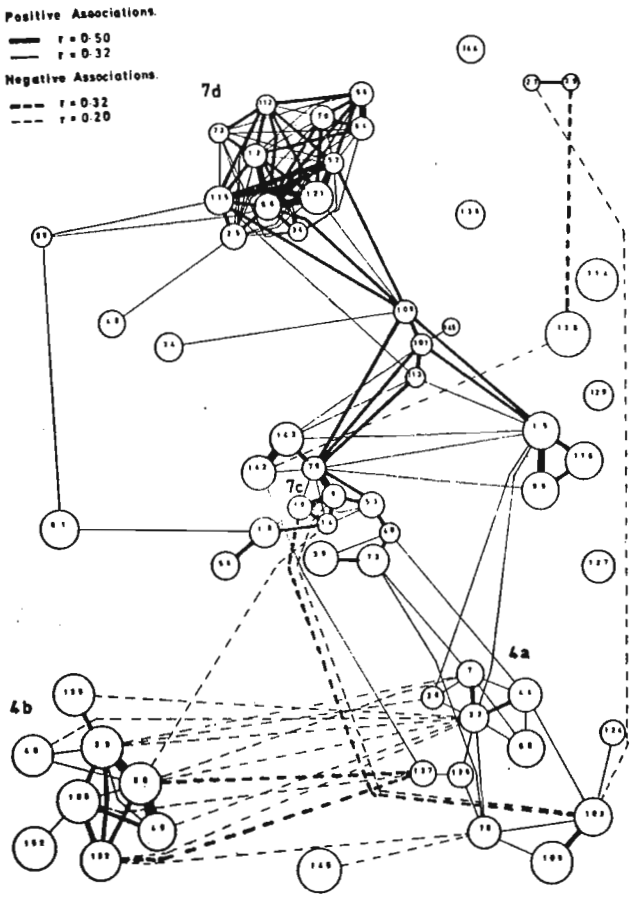

Fig. 1. χ^2 associations of sera tested against human leucocytes.

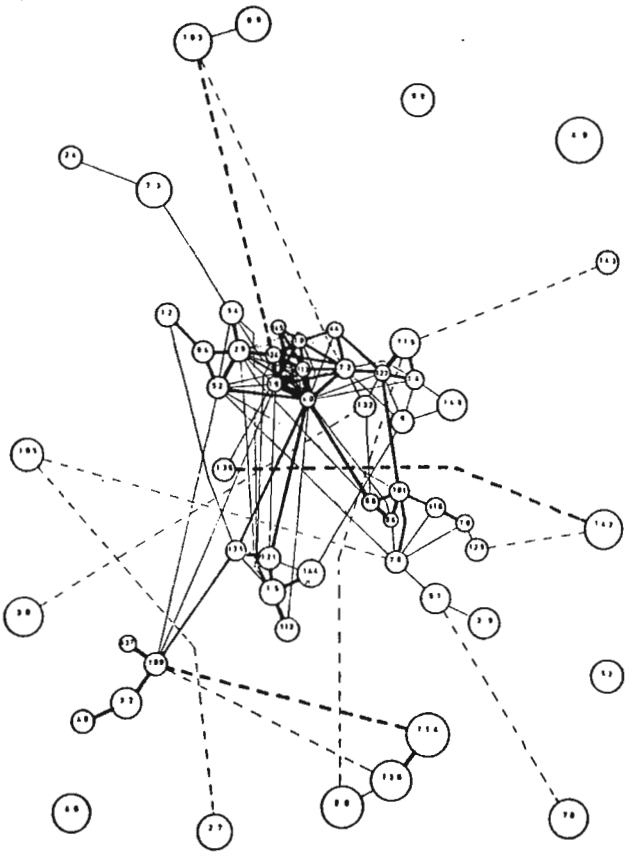

Fig. 2. χ^2 associations of sera with baboon leucocytes.

In Table II the data on the sera making up the antigenic clusters in baboons are summarized for convenience.

The χ^2 map of the comparisons between individual baboons showed only one complex of 19 positively associated individuals. It is not reproduced here.

DISCUSSION

If a human antiserum that detects an antigen, say 8a, in man is tested in baboons it may well react with the cells of some individuals. This does not mean, however, that these individual baboons possess the equivalent of the 8a antigen. For this to be so, we must find that the same sera that fall into the anti-8a cluster in the human χ^2 map are associated in an equivalent cluster in the baboon analysis. Even in different human populations, for example in Caucasians and Bantu, it has been found[3,4] that a serum that identifies a certain factor in one population does not necessarily do so in another. It would thus be surprising if there was much similarity between the leucocyte groups of human beings and baboons, and this study in fact shows that there is little.

The χ^2 map of the baboon tests does show, however, a number of clusters, of which only the central one composed of sera 10, 19, 40, 113, 124 and 145 is well defined.

The χ^2 map shows that all the clusters are in fact subgroups of one major cluster, and it is interesting to note from Table II that almost all the sera composing them have anti-7c or anti-7d affiliations in the human panel. In the tight central cluster of the baboon analysis most of the sera fall into the anti-7c group in human subjects. This strongly suggests that baboons have a leucocyte group that somewhat resembles 7c (HL-A7). The other clusters seen in the baboon χ^2 map may represent antigenic complexes that have no homologues in man. It appears unlikely that the baboons tested have anything equivalent to the human 4a or 4b complexes. We can say nothing about the 8a complex since only one pure anti-8a serum, from an impeccable source, was included in the study. It worked very well in the human panel but did not react with the cells of the baboons at all.

Further studies of baboon leucocyte groups would be of great theoretical interest, but should be carried out with sera derived from parous or transplanted baboons rather than from human subjects. After some well-defined groups have been detected in this way we may be able to investigate further their relationship to the leucocyte groups of man. If methods of immunosuppression improve enough in the future to make baboon-to-man transplants practicable, such studies will be of great practical importance.

ADDENDUM

The results with our anti-7d sera should be accepted with reserve, since we have subsequently shown that the human

Leukocyte Antigens of Baboons

By H. J. Downing, P. Brain, M. G. Hammond, G. H. Vos, and G. R. Webb

THE BABOON IS BEING USED in large numbers for transplant programs and it is therefore desirable to be able to identify its tissue antigens. It has been shown that the leukocytes of baboons will react with human leukoagglutinating sera.[1] Using 26 sera, Murphy et al. found that the greater the number of differences in the leukocyte antigens between the donor and the recipient of a skin graft, the shorter was the period of survival of the graft. This suggested that these human antisera were recognizing tissue antigens of the baboon. This was supported by their observation that homogenates of baboon kidneys reacted with the same antisera as did the leukocytes from the same baboon. Unfortunately, however, the sera used had not been previously characterized in man. Even if a serum had been characterized in one species it is difficult to apply it to another species. For example, if a human antiserum that detects an antigen, say, HL-A2, in man, reacts with the leukocytes of some baboons, it does not necessarily mean that these individual baboons posses the equivalent of the HL-A2 antigen. Even in different human populations it has been found that a serum that identifies a certain antigenic complex in one of these populations does not necessarily do so in the other.[2,3] An illustration of this is the serum Willett which has been described as having an agglutinin activity that corresponds exactly with its cytotoxicity activity.[4] This is certainly true for a white population, but it is not the case for Indians and blacks where the agreement between the two tests falls to 20%. Similarly, two sera that give a close correlation in a white population need not necessarily correlate with one another when tested in another population. In a white population the two sera Willett and S71 gave a close agreement with a χ^2 of 27.7, while in a black population there was very little association between these sera, and the χ^2 was reduced to 2.2. The reason for this is that many sera thought to be monospecific contain second antibodies against determinants that are very rare in one race but common in another, and this is far from being a rare occurrence. In a survey of white, Indian, and black populations, Brain and Hammond[3] found that although many leukocyte antisera appeared in the same tightly associated groups in all three populations, other sera closely associated in one race group were not associated in one of the other race groups. Where the same groups of closely associated sera are found in all three populations, it can be concluded that each of these groups of sera identifies a complex of antigenic factors frequently inherited in association.[5]

On the basis of this principle, human leukoagglutinating sera were used to study the leukocyte groups of baboons.[6] The results are illustrated in Fig. 1. The numbers are the reference number of the sera, the diameter of the circles represent the number of positive tests expressed as a percentage of the total, and the thickness of the lines represents the degree of association as measured by the χ^2 test. This method of drawing maps was first used by Dausset. There is little resemblance between the χ^2 maps for the two species except for one cluster of sera that detect HL-A7 in man and form a corresponding cluster in baboons. This strongly suggests that baboons have a leukocyte antigen that resembles HL-A7, but as this study did not reveal any other antigen shared by humans and ba-

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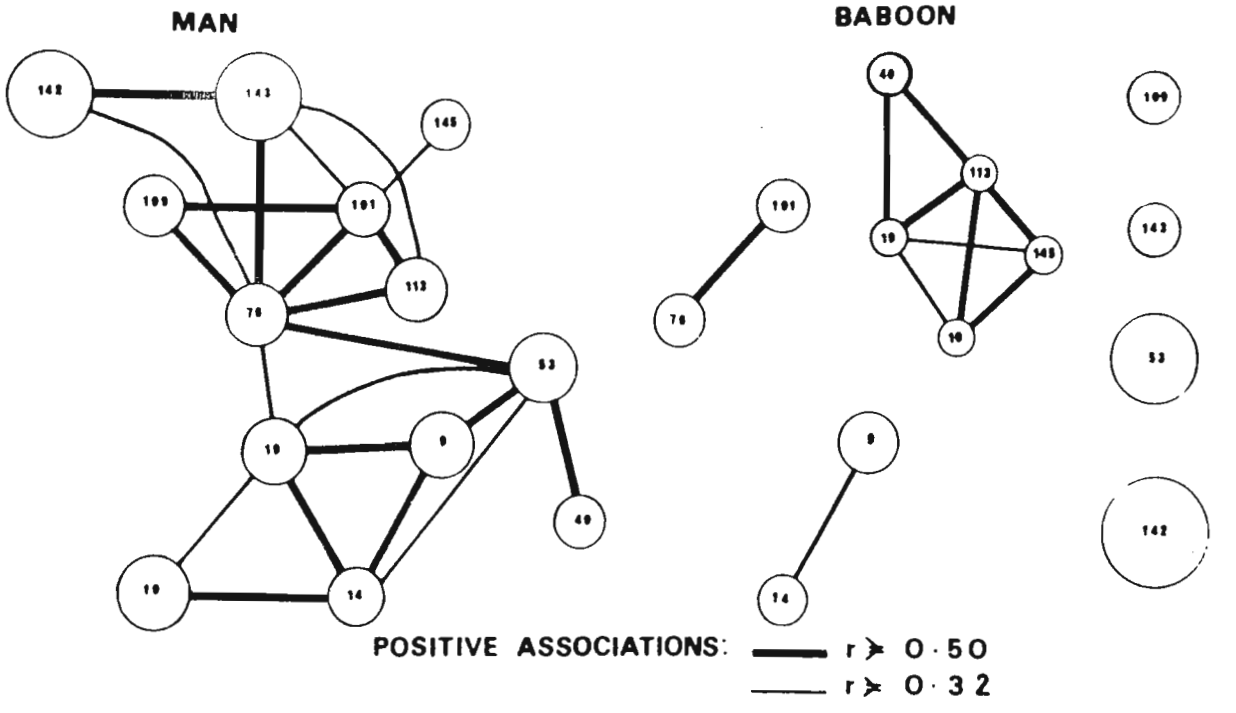

Fig. 1. Relations of 13 sera in man and baboon recognizing 7c complex in man.

boons, an attempt was made to develop isoantibodies in baboons.

Baboons were immunized by skin grafts[7] followed by s.c. booster injections of leukocytes in Freund's adjuvant. Ten days later, samples of blood were taken from the 16 baboons concerned and the sera tested by cytotoxicity against a panel of baboon lymphocytes stored in liquid nitrogen. Of the 16 baboons, 15 gave positive results and were plasmapheresed to give bulk supplies of plasma. The remaining baboon was given a further injection of lymphocytes but again failed to develop antibodies. The

Table 1. Pairs of Baboons With Similar Leukocyte Antigens as Determined by Cytotoxicity Test

CELLS	ANTISERA														
	B2	A3	E5	28	C9	B8	C7	C6	D4	E2	T2	T7	T8	T11	T13
B1	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+
T2	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+
4	+	−	+	−	−	+	+	−	+	−	−	−	+	+	+
5	−	−	+	−	−	+	+	−	+	−	−	−	+	+	+
7	+	+	−	+	−	−	+	+	+	+	+	+	+	+	+
C9	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+
T3	+	−	+	−	−	+	+	+	+	+	−	−	+	+	+
T7	+	−	−	−	−	+	+	+	+	+	−	−	+	+	+
9	+	−	+	−	+	+	+	−	−	−	−	−	+	+	+
C3	+	−	+	−	+	+	+	−	+	−	−	−	+	+	+
C1	+	+	−	+	−	+	+	−	−	−	−	−	+	+	+
C5	+	+	−	+	+	+	+	−	−	−	−	−	+	+	+

results of the cytotoxicity tests between the 15 sera and the lymphocytes from 45 baboons were analyzed by a computer and the χ^2 relationships between sera and between cells determined. The χ^2 values for the sera are shown in Fig. 2. Sera C7 and B8 are from baboons immunized by tissues from the same donor E3 and show a high degree of association. Although baboons A3 and E5 were immunized by donor C3, the sera from these baboons are not associated. Serum E5, however, is related to C7 which is also related to serum T11. Tissues from baboon 7 were used to immunize five baboons, and the sera from these baboons fall into two unrelated groups. Serum T8 is associated with sera T13 and T11, thus forming a group of six sera as shown at left of Fig. 2. The sera from the other two baboons, T2 and T7, that received tissues from baboon 7 are associated with one another but belong to a separate group of 4 sera (T2, T7, 28, and C6) as shown at right of Fig. 2. Outside these two groups of sera are four other sera (plus one serum not shown in Fig. 2) that are not related to any other serum.

Absorption studies have not been performed on any of these sera as the sera were produced by random immunizations and are unlikely to be monospecific. Instead, the sera have been used to compare the lymphocytes from the various baboons in our colony. From the analysis of these results, six pairs of baboons have been selected and are shown in Table 1. The two baboons in the first pair gave identical results with all 15 sera while the members of

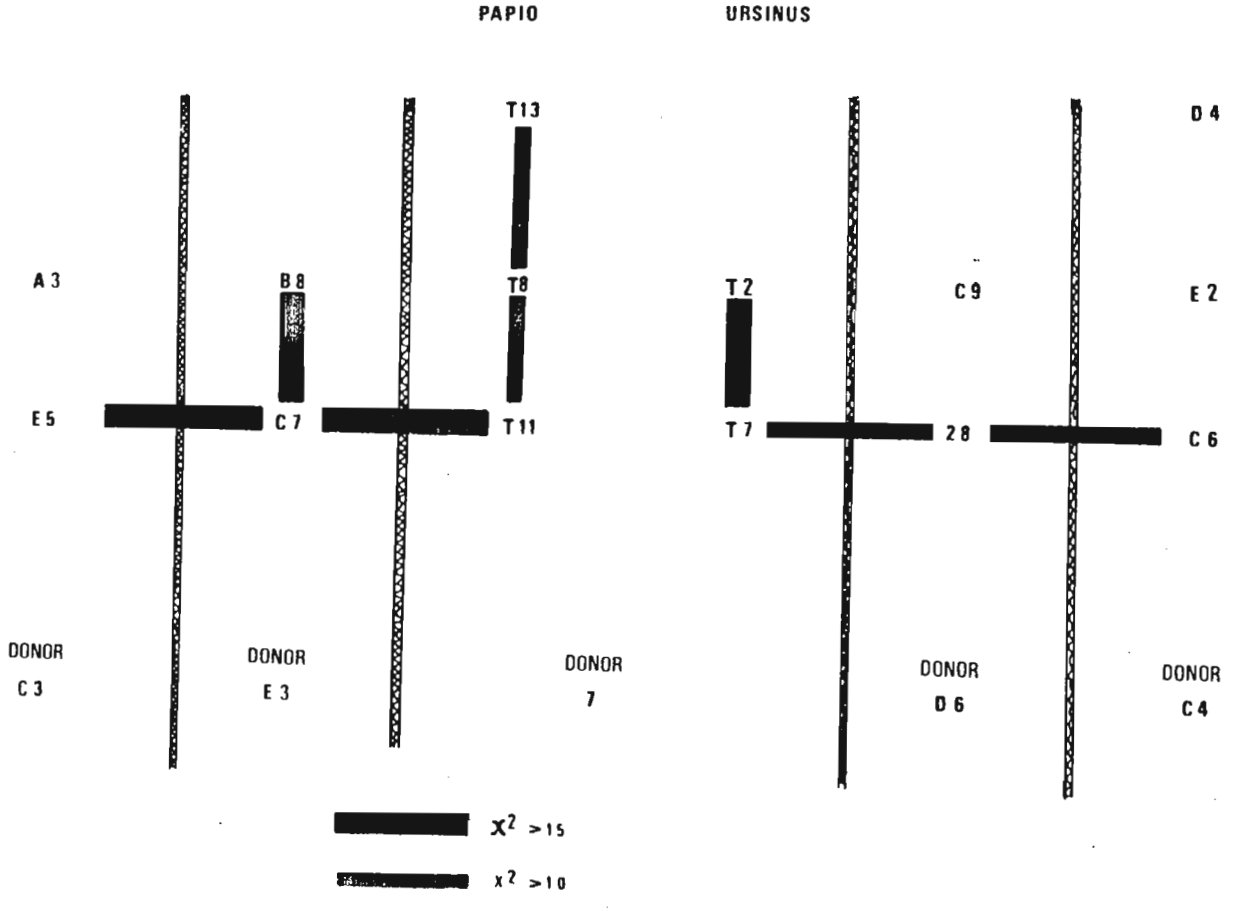

Fig. 2. Relationship between 14 cytotoxic sera produced in baboons by isoimmunization.

the other pairs differ from one another with respect to only one serum.

The next stage of the immunization program will be to exchange skin grafts between the members of each pair in an endeavour to produce more specific sera. These sera will be tested by absorption to see if any of them are monospecific. This work is being performed in one species of baboon, *Papio ursinus*, and it would be of interest to test these sera in other species of baboon. For this reason we hope to collaborate with other laboratories working in this field, especially Dr. Barnes and his colleagues at the University of Birmingham. They have already tested our first batch of sera.

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REFERENCES

1. Murphy, G. P., et al.: S. Afr. Med. J. 43, 1969.

2. Dausset, J., Ivanyi, P., and Ivanyi, D.: Histocompatibility Testing 1965, Copenhagen, Munksgaard, 1965, p. 51.

3. Brain, P., and Hammond, M. G.: Med. Proc. 14:589, 1968.

4. Catalogue of Typing Sera for 1968. Bethesda, Md., National Institute of Health, 1968, p. 130.

5. Amos, D. B.: Transplantation 5:1015, 1967.

6. Hammond, M. G., and Brain, P.: S. Afr. Med. J. 44:380, 1970.

7. Balner, H.: Transplantation 8:206, 1969.

8. Terasaki, P. I., and Singal, D. P.: Ann. Rev. Med. 20:175, 1969.

J. med. Primatol. 7: 174–181 (1978)

An Antigen Resembling HL-A7 on the Leukocytes of Vervet Monkeys[1]

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Key Words. Leukocyte antigens · Vervet monkeys · Tissue typing · HL-A

Abstract. Human leukocyte typing sera of known specificities were used to test the leukocyte antigens of vervet monkeys. The results suggest that these leukocytes contained an antigen resembling the HL-A7 antigen of human leukocytes. This is similar to a previous observation with leukocytes from baboons. These findings are consistent with the suggestion that the 4a/4b complex is the precursor substance from which the other specificities have evolved.

There have been numerous reports in which isoimmune typing sera developed in one species of primate have been used to study platelet and leukocyte antigens of other species. Human sera have been used to investigate the platelet antigens of the chimpanzee, gibbon, orangutan, baboon, rhesus monkey and African green monkey [19]. Other investigators [3–5, 14] have used human sera to test the leukocyte antigens of chimpanzees. In the reverse situation, sera developed in chimpanzees, by immunization with cells from other chimpanzees or human beings, have been used to test human leukocyte antigens [3–5, 14, 18, 19].

Human typing sera have also been used to study the leukocyte antigens of baboons by means of the agglutination technique [8, 15, 16] and the cytotoxicity test [9]. Human typing sera of specificities anti-4a and anti-4b were used to test the leukocytes of rhesus monkeys by the absorption technique [6].

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We describe here an investigation in which human typing sera were used to test the leukocytes of vervet monkeys by the cytotoxicity test. In such studies, however, where sera that have been developed in one species have been used to test leukocytes of another species, the results have to be interpreted with caution [3]. Even within a species, a serum that gives a reliable result in one population will not necessarily do so in another [8, 10]. For this reason we have chosen a number of human sera for each specificity and have analysed the results to see if those sera that show a close relationship when used to test human leukocytes also show a similar correlation when used to test the leukocytes of vervet monkeys. This can be best seen from a diagram showing the relationships between the sera. Sera that cluster together when used to test human leukocytes show a similar cluster when they are used to test the leukocytes of monkeys.

Materials and Methods

Species. The vervet monkey *(Cercopithecus pygerythrus* Cuvier) is a species belonging to the superspecies *C. aethiops* of which the superspecific type species is the grivet monkey. Hill (13) recognises 13 subspecies of *C. pygerythrus.* No attempt, however, has been made in this paper to classify beyond species although the geographical distribution suggests that the specimens concerned were of the subspecies *C. pygerythrus.* The 80 individuals tested were housed at the NII Primate Centre, and had been collected from Natal and Northern Transvaal.

Anaesthetics. The monkeys were anaesthetized by intramuscular injection of ketamine hydrochloride ('Ketalar', Parke-Davis, Detroit, Mich.) at a dose of 15 mg/kg body mass. Blood samples, 5 ml, were collected from the femoral vein and defibrinated.

Cytotoxicity test. The lymphocytes were obtained by density gradient separation using ficoll-hypaque. The test was a modification of the microcytotoxicity test of SINGAL *et al.* [20] in that trypan blue was used instead of eosin to eliminate the need for phase contrast optics. The specificities, and the number of sera for each, are shown in table I.

Analysis of results. The results were analysed at the Computer Centre of the University of Natal using a program designed by G.R. WEBB to calculate X^2 between each pair of sera in turn [11].

Results

Only associations between sera where the χ^2 was equal to or greater than 30 have been considered. These results have been summarized in table II and have been represented diagrammatically in figure 1 where the strength of the associations is shown by the thickness of the lines connecting the various

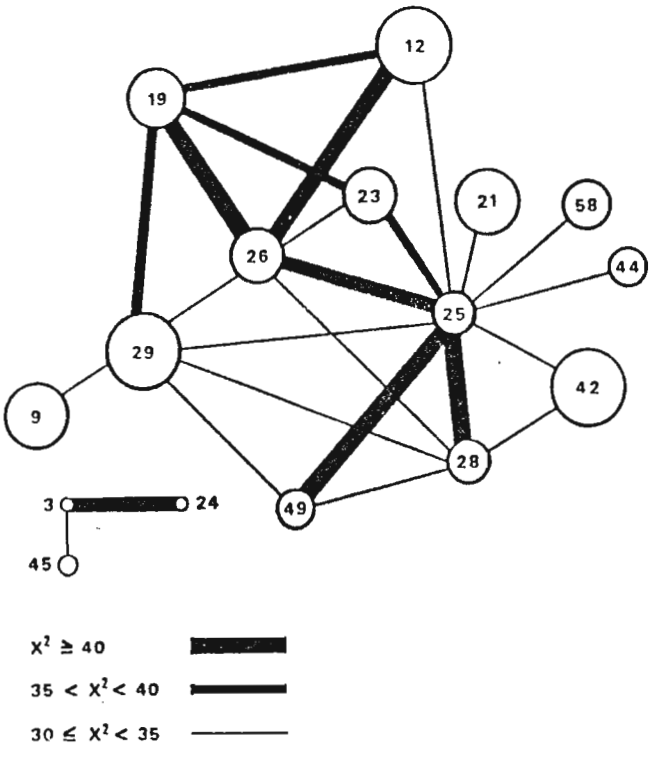

Fig. 1. Associations between human leukocyte-typing sera when tested with leukocytes from vervet monkeys. Diameter of circle represents percentage of positive results. Thickness of line represents strength of association. Numbers are the identification numbers of the sera.

pairs of sera. The percentage of positive results is indicated by the size of the circle representing each serum.

As there were 80 sera tested and compared with each other, there were 3,160 pairs of sera, but of these pairs only 24 had X^2 values equal to or greater than 30 (table II). These 24 pairs were made up from only 16 of the original 80 sera (fig. 1). The specificities of these sera were 4 HL-A7 + W22, 3 HL-A7, 2 HL-A12 and one of each of HL-A1, HL-A2, HL-A3, HL-A5, HL-A13, HL-A17, and W15. When this list is compared with the number of sera of each specificity (table I), it can be seen that of the five sera with specificities HL-A7 + W22 there are four that show relationships with other sera. Similarly, of all four HL-A7 sera, three are associated with other sera. Of these seven sera, one (No. 24) is associated with an HL-A1 serum (No. 3) while the remaining six are related to one another and to six other sera as shown in figure 1. The specificity that occurs most frequently in this cluster of sera is therefore HL-A7, and only the sera of

Table 1. The specificities and number of sera for each specificity

Specificity: anti-	Number of sera
HL-A1	5
HL-A2	6
HL-A3	2
HL-A3 + HL-A11	1
HL-A5	6
HL-A7	4
HL-A7 + W22	5
HL-A8	2
HL-A9	6
HL-A10	2
HL-A11	1
HL-A12	4
HL-A13	1
HL-A13 + W10	2
HL-A14	2
HL-A17	2
HL-A28	1
W5	4
W10	3
W15	1
Total	60

anti-HL-A7 specificity, with and without anti-W22, show any tendency to cluster among themselves as they do if tested with human lymphocytes.

Discussion

The cluster of sera with HL-A7 specificity that has been observed when human typing sera were used to test the leukocytes of vervet monkeys is similar to the earlier observation when a panel of human sera was used to test baboon leukocytes [12]. In this earlier paper the sera were associated in one major cluster that had some subclusters. In the tight central cluster that was formed when the baboon cells were tested, most of the sera belonged to the anti-7c (anti-HL-A7) group when tested with human leukocytes. This strongly suggested that baboons have a leukocyte group that somewhat resembles HL-A7. The results of the present investigation, which

Table II. Comparison of results obtained between pairs of human cytotoxic sera when tested with leukocytes of vervet monkeys

Serum 1		Serum 2		Results				χ^2
No.	specificity	No.	specificity	−−	−+	+−	++	
3	HL-A1	24	HL-A7	72	3	0	5	48.00
		45	HL-A13	68	7	0	5	30.22
9	HL-A2	29	HL-A7 + W22	40	9	5	25	32.04
12	HL-A3	19	HL-A5	42	3	10	25	36.30
19	HL-A5	23	HL-A7	48	4	7	21	38.38
		25	HL-A7 + W22	49	3	10	18	32.19
		26	HL-A7 + W22	49	3	7	21	41.54
		29	HL-A7 + W22	42	10	3	25	36.30
21	HL-A7	25	HL-A7 + W22	48	3	11	18	30.15
23	HL-A7	25	HL-A7 + W22	52	3	7	18	39.31
		26	HL-A7 + W22	49	6	7	18	30.55
25	HL-A7 + W22	26	HL-A7 + W22	53	6	3	18	42.09
		28	HL-A7 + W22	57	2	4	17	51.45
		29	HL-A7 + W22	44	15	1	20	30.67
		42	HL-A12	44	15	1	20	30.67
		44	HL-A12	56	3	8	13	31.25
		49	HL-A17	56	3	5	15	41.50
		58	W15	54	5	6	15	32.74
26	HL-A7 + W22	28	HL-A7 + W22	53	3	8	16	34.87
		29	HL-A7 + W22	43	13	2	22	31.99
28	HL-A7 + W22	29	HL-A7 + W22	45	16	0	19	32.04
		42	HL-A12	45	16	0	19	32.04
		49	HL-A17	56	5	5	13	32.38
29	HL-A7 + W22	49	HL-A17	45	0	16	18	30.85

used cytotoxicity rather than agglutination, suggest that this is also the situation with vervet monkeys.

These two sets of observations, in which human HL-A7 sera react with the cells of baboons and vervet monkeys, are similar to the findings of BALNER *et al.* [3]. These investigators found that certain of their chimpanzee sera (group 3) showed 7c- or HL-A7-like reactivity. They postulated that these sera might have a specificity related to AA (or W22) which is known to cross-react with HL-A7 antigens when used with human cells. Our findings in which the W22 specificity was associated with four of the six HL-A7 sera in the cluster are consistent with BALNER's suggestion.

Further evidence that there are cross-reactions is offered by our observations that not all our HL-A7 were associated when tested with leukocytes of vervet monkeys. Although six of the sera were associated, three (No. 22, 24 and 27) were not. Furthermore, six of the sera (No. 23, 24, 25, 26, 28, 29) were each found to be associated with at least one serum of another specificity. The antigens on the leukocytes of the vervet monkey are therefore different from the human HL-A7 antigen which they resemble. This is similar to the situation with the chimpanzee where BALNER *et al.* [4] have used human cells to absorb chimpanzee sera and have shown that there are differences between the HL-A7 antigens on human leukocytes and their apparent counterpart on chimpanzee leukocytes. BALNER *et al.* concluded therefore that the '7c' on the chimpanzee cells consists of an additional specificity, or that the 7c (chimp) and 7c (human) have different configurations but are cross-reactive.

BALNER [1] has also identified antigens on rhesus leukocytes that may well be the counterparts of the human 4a and 4b antigens.

These observations have led to the suggestion [2, 3, 17] that the basic substances from which the other antigens evolved are the 4a/4b antigens. As this complex is closely related to the seven series of antigens, our findings concerning the HL-A7 antigen in vervet monkeys are consistent with the suggestion that the 4a/4b antigens are the precursor substances from which the other specificities have evolved.

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References

1 BALNER, H.: Leukocyte antigens of man and subhuman primates; in Proc. XIth Eur. Conf. on Animal Blood Groups and Biochemical Polymorphism, Warsaw 1968, pp. 67–87 (Junk, The Hague 1963).

2 BALNER, H.; DERSJANT, H.; VREESWIJK, W. VAN; LEEUWEN, A. VAN, and ROOD, J.J. VAN: Identification of chimpanzee leukocyte antigens (ChL-A) and their relation to HL-A. Transplantation *11:* 309–317 (1971).

3 BALNER, H.; CRABB, B.W.; DERSJANT, H.; VREESWIJK, W. VAN; LEEUWEN, A. VAN, and ROOD, J.J. VAN: Heterologous antisera for human histocompatibility testing. Transplantn Proc. *3:* 1088–1098 (1971).

4 BALNER, H.; LEEUWEN, A. VAN; DERSJANT, H., and ROOD, J.J. VAN: Chimpanzee isoantisera in relation to human leukocyte antigens; in histocompatibility testing 1967, pp. 257–265 (Munksgaard, Copenhagen 1967).

5 BALNER, H.; LEEUWEN, A. VAN; DERSJANT, H., and ROOD, J.J. VAN: Defined leukocyte antigens of chimpanzees: use of chimpanzee isoantisera for leukocyte typing in man. Transplantation *5:* 624–642 (1967).

6 BALNER, H.; DERSJANT, H.; LEEUWEN, A. VAN, and ROOD, J.J. VAN: Identification of two major leukocyte antigens of rhesus monkeys and their relation to histocompatibility; in histocompatibility testing 1967, pp. 267–276 (Munksgaard, Copenhagen 1967).

7 BOYUM, A.: Separation of leukocyte from blood and bone marrow. Scand. J. clin. Invest. *21:* suppl. 97, p. 7 (1968).

8 BRAIN, P. and HAMMOND, M.G.: Leukocyte antigens in three race groups. Med. Proc. *14:* 589–592 (1968).

9 COHEN, E.; GREGORY, S.G.; DOZIER, A.; GROENEWALD, J.H., and MURPHY, G.P.: Human-type erythrocyte A-B-O group and leukocyte antigens of *Papio ursinus,* South Africa; in GOLDSMITH and MOOR-JANKOWSKI Medical primatology 1970. Proc. 2nd Conf. exp. Med. Surg. Primates, New York 1969, pp. 148–152 (Karger, Basel 1971).

10 DAUSSET, J.; IVANYI, P., and IVANYI, D.: Tissue alloantigens in human-identification of a complex system (Hv-1); in Histocompatibility testing 1965, pp. 51–62 (Munksgaard, Copenhagen 1965).

11 DOWNING, H.J.; SCHLEYER, M.E.; SAIKOOLAL, A., and POONIAH, P.: Leukocyte antigens in vervet monkeys *(Cercopithecus pygerythrus* Cuvier). S. Afr. J. Sci. *69:* 84–85 (1973).

12 HAMMOND, M.G. and BRAIN, P.: Leucocyte groups in baboons tested with human antisera. S. Afr. med. J. *44:* 380–382 (1970).

13 HILL, W.C.O.: Primates – comparative anatomy VI (Edinburgh University Press, Edinburgh 1966).

14 METZGAR, R.S. and ZMIJEWSKI, C.M.: Species distribution of human tissue isoantigens. I. Detection of human tissue antigens in chimpanzees. Transplantation *4:* 84–93 (1966).

15 MURPHY, G.P.; BOTHA, M.C.; BREDE, H.D.; WEBER, H.W.; RETIEF, F.P.; ZYL, J.J.W. VAN; ZYL, J.A. VAN, and KLERK, J.N. DE: Correlation of tissue-typing tests in baboon renal allotransplants. J. surg. Res. *9:* 19–28 (1969).

16 MURPHY, G.P.; BREDE, H.D.; WEBER, H.W.; RETIEF, F.P.; ZYL, J.J.W. VAN; ZYL, J.A. VAN, and KLERK, J.N. DE: Correlation of tissue-typing tests in baboon renal allotransplants – a preliminary study. S. Afr. med. J. *43:* 689–694 (1969).

17 ROOD, J.J. VAN; LEEUWEN, A. VAN, and ZWEERUS, R.: The 4a and 4b antigens: do they or don't they? in Histocompatibility testing 1970, pp. 93–104 (Munksgaard, Copenhagen 1970).

18 SHULMAN, N.R.; MARDER, V.J.; HILLER, M.C., and COLLIER, E.M.: Platelet and leukocyte isoantigens and their antibodies: serologic, physiologic and clinical studies. Prog. Haematol. *4:* 222–304 (1964).

19 Shulman, N.R.; Moor-Jankowski, J., and Hiller, M.C.: Platelet and leukocyte isoantigens common to man and other animals; in histocompatibility testing 1965, pp. 113–123 (Munksgaard, Copenhagen 1965).

20 Singal, D.P.; Mickey, M.R., and Terasaki, P.I.: Serotyping for homotransplantation. XXIX. Two new HL-A antigens. Transplantation *8:* 235–240 (1969).

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Histocompatibility Testing 1972

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Frequency of HL-A Antigens in South African Bantu, Indians and Caucasians

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Description of the Populations

There are three distinct race groups in Durban: Caucasian, Bantu and Indian.

The Caucasian population is of Western European origin. Bantu are mostly of the Zulu tribe. Indians are the descendants of immigrants who arrived about a century ago, mostly from the Madras Presidency. The Indians in Durban can be subdivided according to language, by their names; the proportions are approximately: Tamil 36, Hindi 28, Telegu 15, Other 21 (including those with Muslim names). The series tested here, however, was not classified in this way.

The three races are about equally represented in the population of Durban (about 650,000). There is a small Coloured (mixed Caucasian and Bantu) population that was not studied.

The Bantu and Indian populations of the city maintain a Western way of life with little or no mixture between the races.

Materials and Methods

One hundred and fifty unrelated individuals of each race were tested for the antigens listed in Table I.

Lymphocytes were isolated by the method of Boyum (1968) and the cytotoxicity test was performed in Falcon microtest trays using the two-stage procedure recommended by the N. I. H.

Sera were obtained from the N. I. H. bank and elsewhere and used in parallel with sera obtained from the screening of over 50,000 parous women and standardised in this laboratory. In an intensive search for antisera that might detect new antigens in the Bantu and Indian populations, samples were taken from 1,000 parous women of each race and tested against the lymphocytes of at least 40 random donors of the same race. Table I shows the number of sera used to detect each antigen, and their origins.

Results

Table II shows the frequencies of the various antigens in the three population groups. Table III shows the gene frequencies in the three population groups. Figure 1 illustrates the reaction pattern of HL-A 7 and HL-A 7 + W 22 antisera with 50 Bantu and Figure 2 with 150 Bantu. Figure 3 shows the reaction pattern of sera with, or included in, HL-A 12 in 150 Bantu. Figure 4 illustrates the reaction pattern of sera included in HL-A 11 and Figure 5 that of sera associated with the HL-A 5, W 5 complex. Table IV shows the phenotype frequencies of other polymorphisms.

Discussion

Our earlier studies on the Bantu showed many individuals who had no detectable antigens at the first locus. When we obtained sera against Te 63 and Te 66, however, we found that these specificities accounted for

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After the Vth International Histocompatibility Conference the WHO Committee on HL-A Nomenclature agreed on the following equivalents: Te 63 = W 29; Te 66 = W 30 + W 31.

most of the blanks. Both have a far higher incidence in the Bantu than in the other races, and the difference in incidence, here and with other sera, between Bantu and Indians shows clearly that the population groups are, for practical purposes, unmixed with each other.

The Bantu are otherwise distinguished by low frequencies of HL-A 1, 11, 5 and 7 and high frequencies of HL-A 10, W 22 and W 17. The Indians are remarkable for low frequencies of W 19, Te 63 and Te 66 (lower than in Caucasians); HL-A 8 and W 14; and for high frequencies of HL-A 11, HL-A 9, HL-A 5 and W 10. The Indians, unlike other non-Caucasian populations, have a frequency of 22 % for HL-A 1 but this may be the result of a common Indo-European ancestry.

In an earlier published study (Hammond & Brain 1971) we said that the frequency of HL-A 7 in the Bantu was about 28 %. We did not then realise that many of our sera contained antibodies for both HL-A 7 and W 22. Figure 1 illustrates the reaction pattern obtained with these sera in the first panel of 50 Bantu. Six sera including N. I. H. serum D 66-15903 and Te 5691.1, gave identical reaction patterns and all have been previously characterised as anti-HL-A 7. They reacted with only 4 out of 50 donors. Four sera gave identical reactions to K-N8498 from Kissmeyer-Nielsen which is anti-HL-A 7 + W 22 and were positive with 22 out of 50 donors. Another 11 sera including Te 3186.0 (HL-A 7 + W 22) all had a coefficient of correlation ($r = \sqrt{\chi^2/N}$) greater than 0.87 with K-N 8498. Eight sera appear to identify W 22 with some variations.

We then tested a further 100 Bantu using two anti-HL-A 7 sera and two anti-HL-A 7

TABLE I

Numbers and sources of antisera used to identify HL-A antigens

Antigens	Antisera			
	Natal Institute of Immunology	N. I. H. Serum Bank	N. I. H. Tray N 621	Total
HL-A 1	9	1	3	13
HL-A 2	14	1	3	18
HL-A 3	3	3	3	9
HL-A 9	2	3	2	7
HL-A 10	4	1	4	9
HL-A 11	1	1	3	5
W 28	4	3	2	9
W 19	1	2	2	5
Te 63	1	0	2	3
Te 66	0	1	2	2
HL-A 5	6	2	3	11
W 5	3	1	3	7
HL-A 7	9	2	4	15
W 22	3	1	1	5
HL-A 8	4	4	3	11
W 14	3	1	3	7
HL-A 12	11	0	3	14
HL-A 13	4	2	2	8
W 15	3	0	2	5
W 17	5	1	3	9
W 10	3	1	3	7
W 27	1	1	0	2
Total	94	32	56	182

TABLE II

Percentage frequency of antigens in Caucasians, Bantu and Indians

Antigen	Caucasian	Bantu	Indian
HL-A 1	26	5	22
HL-A 2	52	20	31
W 28	7	19	15
HL-A 3	33	12	15
HL-A 11	12	0	32
HL-A 9	14	17	27
HL-A 10	9	23	7
W 19	11	17	3
Te 63	6	13	1
Te 66	5	31	2
HL-A 5	10	4	42
W 5	19	9	27
W 15	18	16	16
HL-A 7	27	11	14
W 27	9	3	3
W 22	3	34	5
HL-A 8	21	13	6
W 14	5	7	1
HL-A 12	31	22	11
HL-A 13	9	5	8
W 10	15	5	19
W 17	6	29	21

TABLE III

HL-A gene frequencies in Caucasians, Bantu and Indians

Gene	Caucasian	Bantu	Indian
HL-A 1	.140	.025	.117
HL-A 2	.307	.106	.169
W 28	.036	.100	.078
HL-A 3	.182	.062	.078
HL-A 11	.062	.000	.175
HL-A 9	.073	.089	.146
HL-A 10	.047	.123	.036
W 19	.057	.089	.015
Te 63	.031	.067	.005
Te 66	.025	.169	.010
HL-A 5	.051	.020	.239
W 5	.100	.047	.146
W 15	.100	.084	.084
HL-A 7	.146	.057	.073
W 27	.047	.015	.015
W 22	.015	.188	.025
HL-A 8	.111	.067	.031
W 14	.025	.036	.005
HL-A 12	.169	.117	.057
HL-A 13	.046	.025	.041
W 10	.078	.025	.100
W 17	.031	.157	.111

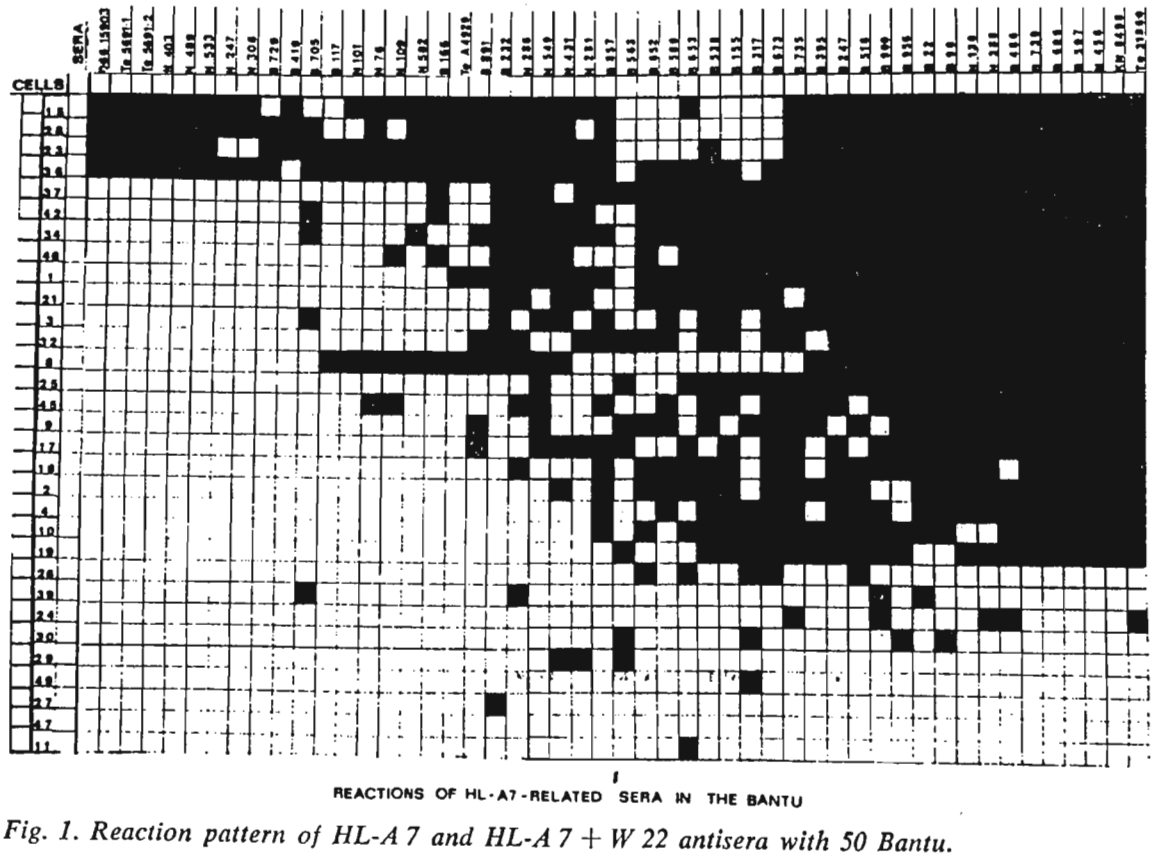


Fig. 1. Reaction pattern of HL-A 7 and HL-A 7 + W 22 antisera with 50 Bantu.

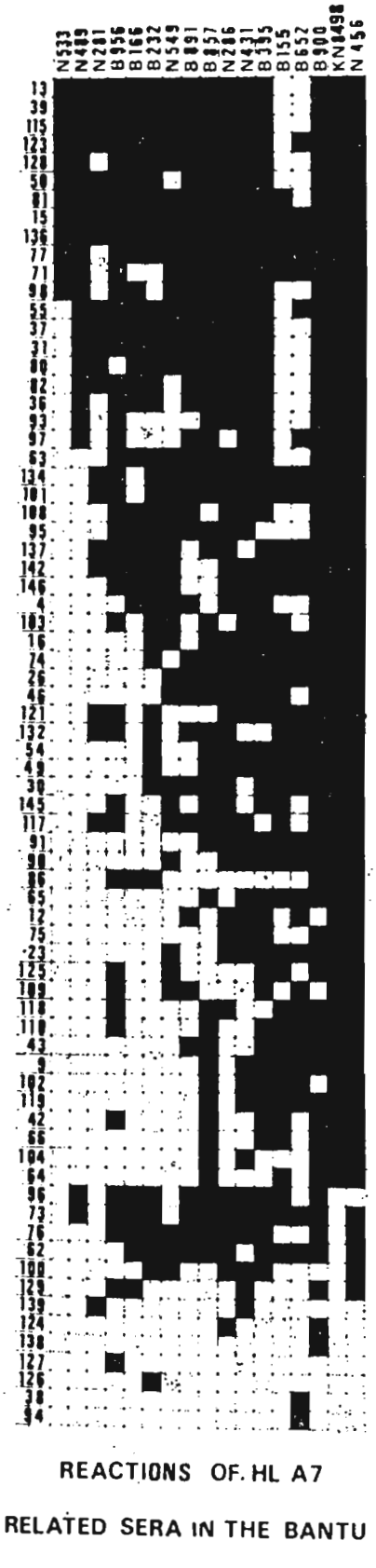


Fig. 2. Reaction pattern of HL-A 7 and HL-A 7 + W 22 antisera with 150 Bantu.

+ W 22 sera and 12 sera that showed associations with this complex. Figure 2 shows the overall pattern of reactions of these sera with 150 Bantu. Two sera, B 155 and B 652, showed some agreement and appeared to recognise W 22 only. Three cell donors appear to possess both HL-A 7 and W 22. It is clear that this complex must consist of more than two or even three antigens. Kissmeyer-Nielsen (personal communication) has found that the antigen AA (W 22) can be subdivided into two categories which he calls AA* and AA-AJ.

When we tested these same sera with 150 Caucasians we found only 3 % positive for W 22 and 27 % for HL-A 7. This makes it extremely difficult to differentiate this complex by testing the Caucasian population; but, as we have shown here, the Bantu population has a high frequency of these antigens and this will make further investigations easier.

Figure 3 shows the reaction pattern of sera associated with or included in HL-A 12 in 150 Bantu. It appears that HL-A 12 is a heterogeneous antigen which can be subdivided into at least two parts. The sera on the left apparently recognise the shortened HL-A 12 and those on the right the short HL-A 12 plus another component. Three sera seem to react only with this new antigen or part of it. Svejgaard *et al.* (1970 a, b) have described an antigen EL* which is defined by an antiserum that reacts with HL-A 12 cells and with cells that are EL* positive. However, the variation in the Bantu appears to be within HL-A 12 because sera that have given identical patterns of reaction in Caucasians show differences in the Bantu. Serum N 320 has only 10 positive reactions. This is not a weak serum that reacts only with those cells that are homozygous for HL-A 12, because two of the cell donors have another antigen present at the second locus. Colombani *et al.* (1971) has recently described a subdivision of HL-A 12 into HL-A 12′ and HL-A 12″ and this may explain the differences in the Bantu.

Several interesting points have emerged from the studies of the Indian population with sera obtained from Indians. There were 57 sera reacting with the cells of less than 10 % of the population, that showed

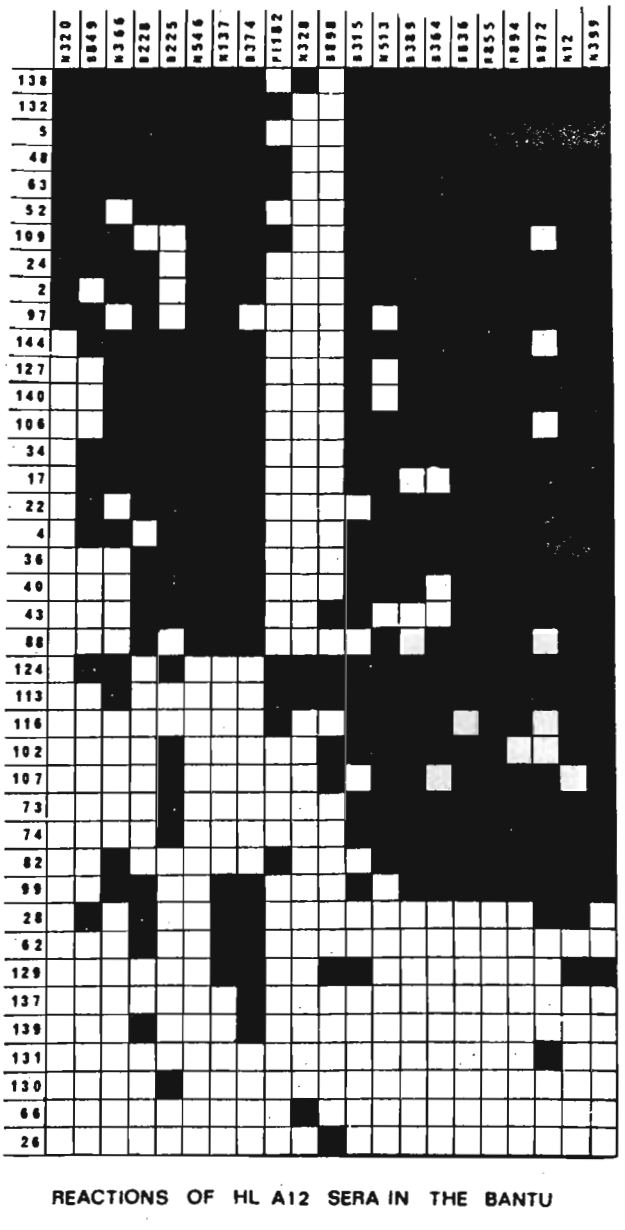


Fig. 3. Reaction pattern of sera associated with HL-A 12 in 150 Bantu.

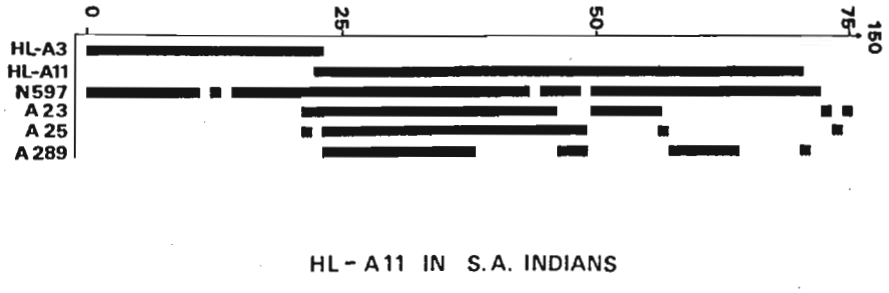
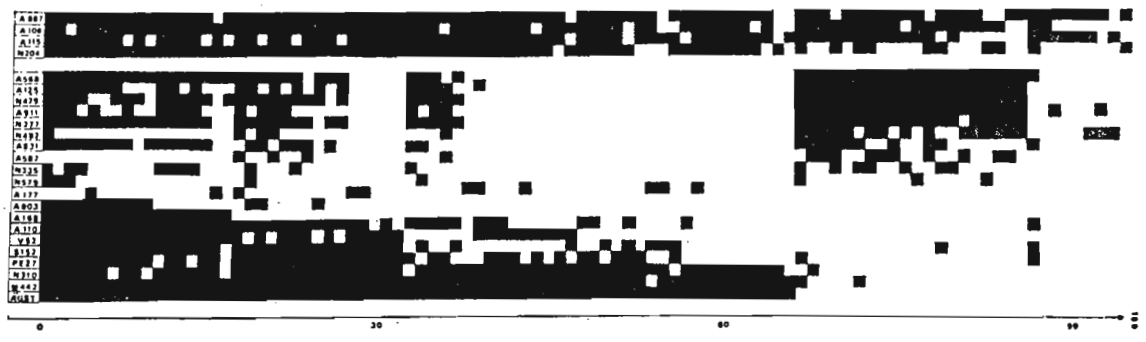


Fig. 4. Reaction pattern of sera included in HL-A 11 in 150 Indians.



HL-A5 AND W5 IN S.A. INDIANS

Fig. 5. Reaction pattern of sera associated with HL-A 5 and W 5 in 150 Indians.

no correlation with any of the known antigens, nor were their reactions included in those of any known antigens. It does not seem possible that they are all recognising specific HL-A antigens or combinations of rare ones, and one explanation is that they may be recognising HL-B antigens, as described by Singal *et al.* (1970), although they found HL-B antibodies primarily as extra antibodies in HL-A antisera. The analysis of 1,004 sera from Bantu women showed 59 sera that had no correlation with known antigens.

TABLE IV

Phenotype frequencies of other polymorphisms in Caucasians, Bantu and Indians

	Caucasian	Bantu	Indian
A	37.2	29.7	21.0
B	11.3	19.0	32.3
O	47.9	44.1	37.1
AB	3.6	4.4	8.9
Weak A	0.0	1.8	0.2
Weak AB	0.0	0.9	0.5
Rh +	85.5	96.4	95.2
Rh −	14.5	3.6	4.8
Le (a+b−)	17.2	24.0	27.1
Le (a−b+)	76.8	55.9	61.4
Le (a−b−)	6.0	20.1	11.4
MMS	21.3	8.1	21.4
MsMs	11.1	16.1	14.3
MNS	24.8	12.9	34.5
MsNs	23.9	33.9	19.0
NNS	5.2	9.7	7.1
NsNs	13.7	19.4	3.6
P +	78.2	93.5	69.7
P −	21.8	6.5	30.3
K +	9.3	0.0	2.2
K −	90.7	100.0	97.8

The Indian population has a higher frequency of HL-A 11 than either Caucasians or Bantu, but the sera we used were significantly different. Serum N 597 contains antibodies to HL-A 3 and HL-A 11 and when characterised in Caucasians there were no positive reactions outside these two specificities. In the Indians this serum has a frequency of 42 %, of which 32 % appeared to be HL-A 11. Three antisera obtained from Indian women gave a reaction pattern which was included in HL-A 11 but they had no significant correlation with each other. This is shown in Figure 4.

The most interesting results, however, are those concerning the antisera recognising HL-A 5 and W 5. The relatively high frequency of these antigens in the Indian population emphasises variations in reaction patterns of the different sera. Figure 5 shows the reaction pattern of several sera that show associations with this complex. It seems that there may be more factors involved than the three described in the 1970 workshop data, viz. HL-A 5, W 5 and W 18. N 310 and AGST appear to be operationally monospecific anti-HL-A 5 sera in Caucasians and in the Indian series they agree very well. V 52 is also a good anti-HL-A 5 serum in Caucasians but in Indians V 52

reacts with only 26 of the 42 HL-A 5 cells. Serum D 66-6222 from the N. I. H. is also a good HL-A 5 reagent in Caucasians but reacts with only 15 of the cells that are positive with V 52. Three other sera (PE 27, S 152 and N 442) also have patterns that are included in HL-A 5, W 5 and W 18.

We have previously shown (Hammond & Brain 1971) that an antiserum that is apparently monospecific when characterised in Caucasians may give very different results if tested in another population, and this has been the experience of other workers also. For the purely practical purposes of tissue typing in South Africa, therefore, it is necessary that every serum used should be characterised and found suitable in each population group. The work we have put into this has produced quite a lot of information that may be of interest to anthropologists.

References

Boyum, A. (1968) Separation of Leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21**: Supp 97, 7.

Colombani, J. D'Amaro, J., Gabb, B., Smith, G. & Svejgaard, A. (1971) International agreement on a platelet complement fixation micro technique. *Transplant. Proc.* **3**, 121.

Hammond, M. G. & Brain, P. HL-A antigens in three populations. (1971) *Vox Sang. (Basel)* **20**, 492.

Singal, D. P., Sengar, D. P. S. & Terasaki, P. I. (1970) Detection of non-HL-A antibodies. *Histocompatibility Testing 1970.* Munksgaard, Copenhagen.

Svejgaard, A. & Kissmeyer-Nielsen, F. (1970 a) Complement fixing Platelet Iso-antibodies V HL-A typing. *Vox Sang (Basel)* **18**, 12.

Svejgaard, A., Kissmeyer-Nielsen, F. & Thorsby, E. (1970b) HL-A typing of platelet. *Histocompatibility Testing 1970.* Munksgaard, Copenhagen.

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HL-A Antigens in Bantu and Indians

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This investigation served a dual purpose. The workshop sera were used to type unrelated individuals from each race to determine the antigen and gene frequencies of each population. At the same time 'difficult' antigens could be studied because the Bantu and Indian populations have high frequencies of these antigens.

Materials and Methods

The Indians of Natal are the descendants of immigrants who arrived about a century ago to work on the sugar plantations. They can be grouped firstly into Tamil and Telegu speakers from Southern Indian, both Hindu by religion but subdivided here by language, and secondly into two groups from the north, Hindus from the north-east and Moslems from the north-west. In this study fifty unrelated Tamil and fifty unrelated Telegu speakers as well as one hundred Bantu, all of the Zulu tribe, were tested.

The workshop sera were tested in parallel with our own battery of 180 selected typing sera. Lymphocytes were isolated by the method of Boyum (1968) and the cytotoxicity test was performed in Falcon mictrotest trays using the N.I.H. technique stipulated for the workshop.

Results and Discussion

Table I shows the antigen frequencies at the SD I locus. The division of HL-A9 into W23 and W24 was well defined. The Bantu are predominantly W23 while nearly all the Indians are W24.

TABLE I

SD I antigen frequencies in %

Antigen	Tamil	Telegu	Indian	Bantu
HL-A1	32	42	37	6
M0*	0	0	0	1
Hl-A2	20	36	28	18
W28	18	12	15	19
HL-A3	12	6	9	12
HL-A11	34	28	31	1
W23	0	2	1	18
W24	36	14	25	4
W25	2	0	1	9
W26	8	6	7	13
W29	0	0	0	16
W30	6	8	7	39
W31	0	2	1	13
W32	6	2	4	2
W19.6	8	10	9	0
W19 NEW	0	0	0	8

TABLE II

SD 2 antigen frequencies in %

Antigen	Tamil	Telegu	Indian	Bantu
HL-A5.1	14	24	19	3
HL-A5.2	12	8	10	0
HL-A5.3	6	2	4	0
HR	4	6	5	3
W5	16	22	19	4
W18	4	4	4	6
W15	6	4	5	2
W16	2	4	3	4
W21	0	0	0	1
HL-A7	18	16	17	14
W22	0	0	0	0
MWA	0	0	0	35
W27	0	0	0	0
407*	0	0	0	1
HL-A8	6	6	6	13
HL-A14	0	0	0	4
HL-A12	14	4	9	14
TT	0	0	0	8
HL-A13	4	8	6	8
W10.1	20	18	19	0
W10.2	14	20	17	0
Sabell	0	0	0	1
Da34	2	0	1	0
Da35	2	0	1	1
TY	10	2	6	0
HS	0	0	0	0
W17	28	32	30	39

The components of W19 were more difficult to distinguish. Figure 1 illustrates the reaction pattern of all the sera involved. A new antigen which we have called W19 NEW appears to be included. It is defined by positive reactions with two of the W29 workshop sera (W034 Fabre, W036 abs. 8.53) and negative reactions with the other two W29 sera (W033 Fe71, W035 12385.1). In addition W114 RC and W142 HIB are negative with W19 NEW but positive with W29. Workshop serum W040 Fe 51A is positive with W30 + W19 NEW and negative with W29. W19 NEW was only present in the Bantu. W30 has a frequency of 39% in the Bantu and workshop sera W032 Nakumura and W048 SAL may define subdivisions of W30 judging by their reaction patterns which are almost completely included in W30 (Fig. 1). The workshop sera did not define W31 but two sera from the NIH (Thompson and Quinones) which both react with W31 + W32 were used on our own trays. W19.6 was not detected in the Bantu but had a frequency of 9% in Indians.

Table II shows the antigen frequencies of the SD 2 locus. We have confirmed the high frequency of the MWA antigen and the absence of W22 in the Bantu. Neither of these antigens was detected in Indians. The antigen TT was present in 8% of the Bantu but was absent from the Indians tested. W17 has a high frequency in the Bantu (39%) and in Indians (30%). Figure 2 shows how HL-A5 can be subdivided into three parts. HL-A5.1 is defined by the two workshop sera W129 298E and W130 PA 101.11. The other workshop serum (W128 191E) that was submitted as a short HL-A5 reacted as a 'standard' HL-A5 as did serum W119 Bechard. The difference

between the 'standard' HL-A5 sera and W120 Eiden may define a further subgroup, HL-A5.3.

Serum W120 Eiden is positive with HL-A5.1 + HL-A5.2. Six local sera reacted similarly to Eiden. In a previous investigation (Hammond et al. 1974) we showed four subdivisions of HL-A5 in the Indian population, one of which appeared to be confined to Indians from the north of India. In this study however, we tested only Indians from the south of India. Figure 3 illustrates the reaction pattern of antisera associated with W10 and HL-A13. W10.1 is defined by serum W075 2608/72. Serum W078 10234.1 appears to be HL-A7 + W10.1. Only the anti-HL-A13 sera show good agreement, however, and the W10 complex needs further study especially in the Indian population. The antigen TY had a frequency of 6% in Indians but was not detected in the Bantu.

TABLE III

SD 3 antigen frequencies in %

Antigen	Tamil	Telegu	Indian	Bantu
T1	8	2	5	0
T2	0	0	0	15
T3	8	14	11	10
T4	14	16	15	14
T5	2	2	2	4

Table III shows the antigen frequencies at the SD 3 locus. T1 was not found in the Bantu and T2 was absent in the Indians. The associations between antigens of the SD 3 series and the SD 2 series are quite different in these populations compared with Caucasians, except for the association between T5 and HL-A12 in all three races and T4 and W5 in Indians and Caucasians. An interesting association is that between T2 and the blanks in the Bantu, which may indicate the presence of an undefined antigen more common in the Bantu and associated with T2. Table IV shows the haplotype frequencies, standard errors and delta values in the four population groups. Significant delta values are underlined. Also shown are the delta-haplotype ratios suggested by Thomsen et al. (1974) which indicate how much the delta value contributes to the haplotype frequency. The correlation coefficients (r) between the antigens are also shown in Table IV.

These associations show that is easier to characterise antisera by using panels from all three race groups, and that the identification of complex antigens may only be possible in this way.

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References:

Boyum, A. (1968) Separation of leucocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. 21 Supp. 97, 7.

Hammond, M.G., Appadoo, B. & Brain, P. (1974) Subdivision of HL-A5 and comparative studies of the HL-A polymorphism in South African Indians. Tissue Antigens 4, 42.

Thomsen, M., Dupont, B., Jersild, C., Hansen, G.S., Staub Nielsen, L., Platz, P., Ryder, L., & Svejgaard, A. (1974) Linkage disequilibrium within the major histocompatibility complex (MHC) in man. Tissue Antigens 4, 400.

TABLE IV

Haplotype frequencies and correlation co-efficients

		TAMIL					TELEGU					INDIAN					BANTU				
SD 1	SD 2	HF	SE		/HF	r	HF	SE		/HF	r	HF	SE		/HF	r	HF	SE		/HF	r
HL-A1	W17	75	33	49	.65	.34	135	39	93	.68	.55	105	26	71	67	.45	18	12	11	.61	0.14
BLANK	W17	58	27	55	.94	.40	44	36	27	.61	.08	52	21	42	.80	.27	0	24	-17	-	0.19
HL-A2	W5	2	20	-7	-	.08	91	32	68	.74	.51	50	20	35	.70	.27	0	6	-4	-	0.10
HL-A1	TY	51	22	42	.82	.49	10	10	8	.80	.17	31	12	24	.77	.33	-	-	-	-	-
BLANK	HL-A12	50	23	49	.98	.56	8	13	6	.75	.08	29	14	26	.89	.30	0	13	-7	-	0.13
HL-A1	HL-A5	44	36	13	.29	.08	0	50	-90	-	-.44	4	30	-33	-	-.18	5	5	4	.80	0.20
W19-NEW	HL-A13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	13	34	.94	0.86
W25	HL-A12	0	5	-2	-	.06	-	-	-	-	-	0	3	-1	-	-.03	30	13	26	.86	0.48
W24	HL-A7	56	29	37	.66	.30	5	17	-1	-	-.02	29	17	17	.58	.17	20	10	19	.95	0.50
SD 2	SD 3																				
W5	T4	73	27	67	.91	.92	95	30	83	.87	.88	84	20	75	.89	.90	4	7	2	.50	.06
W17	T3	29	19	23	.79	.31	60	26	48	.80	.46	45	16	35	.77	.40	4	17	-9	-	.08
W10	T1	41	20	33	.80	.41	10	10	8	.80	.18	25	11	20	.80	.31	-	-	-	-	-
W5	BLANK	84	28	14	.16	.06	0	96	-178	-	-.45	27	59	-56	-	-.21	0	28	-17	-	-.16
HL-A12	T5	0	5	-2	-	.06	10	10	10	1.00	.70	5	5	4	.80	.20	15	9	13	.86	.36
BLANK	T2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45	19	34	.75	.25

HF = Haplotype frequency SE = Standard error = delta All values x 1 000

r = Correlation co-efficient

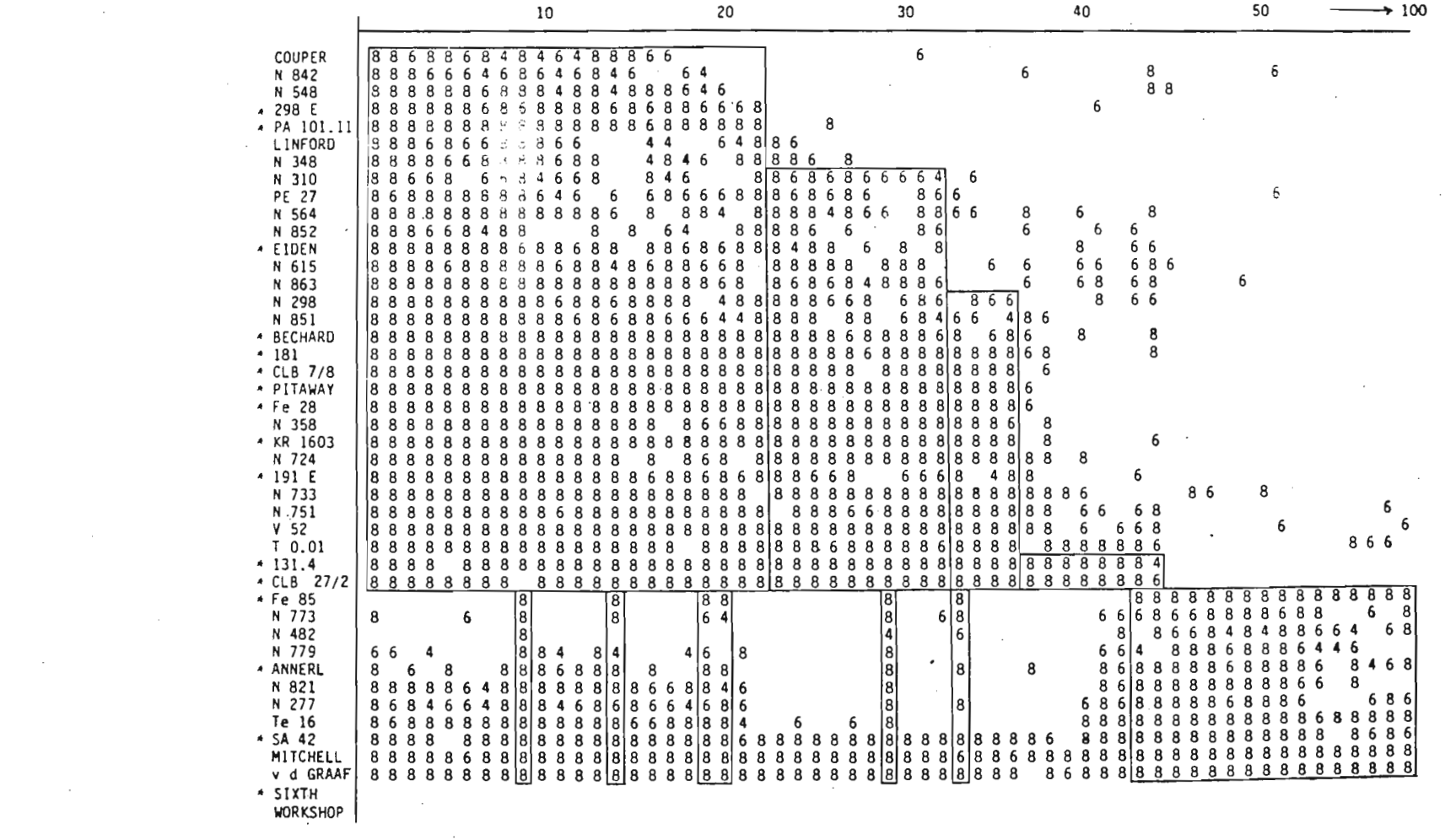

Figure 2 Reaction pattern of HL-A5, HR and W5 antisera.

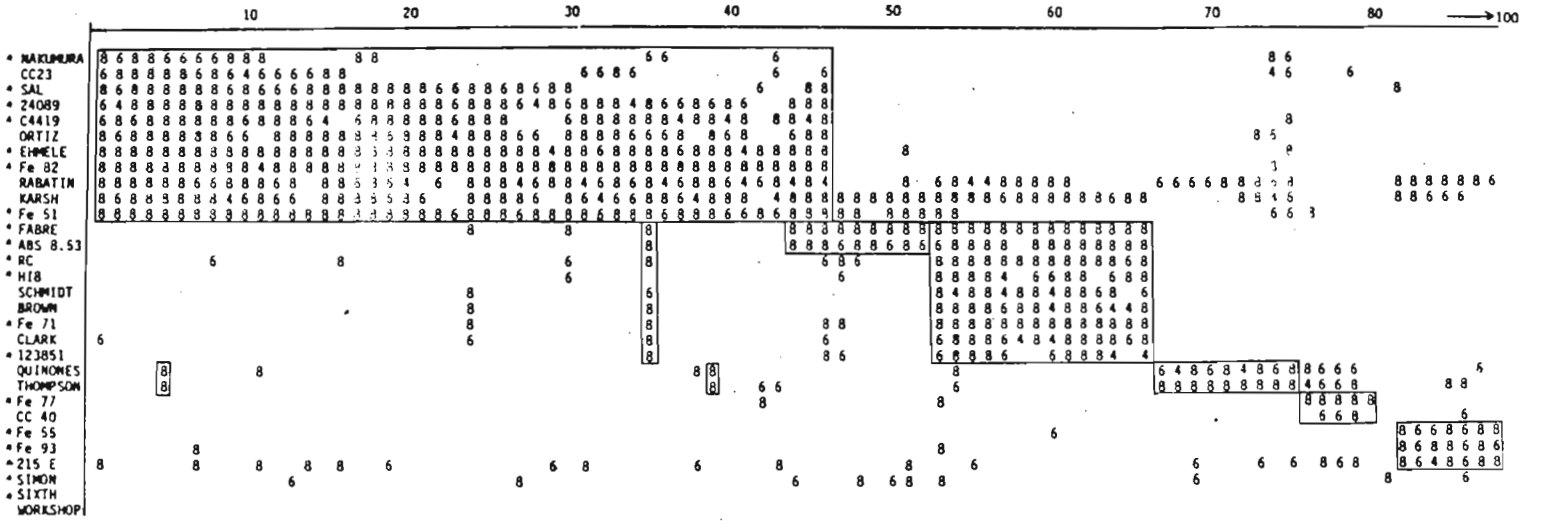

Figure 1 Reaction pattern of W19 related antisera.

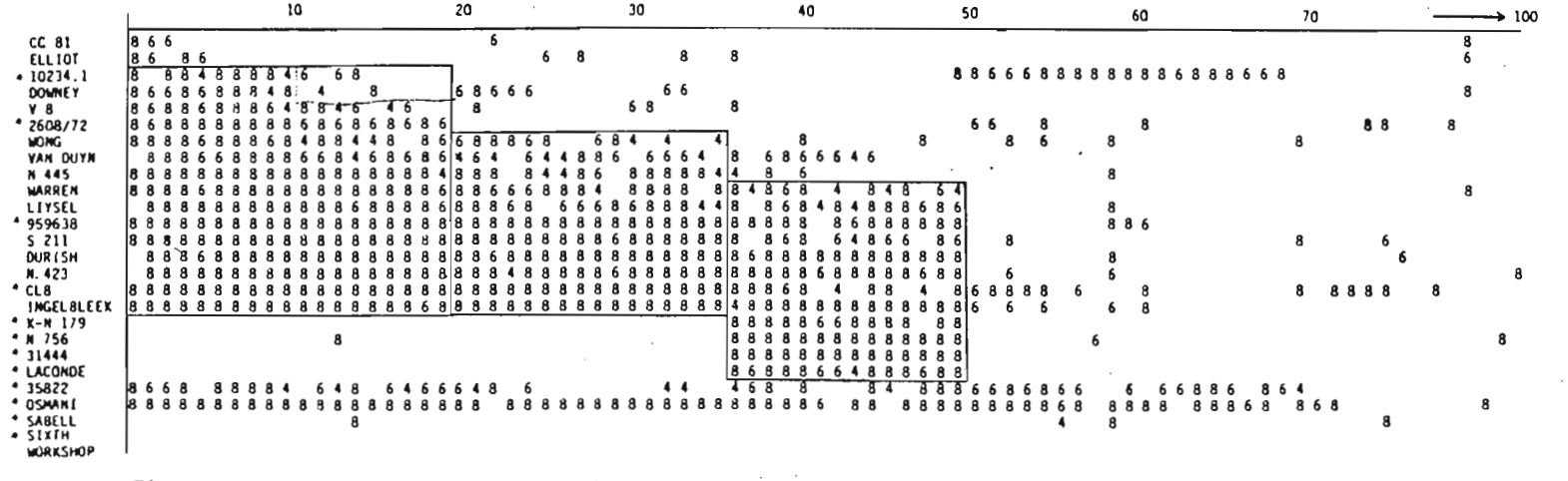

Figure 3 Reaction pattern of W10 and HL-A13 antisera.

Histocompatibility Testing 1977

Report of the 7th International Histocompatibility Workshop and Conference

The Workshop Conference took place
in Oxford, England from
4-10 September, 1977

Editors :
W.F.Bodmer
J.R.Batchelor
J.G.Bodmer
H.Festenstein
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MUNKSGAARD

HLA IN NON-CAUCASIAN POPULATIONS

M.G. Hammond, B. Appadoo, and Peter Brain, Natal Institute of Immunology, Durban, South Africa.

B5 + HR: B5.1, B5.2, B5.3, B5.4 and HR all present in Indians. B5.1 and HR in Zulus. All groups included in W4. Comparison with B5 patterns in Dutch Caucasians are presented in table:

B5 PATTERNS WITH 7W SERA

430	432	428	433	434	435	436	429	432	437	431	440	438	439	Zulu N	Zulu spec	Indian N	Indian spec	Dutch Cauc. N	Dutch Cauc. spec
+	+	++	+	+	-	+	++	+	+	+	+	+	1	B5.1	10	B5.1	16	B5.1
-	-	++	+	+	-	±	±+	+	+	+	+	+	0		4	B5.2	0	
-	-	±-	±	++	±	±	++	+	+	+	+	+	0		0		5	B5.2
-	-	--	-	-+	±	±	++	+	+	+	+	+	0		3	B5.3	0	
-	-	--	-	--	-	-	--	-	+	+	+		0		1	B5.4	0	
-	-	--	-	--	-	-	--	-	-	-	+		2	HR	0		0	
-	-	--	-	--	-	-	--	-	+	-	+		0		1	HR	0	
-	-	--	-	--	+	±	--	-	+	-	+		0		0		1	HR

Bw40: heterogeneous in Indians (see Schreuder and Bos).
Bw15) 'Short' Bw15, only present in Indians; included in W6.
Bw17) 'Long' Bw15: 7W436 and BW17 sera reacted with both Indians and Zulus; local sera recognized two patterns; inclusion in W4. Bw17 was well defined with 7W sera; included in W4.
B cell serology: Dw1, Dw4 and Dw6 could not be defined.
Dw2: heterogeneous in Indians; 5 sera reacted with all 10 Dw2 individuals whereas 9 only reacted with 6 of them.

ASSOCIATIONS BETWEEN HLA-A, -B AND -D LOCI AND DIABETES IN SOME SOUTHERN AFRICAN POPULATIONS

M.C. Botha, B.R. Briggs, E.D. du Toit, E. Campbell, D. Taljaard, W.P.U. Jackson*. Provincial Blood Grouping Laboratory and *Department of Medicine, Cape Town, South Africa.

The known increases of A2, B8 and Bw15 and decreases of B7 in European juvenile onset diabetes (JOD) were confirmed (n=29). Of most interest were the Xhosa maturity onset diabetes (MOD) in which the following antigens demonstrated significant increases in two consecutive studies:

ANTIGEN	CONTROLS (n=76) pos.	(freq)	FIRST STUDY (n=20) pos.	(freq)	p (fisher)	SECOND STUDY (n=30) pos.	(freq)	p (fisher)
A2	19	(.25)	9	(.45)	.053	15	(.50)	.011
B8	8	(.11)	6	(.30)	.035	8	(.27)	.041
Bw35	1	(.01)	3	(.15)	.027	6	(.20)	.001

In a small group of 11 Xhosa JOD Bw35 was increased with a frequency of .30 and p = .001. For Dw3 (the only antigen for which typing was done) the frequency was 0.73 as compared to .33 in the control group (p = .020).
No significant deviations were noted in 30 non-Malay Coloured MOD's and in two consecutive studies of respectively 24 and 35 Malay Coloured MOD's.

SV=PR: A NEW B LOCUS ANTIGEN DISCOVERED

Ieke Schreuder, Alie Bos. Dept. of Immunohaematology, University Hospital Leiden, Holland.
Ella van den Berg-Loonen. Central Laboratory of the Dutch Red Cross Bloodtransfusion Service, Amsterdam, Holland.

Two sera recognized the same specificity:
Serum VR30087: B5, B18, Bw15.2, Bw35, HR, Bw21, SV; Leiden.
Serum CLB23 : B5, B18, Bw15, Bw35, HR, Bw37, PR; Amsterdam.

SV=PR found in 2 out of 200 individuals used for cell exchanges:

TB: Aw36, Aw23, B12, SV=PR, W4, W6 VR30087 + CLB23 +
JM: A3, A28, Bw22, SV=PR, W6 VR30087 + CLB23 +

SV and PR were found to segregate in families.
SV=PR is a new B locus specificity with a very low gene frequency (<1%) and is included in W6.

HLA-BW40 IS HETEROGENEOUS

Ieke Schreuder, and Alie Bos. Dept. of Immunohaematology, University Hospital Leiden, Holland.

Several HLA-Bw40 related patterns were observed with local sera in 239 out of 1350 HLA typed blood donors.

PATTERN	SERUM: 1	2	3	4	5	6	N=239
1. Bw40-W6-Cw3	+	+	+	+	+	+	177
2. Bw40-W6-Cw2,Cneg	+	+	±	+	+	-	43
3. KSO-W6	+	±	±	+	-	-	(2)
4. Bw40-W4=407*	+	±	-	-	-	-	3
5. Bw41-W6	-	-	-	-	-	+	16

SERUM ORIGIN:	
nr.1, 2 VR:	w40+13
nr.3, VR:	w40+7
nr.4, USSR:	w40
nr.5, Japan:	w40
nr.6, Nij:	w40+w41

Pattern 1 and 2 are distinct and associated with the presence of Cw3 and Cw2 (or occasionally C negative) respectively.
Pattern 3 was only seen on two cells send to us by Dr. Kissmeyer-Nielsen.
Pattern 4, 407* in the Dutch population:
1. segregates in families.
2. is included in W4.
3. has a gene frequency of less than 1%.
4. is in link. diseq. with A3: hapl.freq.=.0035;delta =.003.
5. is mainly found together with T7 (Cw7).
6. can be recognized by several Bw40 and B27 antisera.
7. is best defined by serum 7W387-anti Bw40C.

Bw40 patterns with 7W sera as observed in Dutch Caucasians, South African Zulus and in Asian Indians (M.G. Hammond et al).

457	455	460	456	391	459	383	390	389	458	453	454	382	385	386	384	388	452	387	Dutch cauc. N	Dutch cauc. spec.	Zulu N	Indian N	Indian spec.
+	+	+	+	+	+	+	+	+	+	+	+	+	±	-	-	-	-	-	14	w40-Cw3	1	2	w40.1
-	±	+	±	+	+	+	+	+	+	+	+	+	±	-	-	-	-	-	10	w40-Cw2	0	3	w40.2
-	-	-	-	+	+	+	+	+	+	+	±	+	-	-	-	-	-	-	0		0	7	w40.2
-	-	-	-	-	-	+	+	+	+	±	±	±	-	-	-	+	+	+	4	407*	0	0	
+	-	-	-	-	-	-	-	-	-	-	-	±	+	+	+	-	-	-	0		0	3	w41
+	-	-	-	-	±	-	-	-	-	±	+	±	+	-	+	-	-	-	5	w41	0	0	

Serum 322 was positive with many American Black and Japanese Aw33 cells but did not appear to give such strong correlations in other groups. There was a suggestion from the French data which was analysed separately, that a "short" version of Aw33, comparable to the behaviour of Fe55 in other Workshops may exist and that cells +ve with 328 and -ve with 331 are a variant of Aw33, which is related to the A19 cross reactive group (CREG) and in linkage disequilibrium with B14.2. The remaining Aw33 cells, +ve with 331 and 328 appear to be more closely related to the A28 CREG and show some resemblance to the antigen previously described as "Malay" (Joysey et al., 1972 in Histocompatibility Testing 1972) with a high △with B12.

Working Party : As A10.

The "Black" antigens : Aw36, Aw34, Aw43 and Bw42 (Table 4.6)

Aw36

Aw36, found only in Blacks, was originally defined by unexpected extra reactions in some A1 sera(Histocompatibility Testing 1972). Aw36 could not be clearly defined with the 1977 Workshop sera. There was a weak and therefore unreliable Aw36 component in sera 301 and 473. Serum 303, reference serum for A1 in previous Workshops, does not react with Aw36 positive cells.

Aw34

Using the 1977 Workshop sera, this specificity could only be defined in the absence of A25 and A26. Sera 317, 331 and 423 had strong Aw34 activity, whereas sera 316 and 384 reacted weakly with some of the Aw34 positive cells tested. Sera 310, 311, 312, 313, 314, 327, 420, 421 and 422 were negative with Aw34 and Aw43 cells.

Aw43

Dr. Botha's laboratory submitted 10 Aw43 positive cells of Xhosa origin. The definition of Aw43 on these cells was principally based on local sera not included in this Workshop. Serum 315, used in the 1975 Workshop to define Aw43, was positive with these cells as were sera 316, 317 and 423.

Bw42

In the African Blacks, there was good agreement in defining Bw42 by the different laboratories in spite of the heterogeneity of the Bw22/Bw42 sera used in this Workshop. The apparent absence of Bw22 in this ethnic group made the definition of Bw42 easy. In contrast, Bw22 was present in the American Blacks, which may complicate the interpretation of Bw42. The results obtained from the African and the

TABLE 4.6

Reaction Pattern with A1 and Aw36 Positive Cells

	301	303	473	
A1	+	+	+	
Aw36	(+)	-	(+)	(weak reactions) (ie. 30-40%)

Aw34, Aw43

	315	316	317	331	384	423
Aw34	-	-/+	+	+	-/+	+
Aw43	+	+	+	-	-	+

Bw42. Reactivity of 7w sera

	7w Sera
+ve (50-100% kill)	373, 374, 377, 378 (457)*
+ve (30-50% kill)	416, 442, 443, 446, 449, (459)*
± (30% kill)	372, 376, 445, 447, 450, (455)*

* These sera are primarily anti B40.

American Blacks showed that there was a strong Bw42 component in sera 373, 374, 377, 378 and 457. In addition, sera 416, 442, 443, 446, 449 and 459 reacted strongly with Bw42 positive cells from African Blacks, but gave equivocal results in the American Blacks. Some other sera (372, 376, 445, 447, 450 and 455) seemed to contain a weak Bw42 component.

When analysing sera which have such complex reaction patterns as the Bw22 and B40 sera used in this Workshop, negative reactions can be extremely informative. Serum 416, together with the heterogenous B40 sera 457 and 459, may help in differentiating between Bw42 and Bw22. All three sera reacted with B7 and Bw42 positive cells but not with any Bw22 positive cells (Table 4.6).

Editorial Note :- This report on Bw42 should be read in conjunction with the report on Bw22.

Working Party : A. Biegel,M.C. Botha, C. Bouysou, B. Briggs, B. Hasty, S. Herbert, M. Pollack, E. Wolf (data also from R. Duquesnoy and M. Hammond).

Additional note, contributed by Dr. A. Biegel, on B7 - Bw22 - Bw42 Group in American Blacks.

In Region US1, 170 American Blacks were typed ; in addition to the conventional B7, Bw22.1 and Bw42, two variants were found, and out of a total of 51 cells 12 remain unclassified with regard to this antigen group.

The first variant is a "short" Bw42 ("42.2" ; N = 4) differing from Bw42 by negative reactions for sera 373, 374, 377, 416, 446, 447 and by positive reactions in 379 and 455. These cells were not B7, as shown in this and previous workshops ; they carried neither Cw1 nor Cw3.

The second variant (N = 2) is a variant of Bw22.1, with negative reactions in 376, 444 and 448 ; and positive in 379 and 457, distinct from the standard Bw22.1 pattern. Bw22.1 in American Blacks is rare, but when present is Cw3 associated. This variant is negative for Cw1 and Cw3, as is Bw22.2 in this population group. For each of these variants, the antigen assignment for one or more cells was confirmed by family analysis. For additional details, see the US1 regional report (this volume).

B5, Bw51, Bw52, Bw53, Bw35 (formerly B5, B5.1, B5.2, HR and Bw35)

The specificities within the B5 - Bw35 complex have in this Workshop become more clearly delineated. For the first time agreement can be reached on the definition of Bw53 (formerly HR) in the five populations studied. However, no monospecific reagent for Bw53 was detected. Bw51 (formerly B5.1) was differentiated from Bw52 (formerly B5.2) ; monospecific

TABLE 4.7

Sera for B5-Bw35 complex

	332	333	334	335	336	337	338	339	340	341	342	343	428	429	430	431	432	470
Bw51	+	+	+	+	-/+	+	+	+	+	-	-	-	+	+	+	+	+	-
Bw52	-	-	-	+/-	+	+/-	+/-	+	+	-	-	-	-	+/-	-	+/-	+/-	-
Bw35	-	-	-	-	-	-	-	-	+/-	+	+	+	+/-	+/-	-	+/-	-	+
Bw53	-	-	-	-	-	-	-	+	+	+	-	+	+/-	+	+/-	+	-	-
Other specificities present		B8					Bw21.2 Bw15A			Bw21.1								

TABLE 4.8

Antigen Frequencies (%) from the Patterns of 20 Selected Laboratories

(N = population size)

	Europ. Caucas. (N = 363)	N. Amer. Caucas. (N = 358)	Amer. Blacks (N = 221)	African Blacks (N = 102)	Japanese (N = 374)
Total B5	15.55	10.61	14.93	ND	32.89
Bw51	9.91	5.03	7.24	ND	12.30
Bw52	1.65	1.68	0.45	ND	9.36
Bw35	16.53	ND	14.48	10.78	10.43
Bw53	0.55	ND	5.88	1.96	1.87

ND = Not done

TABLE 4.9

B5

	337	432	335	338	334	333	336	Europ. Caucas.	N. Amer. Caucas.	Amer. Blacks	African Blacks	Japanese
B5	+	+	+	+	+	+	+	5	6	6	0	18
Bw51	+	+	+	+	+	+	-	28	13	9	2	37*
	+	+	+	+	+	-	-	3	2	1	0	0
Bw52	+	+	+/-	+/-	+/-	-	+	4	3	0	2	21
	+	+	+	+	+	-	+	0	2	0	0	0

* Serum 337 was negative in 3 Japanese cells.

examples of anti-Bw51, Bw52 and Bw35 sera were found among the submitted sera. There was suggestive evidence, but not clear definition, of a further possible split of B5 in two populations, namely Black and Asian Indians, but the patterns for these are not provided since more study is required. The patterns of serum reactions shown represent a summation based on all the cells from 20 selected laboratories.

Bw51

The serum pattern for the Bw51 specificity was defined by 13 sera. The pattern was essentially the same in the European Caucasoids, North American Caucasoids and Blacks, African Blacks and Japanese (Table 4.7). The four sera, 332, 333, 334 and 335, were all specific for Bw51 (333 also contained anti B8). Bw51 was a frequently occurring antigen in all populations with a range from around 11% - 34% (Table 4.8). This is a rather conservative estimate. This also applies to all the calculations for the other specificities in this table.

Bw52

Nine sera could be used to define Bw52. Only serum 336, of Japanese origin, was almost monospecific for Bw52. This serum in a few laboratories gave some weak reactions in single typings of individuals with Bw51 cells (defined by the Workshop criteria). However, this may be the consequence of the cross-reactivity since Bw52 cells can absorb anti-Bw51 antibody. The frequency of Bw52 was highest in the Japanese population, and occurred with a lower frequency in the other populations with the exception of the African Blacks (Table 4.9).

Bw35

There were eight sera that could be employed to define Bw35. Serum 342 was monospecific (Table 4.7). Serum 344, submitted as anti-Bw35, was actually anti-Cw4 serum. Serum 433, submitted as having Bw35 specificity, was non-reactive in most laboratories. Sera 341 and 343 both had activity for Bw35 and Bw53 together. The remaining sera in the pattern gave $\pm$ reactions, i.e. these did not react with all Bw35 cells. Serum 470, submitted as Cw4, also gave $\pm$ reactions with Bw35 cells which may result from the linkage disequilibrium between the two specificities. It is likely to contain Bw35 since this $\pm$ reaction is present in the Japanese population in which Cw4 has a low frequency. No information was available on the relationship of the $\pm$ patterns to the postulated antigens Bw35A and Bw35C.

Bw53

No monospecific Bw53 serum was submitted. There were two groups of Bw53 sera, one with B5 and one with Bw35, with a

TABLE 4.10

Bw53, compared with B5 (Bw51 + Bw52)

	428	429	431	432	339	340	Europ. Caucas.	N. Amer. Caucas.	Amer. Blacks	African Blacks	Japanese
"B5"	+	+	+	+	+	+	40	22	19	4	ND
HR	+/-	-	+/-	+/-	+	+	4	12	17	1	ND

ND = Not done

TABLE 4.11

Bw35/B5

	339	340	429	431	342	343	341	470	403	344	Europ. Caucas.	N. Amer. Caucas.	Amer. Blacks	African Blacks	Japanese
B5 (Bw51)	+	+	+	+	-	-	+/-	-	-	-	34	15	13	6	12
Bw35	-	-	-	-	+	+	+	+	-	+	23	21	4	0	0
	-	+/-	-	-	+	+	+	+	-	+	13	0	0	7	0
Not Bw35*	-	+	-	+/-	+	+	+	+	+	-	0	0	0	0	8

* Cells listed as B5, B15, Bw35 by submitting laboratories

TABLE 4.12

HLA-B12 : Bw44, Bw45 (formerly B12, not TT* and TT*)

	345	346	434	349	347	348	Europ. Caucas.	N. Amer. Caucas.	Amer. Blacks	African Blacks	Japanese
Bw44	+	+	+	+	+	+	4	4	13	2	2
(B12, not TT*)	-	+	+	+	+	+	41	14	7	4	18
	-	+	+	+	+	-	23	20	2	4	8
	-	-	+	+	+	+	6	9	4	1	3
	-	-	+	+	+	-	8	26	5	2	11
Other variants,	+	-	+	+	+	+	1	0	0	0	0
probably Bw44	+	+	+	-	+	+	0	1	0	0	0
	-	+	+	+	-	+	2	0	0	0	0
Bw45	+	+	-	+	+	+	1	1	2	1	0
(TT*)	+	+	-	+	+	-	0	0	3	1	1
	-	+	-	+	+	+	3	0	0	0	0
	-	+	-	+	+	-	2	1	0	0	1
Other variants	+	+	+	+	+	-	0	1	9	5	0

(Patterns with less than three positive reactions are not included)

total of 8 sera. The Bw53 specificity can now be readily defined, the specificity being present in most populations at low frequency except for the Blacks. The disparity between African and American Blacks could perhaps be due to different tribal origins, or misclassification in the pattern defining B5.

B5

In all populations there were cells that reacted with both Bw51 and Bw52 sera. These cells frequently had another well-defined B locus specificity, suggesting that the result may be due to serologic rather than genetic considerations. Perhaps this flows from the antigen density, although in family studies reported elsewhere, the subdivisions are inherited as Mendelian dominants. Cells not defined as Bw51 or Bw52 are referred to in the tables of frequency as B5. Two reports in the literature (Hammond and Payne et al.) indicate the presence of other subdivisions of the B5 - Bw35 complex in restricted populations which were not observed in the Workshop data.

Editorial Note : Tabulations of the PATTERN analysis of the Workshop data are appended to provide additional information on this group of antigens, and comparisons of serum behaviour. (Tables 4.9, 4.10 and 4.11)

Working Party : D.B. Amos, P. Engelfriet, M. Hammond, C. Mazzilli, R. Payne, P. Richiardi, A. Ting.

HLA-B12 : Bw44, Bw45 (formerly B12 (not TT*) and TT*)

There were six sera submitted to the Workshop to define the parts of B12. Two of these appeared to be of special importance in defining the split between the Bw4 associated Bw44 and the Bw6 associated Bw45. Serum 345 reacted as anti Bw45 with a few extra reactions and serum 434 appeared to recognise only the Bw44 antigen in the Caucasian population. The exact definition of Bw44 and Bw45 still presents problems when analysing the total 7th Workshop data. As in the 6th Workshop, the various patterns indicate the heterogeneity of the antigens and the antisera used to determine them. The clearest correlation using 7th Workshop sera is shown in the 2 x 2 tables in the Scandinavian Regional report on a Caucasian population. The majority of B12 typings in all ethnic groups agree with these Scandinavian findings (q.v.). The results for the Bw45 antigen are similar but less clear cut, probably because of the small number of Bw45 cells recognised in the analysis. There is a group of cells of American and African Black origin which are Bw6 associated and show a pattern of reactivity which does not occur in Caucasoids. These cells are recognised by a negative reaction with serum 348 and positive reaction with all the other B12 sera. Japanese cells do not appear to have a definable Bw45

Histocompatibility Testing 1980

REPORT OF THE EIGHTH INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP
HELD IN LOS ANGELES, CALIFORNIA, USA
4-10 FEBRUARY 1980

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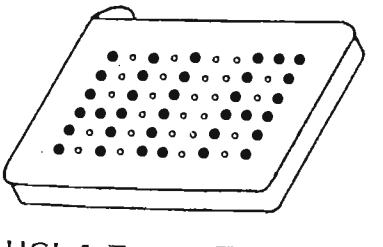

UCLA TISSUE TYPING LABORATORY
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FURTHER SPLITS OF HLA-B5

M.G. Hammond
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Received August 20, 1979

The reaction patterns of 8W sera show a further subdivision of B5.

Table 1 shows the reaction patterns of three families. The mother and five shildren all possess a short BW51. Sera 8W057, 059, 268, and 060 are all negative. The father has 8W64.

The second family has BW52 as defined in the Workshop prescreening specificity patterns. The mother and both children are BW52 positive. The father and one child have BW35. The third family shows the inheritance of a short BW52 from the father to three children. The key sera are 8W338, 8W595, and 8W278. BW35 is inherited from the father by the other two children. Some of the BW35 sera appear to be very weak. The mother appears to be 8W59 positive.

Table 2 shows the reaction patterns of the disease trays. There is no clear-cut split of BW51 although serum 8W502 may be a key serum. Cell 55 is BW52 as defined in the newsletter and the four cells below this appear to be a short BW52 but only two sera (8W133 and 8W782) reveal this split. Cell 65 is 8W66 and is clearly different from these splits.

These findings lent support to my earlier description of four splits of B5 (1). The 19th International Cell Exchange featured splits of B5 and I reported then, on B5.1, B5.2, and B5.3. It would be interesting to see how those cells react with the Workshop sera.

REFERENCE

1. Hammond MG, Appadoo B, Brain P. Subdivision of HL-A5 and comparative studies of the HL-A polymorphism in South African Indians. Tissue Antigens 1974, 4:42.

Table 1. Reaction patterns of families with B5 splits.

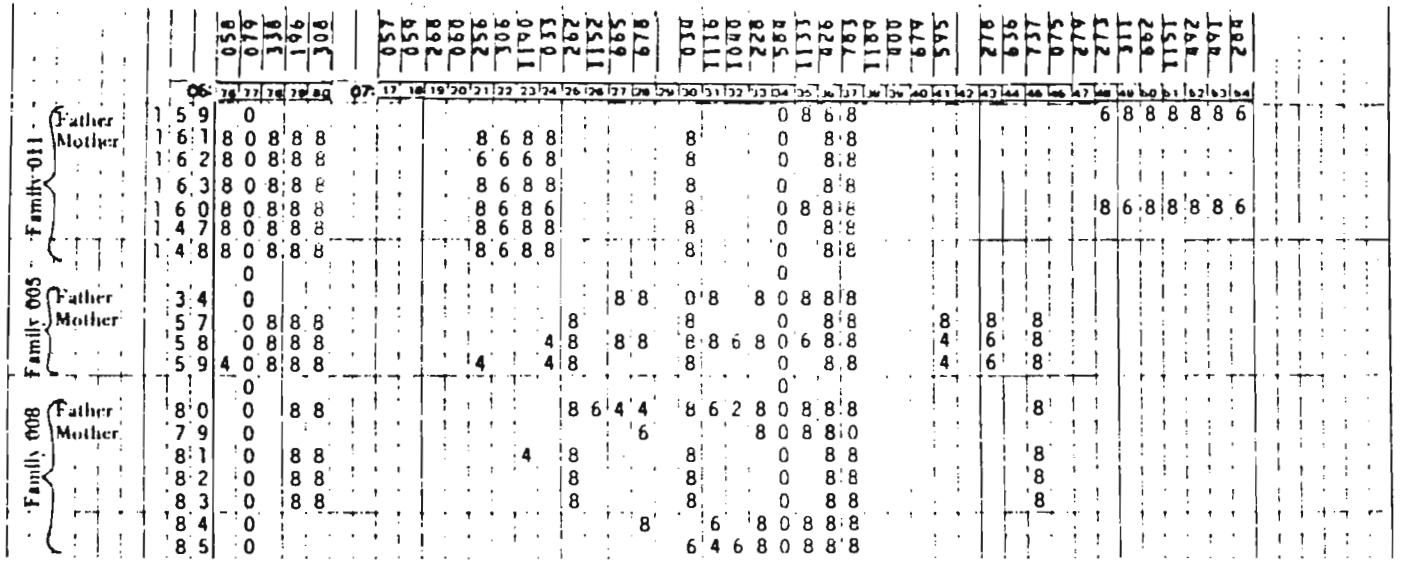
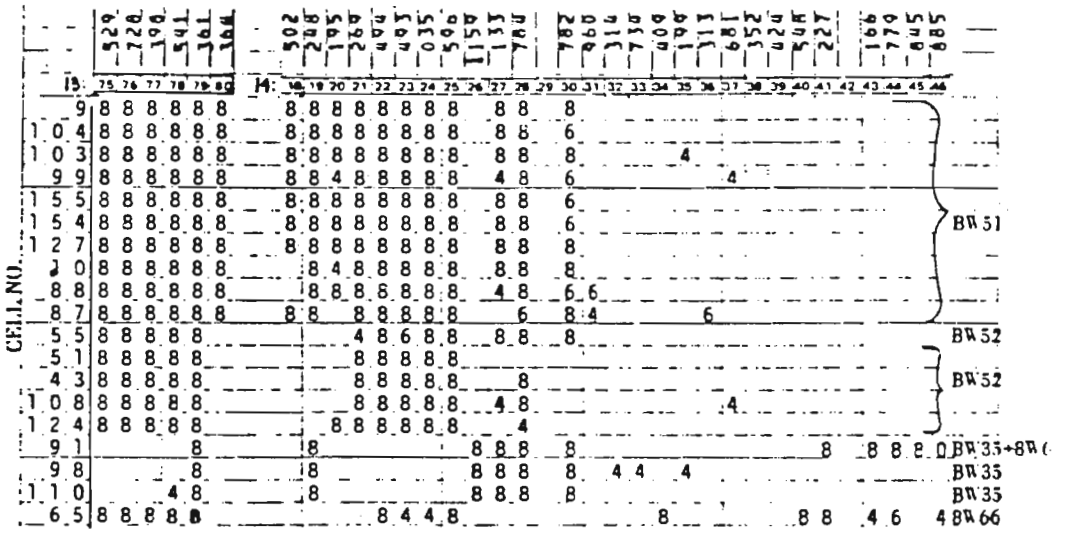


Table 2. Reaction patterns of 'disease' patients with B5 splits.



CONFIRMATION OF ST1 (8W12) IN SOUTH AFRICAN INDIAN FAMILIES

M.G. Hammond and D. Appadoo
Natal Institute of Immunology, Durban, South Africa
Received October 24, 1970

The new DR specificity ST1 reported by Colombe et al (1) is clearly demonstrated in three Asian Indian families and in two other individuals (Table 1). Several interesting points emerged from these studies in a different race.

In all three families the haplotype carrying ST1 also carries the BfF allele. The gene frequency of BfF is 0.357 in Asian Indians (unpublished observations on 380 Indians) and shows significant linkage disequilibrium with HLA-B37. This consistent finding of ST1 and BfF together suggests that the Bf locus is closer to the DR locus than to the B locus.

One family has the B37 antigen on the same haplotype as ST1. The other two have BW35. Family 02 also demonstrates a crossover between the A and C loci and the ST1 travels with B37, CW1, and BfF.

Only one South African Negro was found (cell 62) with the reaction pattern defining ST1. DRW2 was also present but the Bf typing has not been done. The frequency of BfF in South African Negroes is 0.623 and shows strong linkage disequilibrium with the AW30-BW42 haplotype (2).

There is only one discrepancy between serum 691 (BW12) and serum 1097 (BW14) and no conclusions can be drawn. The DC-1 specificity is clearly distinct.

Finally, the rarity of DRW1 in Asian Indians (<2%) has facilitated the definition of ST1 because the antibody is so often found as an extra in DRW1 sera.

REFERENCES

1. Colombe B, Payne R, Cann H. Reactivity to a new DR specificity, ST-1, in selected 8th International Workshop antisera. This volume.
2. Teng YS, Kirk RL, Hammond MG. Linkage disequilibrium between HLA and Bf in Black South Africans. Human Genetics 1979, In press.

Table 1. Eighth workshop sera containing ST-1.

			ST-1								DC1		
			691	1097	1211	611	1044	763	1229	1070	725	788	567
			Bw12	Bw14	7	1	1	1	1	7J	DC1	6+2	1+2+6+X
Family	Members	DR Phenotype											
02	13	4; ST-1	8	8	8	8	6	6	6	6	1	1	1
	14	3; ST-1	6	6	6	6	6	6	0	6	6	6	4
	15	7; ST-1	8	8	6	8	8	8	6	6	1	1	4
	12	3; 7	1	1	1	1	1	1	1	6	1	1	4
	7	3; 4	1	1	1	1	1	1	1	1	1	1	1
	47	3; 4	1	1	1	1	1	1	1	1	1	1	1
07*	* 241	5; ST-1	6	4	6	4	6	6	1	1	6	4	4
6	* 242	-; ST-1	8	6	8	8	6	8	1	0	8	8	4
	64	5; ST-1	8	6	6	8	6	6	1	0	6	1	6
	72	7; ST-1	8	6	6	8	6	8	1	0	6	4	6
	66	2; 7	1	1	1	6	1	1	1	0	6	4	8
	71	2; 5	1	1	1	1	1	1	0	0	8	6	4
08	79	2; ST-1	8	6	1	8	6	6	0	0	6	6	6
	85	2; ST-1	6	6	6	6	4	4	0	0	6	6	1
	193	2; ST-1	8	6	8	6	6	6	8	6	6	1	6
	194	2; ST-1	6	1	8	8	6	6	8	6	6	4	8
	80	2; -	1	1	1	1	4	1	1	1	6	4	8
	81	2; -	1	1	1	1	1	1	4	1	8	4	8
	82	2; -	1	1	1	1	1	1	4	1	8	6	8
	83	2; -	1	1	1	1	1	2	1	1	6	6	6
	84	2; -	1	1	1	1	1	2	1	1	6	6	4
Random	62	2; ST-1	8	6	8	4	8	6	6	6	4	6	4
Indvd.	97	5; ST-1	8	4	6	6	1	1	0	0	4	6	0

Parents are underlined *Brother and sister of 64

HETEROGENEITY OF B40

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Received November 12, 1979

Since our first report on the heterogeneity of B40 (1), successive International Workshops have emphasized the complexity of the crossreactive group of antigens which include B7, B13, BW41, and BW48 (1,2).

Table 1 shows the reaction pattern of the sera used in the family studies. The first cell (195) is the only one which can be classified as 8W60 (B40.1). The other B40 cells are all classified as 8W61 (B40.2) although the reaction pattern reveals further heterogeneity.

Table 1 has been divided to show a possible further split of 8W61, although many of the sera clearly have weak extra antibodies which makes it difficult to decide on a clear-cut split.

All the B40 cell donors are Asian Indians. BW41 is well defined and was only found in Negroes. BW47 and BW48 were not found in families.

Table 2 shows the reaction pattern using the disease trays. The first three cells show the reaction pattern of B13. Five cells are classified as 8W60 and the remaining B40 cells as 8W61. Positive reactions with serum 8W086 seem to define a split of 8W61. The extra reactions of serum 8W346 are with B5 and B7 cells. Three B7 cells with other antigens present at the B locus have been included to show that cell 235 could be either 8W60 or 8W61 and that BW48 can only be assigned to B7 negative cells such as cell 093. Again, all the B40 cells are Asian Indians whereas all the BW41 cells are from Negroes.

REFERENCES

1. Hammond MG, Appadoo B, Brain P. HLA antigens and antibodies in South African Indians. Tissue Antigens 1972, 2:389.
2. Hammond MG, Appadoo B, Brain P. HLA antigens in Bantu and Indians. In *Histocompatibility Testing 1975,* Kissmeyer-Nielsen F, ed, Munksgaard, Copenhagen, 1975, 173.
3. Hammond MG, Appadoo B, Brain P. HLA in non-Caucasian populations. In *Histocompatibility Testing 1977,* Bodmer W, et al, eds, Munksgaard, Copenhagen, 1978, 407.

Table 1. Reaction pattern of B40 antisera.

Tray 09 cells	087 19	365 20	013 21	1115 22	539 23	331 24	261 25	028 26	422 27	260 28	535 30	380 31	317 32	504 33	206 34	1170 35	207 36	1160 37	318 38	731 39	760 40	421 41	537 43	088 44	552 45	Bv 60	Bv 61	Bv 41	Other
195	8	8	o	8	8	8	8	8	8	8	8	8	8				o			8				6	4	+	-	-	8w59
252	8	8	o	4		8	8				8	8	8	8	4		o			8				6	4	-	+	-	8w68
251	6	8	o	4		8	6			4	6						o			4						-	+	-	8w68
189		8	o			8	8			4	8	6	6	8			o			6				8	6	-	+	-	Bw35
059	8	8	o		4	8	8			8	8	4	6	8			o			8						-	+	-	Bw52
060	8	8	o		6	6	8		8	6	6		4		4		o			6						-	+	-	B7
048	8	8	o			6	8			6	8		8	6		4	o			8						-	+	-	B7
005	8	8	o				8			4	8	6	8	8			o	6						4	6	-	+	-	Bw53
012	8	8	o				8				8	6					o			6						-	+	-	B8
015	8	8	o				8			6	8	4	8	6			o			8				6	8	-	+	-	B37
079	6	8	o														o			6						-	+	-	Bw35
081	8	8	o							4	8	4					o			6						-	+	-	<Bw52
082	4	8	o							4							o			4						-	+	-	<Bw52
083		8	o				4			4	8		6	6			o			6						-	+	-	<Bw52
084	6	8	o								4						o			6					4	-	+	-	Bw35
097	6	8	o				8				4						o			8				6		-	+	-	-
169	4	8	o							6	4						o									-	+	-	Bw44
095	8	8	o				6					6	8				o							6		-	+	-	B8
117			o														o			8	6	8	8		8	-	-	+	Bw42
073		6	o			6											o	4		8	6	8	8		6	-	-	+	Bw44
074			o		6	4	8					6					o	6		8	8	8	8		8	-	-	+	Bw42
119			o			4											o			8		8	8		6	-	-	+	Bw44
113		8	o		4	4	8	8	8	8	8	6		4	8		o			8	8	8	8	6	8	-	-	+	B7
131			o				6		8	8	6				6		o			8	6	6	4		6	-	-	+	B7
118			o				8	8	8	8	8				8		o									-	-	-	B7, Bw44
114			o				8		8	6	4						o									-	-	-	B7, B8

Members of the same family are bracketed together.

Table 2. Reaction pattern of B40 antisera.

Tray 15 Cells	996 38	856 39	884 40	086 41	259 43	360 44	346 45	347 46	349 47	084 48	005 49	551 50	652 51	Bw 60	Bw 61	Bw 41	Bw 48	Other
088	8	8	8		8	8	8		4					-	-	-	-	B13, Bw51
037	8	8	8	6	8	8			4					-	-	-	-	B13, -
124	8	8	8		8	8								-	-	-	-	B13, Bw52
094					8	8	8	8	8		6	6		+	-	?	-	-
106					8	8	8	8	8		4			+	-	-	-	Bw51
107					8	8	8	8	8	6				+	-	-	-	B8
150					8	8	8	8	8		8	4		+	-	-	-	Bw44
214					8	8	8	8	8	8	6			+	-	-	-	Bw51
238				8	8	8			6	6				-	+	-	-	Bw44
015				8	8	8			8					-	+	-	-	B37
020				8	8	8			8					-	+	-	-	-
144				8	8	8			8					-	+	-	-	-
041				6	8	6			4					-	+	-	-	8w67
197				4	8	8			8					-	+	-	-	Bw35
087				6	8	8	8		8					-	+	-	-	Bw51
005					8	8	8		8					-	+	-	-	Bw53
090					8	8	6		8			4	8	-	+	-	-	8w68
204					8	8	8		6					-	+	-	-	Bw51
055					8	8	4		8					-	+	-	-	Bw52
025					8	6			8				4	-	+	-	-	8w64
024					8	8			0					-	+	-	-	B8
198					8	8			8	4				-	+	-	-	-
235					8	8	8	8	8					?	?	-	-	B7
226							8	8	8					-	-	-	-	B7, Bw44
105							8	8	8					-	-	-	-	B7, Bw68
175							8	8	8					-	-	-	-	B7, B37
093							8	8	8					-	-	-	+	Bw51
164					4		8	8	8	6	8			-	-	+	-	B7
203					8						8			-	-	+	-	B8
140					8						8			-	-	+	-	B8
052					8						8			-	-	+	-	B8
050					8						8			-	-	+	-	-
248	8	8	8		8	6		4	8		8			-	-	+	-	B13
207	8	8	8	6	8	8	6				8	6	4	-	-	+	-	B13

A/C CROSSOVER IN SOUTH AFRICAN INDIAN FAMILY

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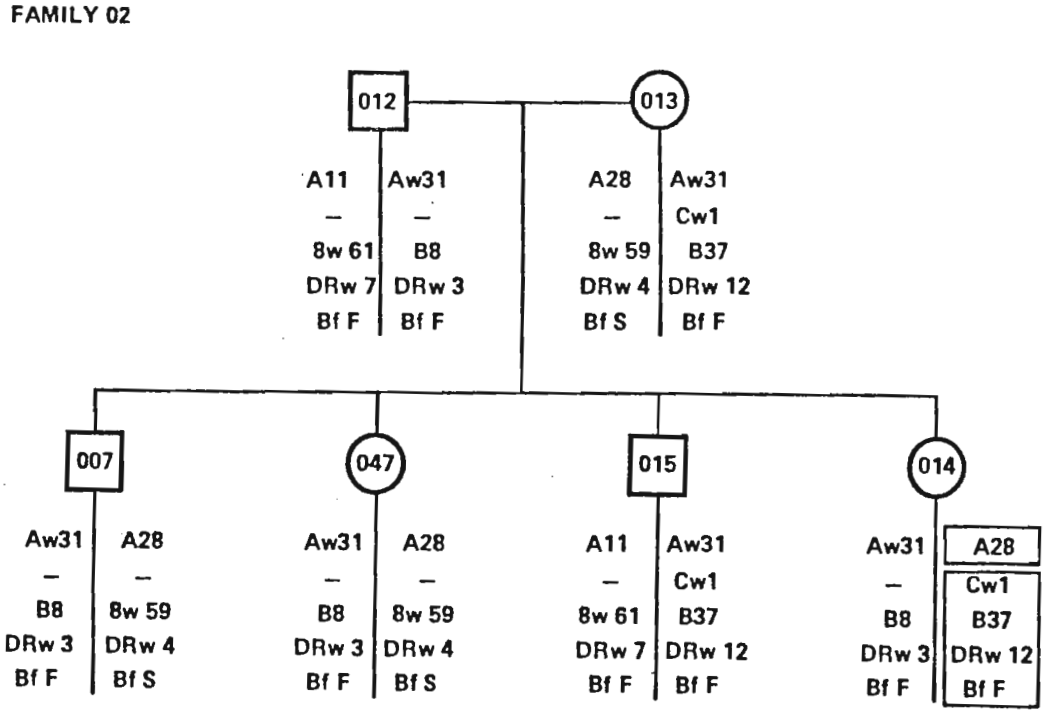
Received December 5, 1979

One of the Indian families typed for the 8th Workshop showed a crossover between the A and C loci. The pedigree is illustrated in Figure 1.

This family was also typed for Bf, C'2, and C'4. The C'2 and C'4 typings were not informative. The Bf^F allele traveled with the C, B, and DR alleles which does not contradict the positioning of the Bf locus between B and D.

Figure I. South African Indian family with a crossover between A and C.



BW53

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History

This antigen was first described by Engelfriet et al in 1972 (1) as the antigen HR. This antigen can only be defined by extra reactions in some B5 and BW35 antisera but during the 7th Workshop it was decided that this definition was clear enough for the provisional designation BW53.

Serology

No monospecific antisera were available for the 8th Workshop but the definition of BW53 was quite clear in the absence of B5 and BW35. The sera used in the disease set gave a better definition than the genetic set. It was impossible to define BW53 in the presence of BW35 using the genetic set (when no other antigen was present) unless the presence of BW4 is taken to indicate that BW53 is present. In the disease set it was impossible to distinguish between BW35 and BW53 in the presence of B5 except by using the absence of BW6 to indicate the absence of BW35.

Linkage

No linkage disequilibrium was evident in the predata analysis but the estimated haplotype frequencies in Caucasians showed that the A28-BW53 haplotype had the highest frequency followed by AW30-BW53. These two A-locus antigens have much higher frequencies in Negroes who also have the highest frequency of BW53.

Conclusions

The definition of BW53 continues to be difficult since monospecific sera are lacking.

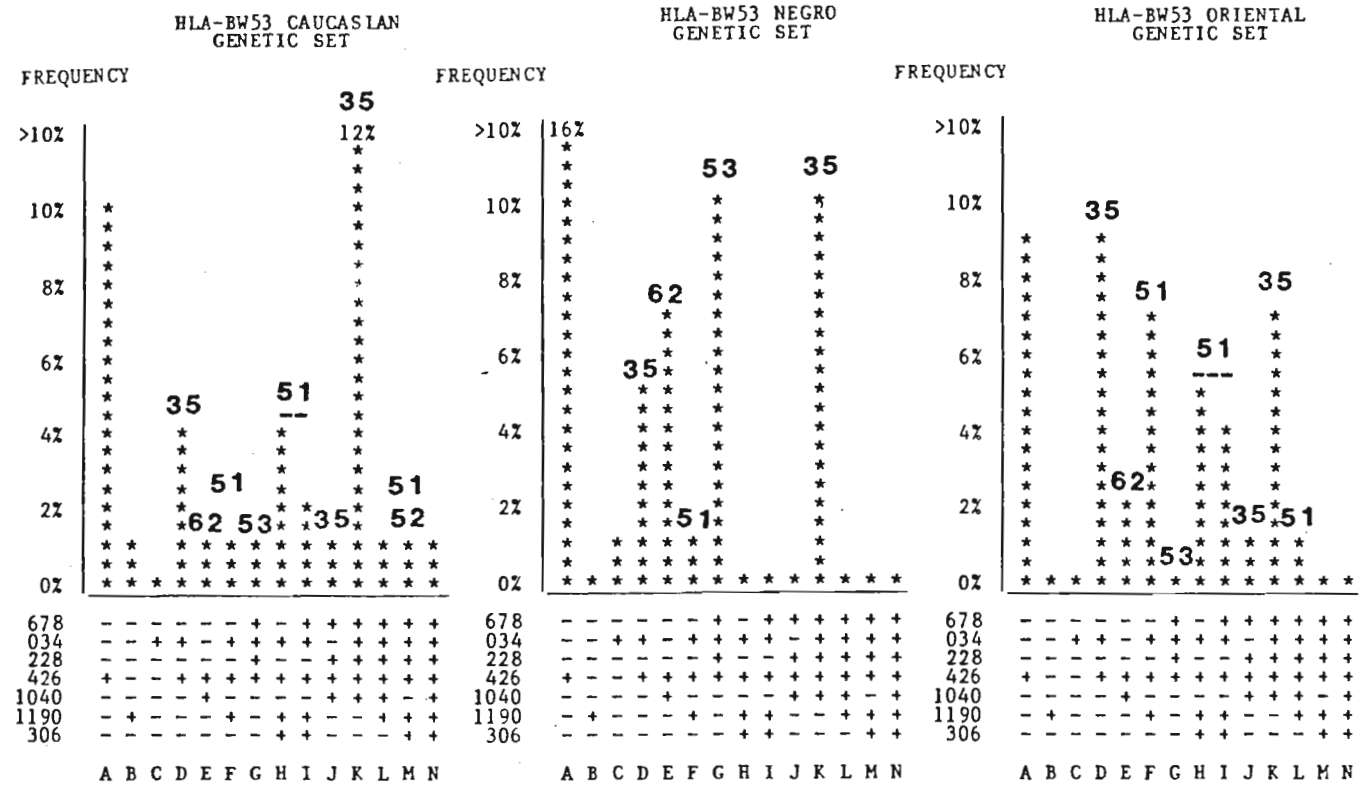
REFERENCE

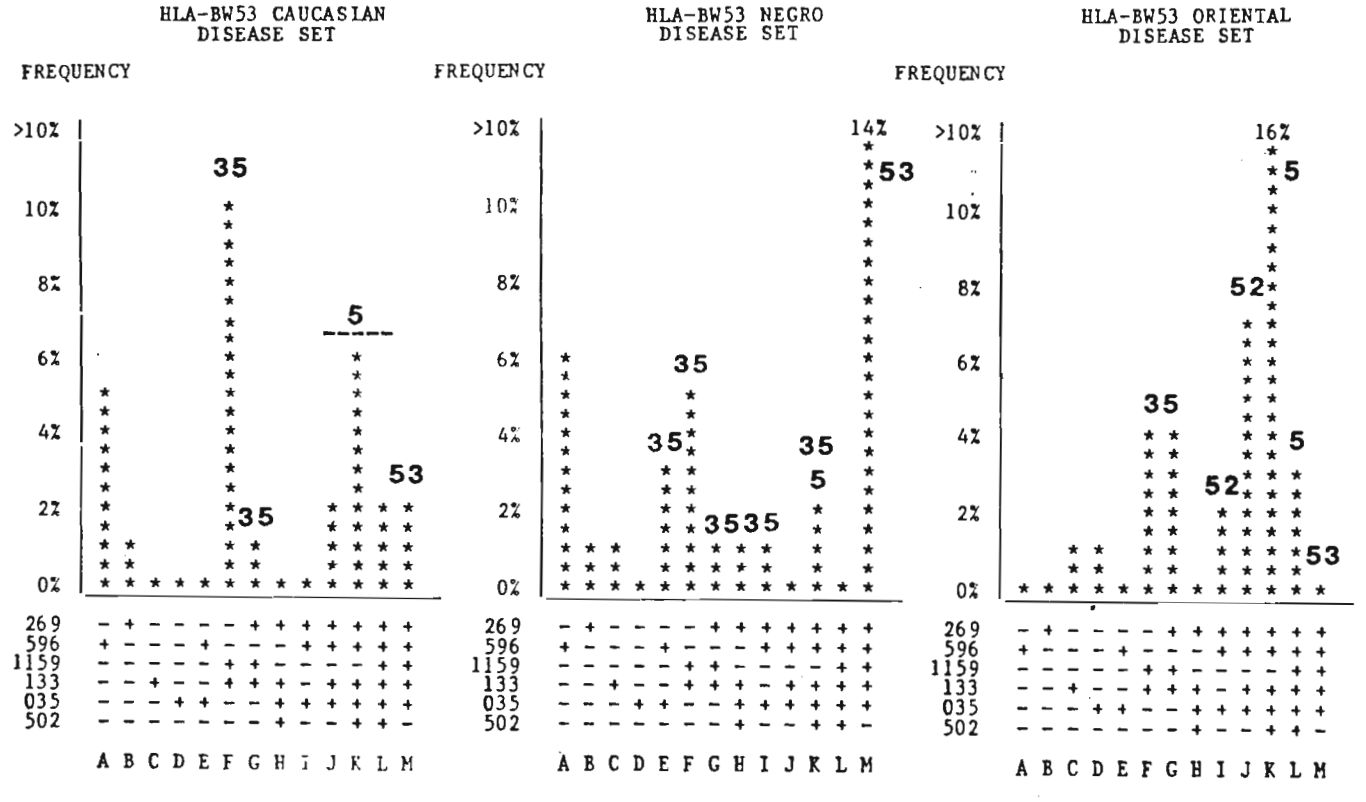
1. Engelfriet CP, Veenhoven von Riesz E, Kort-Bakker M, van den Berg-Loonen PM. Some studies with anti-4c, anti-R, anti-HL-A5, anti-W5, W18 and the description of a new antigen of the four segregant series, called HR. In *Histocompatibility Testing 1970,* Terasaki PI, ed, Munksgaard, Copenhagen, 1972, 475.

BW53

Caucasian: 1.5 Negro: 12.6 Oriental: 0.2

Card Column	Serum Number	Lab	Random Population % Frequency With Antigen C	N	O	r	% 8s	Other Specificities
06-79	196	CAN	25	25	41	39	60	BW51,BW52,B13,BW49, BW59,8W66
07-27	665	CRB	16	27	9	42	60	BW35
07-28	678	GAN	21	27	19	40	77	BW35
07-30	034	GOL	33	37	42	49	85	BW51,BW35,BW52
07-33	228	FES	21	27	16	54	89	BW35
13-78	541	BOT	27	46	37	36	85	BW51,BW52,BW49,BW63, BW57,BW58,BW59
14-19	248	GAZ	25	37	29	46	83	BW35,BW51,CW4
14-21	269	MYR	16	23	35	56	87	BW51,BW52
14-22	494	ENG	20	27	34	55	90	BW51,BW52,BW49,BW63, 8W66
14-23	493	ENG	14	17	29	71	64	BW51,BW52
14-24	035	GOL	15	25	33	71	88	BW51,BW52,8W66
14-25	596	MYE	21	29	32	50	86	BW51,BW52,BW49,BW63, 8W66
14-26	1159	GEL	16	23	13	86	86	BW35
14-27	133	PER	27	30	37	62	100	BW35,BW51,BW52
14-28	784	ENT	37	51	54	55	100	BW35,BW51,BW62,BW52, 8W59
14-30	782	ENT	40	48	51	42	93	BW35,BW51,BW62,BW52, 8W59,BW49


HLA-BW53 CAUCASIAN GENETIC SET
HLA-BW53 NEGRO GENETIC SET
HLA-BW53 ORIENTAL GENETIC SET
FREQUENCY
>10%
10%
8%
6%
4%
2%
0%
A B C D E F G H I J K L M N
678
034
228
426
1040
1190
306


HLA-BW53 CAUCASIAN DISEASE SET
HLA-BW53 NEGRO DISEASE SET
HLA-BW53 ORIENTAL DISEASE SET
FREQUENCY
>10%
10%
8%
6%
4%
2%
0%
A B C D E F G H I J K L M
269
596
1159
133
035
502

Sera Number				
034	55			
678	82	62		
665	75	44	71	
196	08	57	21	00
	228	034	678	665

Sera Number										
784	83									
133	76	82								
1159	60	62	73							
596	39	38	40							
035	42	54	55	14	72					
493	44	54	56	11	70	92				
494	41	39	45	10	87	74	77			
269	51	61	66	27	63	85	87	72		
248	70	78	89	76	32	44	43	33	53	
541	39	36	38	12	80	58	60	76	60	30
	782	784	133	1159	596	035	493	494	269	248

Serum Number	PUR 07 P	P	1	2	3	HLA
1152	-	+	+	-	-	53,35
665	-	+	+	-	-	53,35
678	-	+	+	-	-	53,35
034	-	+	+	-	-	53,35
228	-	+	+	-	-	53,35
426	-	+	+	+	-	53,35
783	-	+	+	-	-	53,35
1116	-	-	-	-	-	35
1040	-	-	-	-	-	35
058	-	-	-	-	-	5
079	-	-	-	-	-	5
338	-	-	-	-	-	5
196	-	+	+	-	-	5
308	-	-	-	-	-	5
256	-	-	-	-	-	51
306	-	-	-	-	-	51
1190	-	-	-	-	-	51

Serum Number	VIL 03 P	P	1	2	3	4	HLA
1152	-	-	-	-	-	-	53,35
665	-	-	-	+	-	-	53,35
678	+	+	+	+	-	-	53,35
034	-	+	+	+	-	-	53,35
228	-	+	+	+	-	-	53,35
426	-	+	+	+	-	-	53,35
783	-	+	+	+	-	-	53,35
1116	-	-	-	-	-	-	35
1040	-	-	-	-	-	-	35
058	-	-	-	-	-	-	5
079	-	-	-	-	-	-	5
338	-	-	-	-	-	-	5
196	-	-	-	-	-	-	5
308,	-	-	0	-	-	-	5
256	-	-	-	-	-	-	51
306	-	-	-	-	-	-	51
1190	-	-	-	-	-	-	51

PROCEEDINGS OF THE SECOND ASIA AND OCEANIA HISTOCOMPATIBILITY WORKSHOP CONFERENCE

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December 1981

Bw35

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Bw35 was well defined by the sera used in this Workshop. Eight sera (see tables) gave strong reactions and together were able to define Bw35 even in the presence of B5 or B15. Sera 249 and 446 reacted with some Bw51 cells, sera 237, 238, 421 and 449 were positive with nearly all B5 splits and sera 447 and 448 were positive with Bw63 cells.

Bw35 is usually associated with Bw6 but there were 2 of 43 Caucasian cells that were Bw35 positive Bw6 negative. In the Japanese 18 of 175 cells were in this category while in Chinese only 5 of 9 Bw35 cells were positive for Bw6. The definition of Bw6 presented some difficulty and I have therefore counted those cells which were negative for all Bw6 sera as being Bw6 negative for this analysis.

Frequency of Bw35 in the three populations:

	Chinese	*Japanese*	*Caucasians*
	N = 164	*N = 992*	*N = 520*
Frequency %	5.5	16.3	7.7

There are marked differences in the frequency of Bw35 associated haplotypes in the different races tested in this Workshop. The well known Bw35, Cw4 haplotype is not common in Japanese and is replaced by Bw35, Cw3. Other differences are shown in Table II. No significant delta values were seen in the Chinese Bw35 association.

Table I

Bw35 Sera in Each Population

Serum Number	Caucasian		Japanese		Chinese		Other
	r	%8+	r	%8+	r	%8+	Specificities
237	64	98	52	91	64	86	Bw51 + 52
238	56	93	32	89	70	100	Bw51 + 52 + 53
249	77	97	46	84	67	100	Bw51
421	53	90	36	94	74	100	B5
446	87	87	73	90	67	100	
447	87	90	69	87	59	100	Bw63
448	79	76	59	69	48	100	Bw63
449	57	84	40	68	39	33	B5

Table II

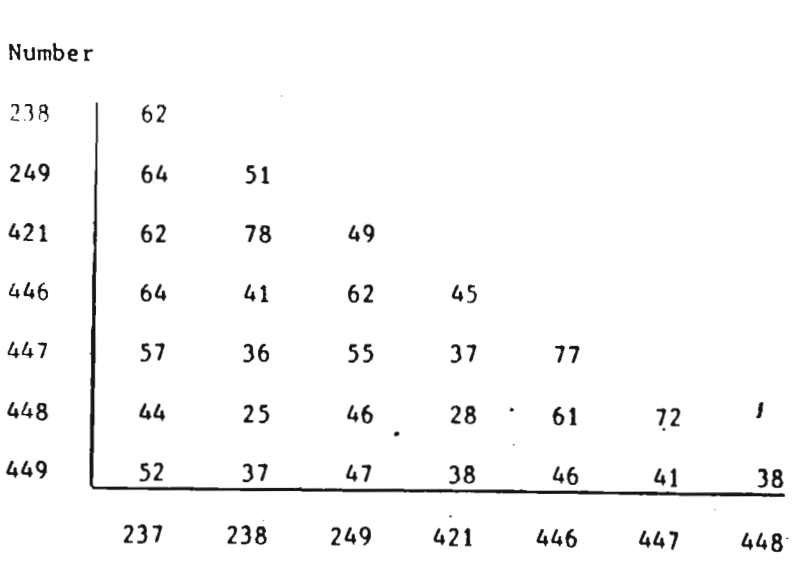
The Most Frequent Bw35 Haplotype ($x10^4$)

	Caucasian N = 688	Japanese N = 994	Chinese N = 164
A2, Bw35	21	275*	80
A3, Bw35	88*	6	0
A11, Bw35	105*	93	35
Aw24, Bw35	68	268	94
Bw35, Cw3	15	600*	77
Bw35, Cw4	325*	23	150
Bw35, DR1	114*	0	0
Bw35, DR4	41	374*	0

* Significant linkage disequilibrium

Figure 1

Bw35 sera x sera r values x 100



Bw53

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It is not possible to define Bw53 with the Workshop sera. The four cells defined as Bw53 in previous workshops gave inconsistent reaction patterns which could not be differentiated from the reaction patterns of the various splits of B5. Three sera were submitted as containing Bw53 antibodies. Serum 241 gave hardly any strong positive reactions. Sera 249 and 446 had many extra reactions besides Bw35, especially in the Japanese, but no consistent pattern could be found in order to define Bw53.

Bw53 is a low frequency antigen in all races except Blacks who were not tested in this Workshop.

Cw4

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Cw4 was very closely defined by five antisera (541, 542, 543, 544, 545) and by serum 551 which also reacted with Cw6 cells. Sera 542 and 544 were not as strong as the others.

Frequency of Cw4

	Caucasian *N 520*	*Japanese* *N 992*	*Chinese* *N 164*
Frequency %	9.8	6.7	14.6

There are marked differences in the associations of Cw4 with other A and B locus antigens in the different races. The well known linkage disequilibrium between Cw4 and Bw35 is present in Caucasians but not in Japanese or Chinese. Instead, Cw4 is associated with Bw62 with a significant delta value. Also noticeable is the lack of an association between A3, Cw4 and B13, Cw4 in Japanese and Chinese.

Table 1 Cw4 Sera in Each Population

Serum No.	Caucasian r	Caucasian %8+	Japanese r	Japanese %8+	Chinese r	Chinese %8+	Other Specificities
541	74	96	64	93	82	100	
542	61	67	65	81	56	50	
543	85	98	73	95	83	82	
544	77	79	73	76	87	72	
545	81	95	75	95	84	95	
551	49	94	57	100	58	91	Cw6

Table 2 Cw4 Haplotypes ($x10^4$)

	Caucasian N = 688	Japanese N = 992	Chinese N = 164
A3, Cw4	109*	0	0
A11, Cw4	119*	157*	214
Aw31, Cw4	6	58*	61
B13, Cw4	62*	0	0
Bw35, Cw4	325*	23	150
Bw56, Cw4	0	50*	0
Bw62, Cw4	75	224*	319*

* Significant linkage disequilibrium

Figure 1 Serum x serum r values x 100 for Cw4 sera

Serum Number	541	542	543	544	545
542	65				
543	85	69			
544	73	71	75		
545	74	59	75	70	
551	61	43	62	52	56

Subdivision of HLA B15 in Indians

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Two splits of B15 have been given official numbers, Bw62 and Bw63. The definition of 8w66 or B15.3 at the Eighth Workshop was not clear enough to be given a W number, nor was the definition of 8w59 which includes BU and SV. The sera used in the Second Asia-Oceania workshop, however, were able to give a better definition of these splits.

Three monospecific sera gave a good definition of Bw62 but Bw63 could only be defined by extra reactions of two B17 sera (404, 405) in the absence of B17 because the broad B15 sera (340, 341, 343) reacted weakly with B17 cells. 8w66 or B15.3 was best defined in the absence of Bw35 by sera 447 and 448 together with the broad B15 sera and sera 337, 344 and 345 which had different extra specificities as listed in Table 1. The number of cells with each pattern in each race was derived from a computer programme, run in Melbourne, utilising all the data and included families, disease data and panel cells but nevertheless the frequency of Bw62 is very low in all the populations tested. Table 2 shows the segregation of 8w66 in an Indian family.

8w59 was only defined by a single serum (356) in the absence of Bw62, Bw63, 8w66, Bw35 and B17, but it is apparent that there is a relatively high frequency of this specificity in Chinese and Indians.

Segregation of 8w59 was shown in another family with the following haplotypes.

No.					
013	Mother	A28, 8w59 // Aw31, B37,Cw1			
077	Father			A11,Bw61	//Aw31,B8
060	Child 1	A28,8w59			Aw31,B8
097	Child 2		Aw31,B37,Cw1	A11,Bw61	
078	Child 3		Aw31,B37,Cw1		Aw31,B8
079	Child 4		Aw31,B37,Cw1		Aw31,B8

Table 1

Reaction pattern of sera used to define splits of B15

Serum Number	Bw62	Bw63	Bw66	8w59	Other Specificities
349	+	–	–	–	
339	+	–	–	–	
338	+	–	–	–	
347	+	±	–	–	
346	+	±	–	–	Cw1
348	+	–	±	–	
337	+	–	±	–	B13
345	+	–	±	±	Cw1 + B7
344	+	–	+	±	
342	+	+	+	–	
340	+	+	+	–	B17
341	+	+	+	–	B17
343	+	+	+	–	B17
508	±	±	±	–	Bw46
447	–	–	+	–	Bw35
448	–	–	+	–	Bw35
404	–	+	–	–	B17
405	–	+	–	–	B17
356	+	+	+	+	Bw35 +B17
CAUC	34	4	1	4	452 cells
JAP	112	0	11	59	1124 cells
CHIN	25	1	13	34	218 cells
INDIAN	3	2	6	16	122 cells

Table 2

Segregation of Bw66 (15.3)

	M 064	F 063	C1 065	C2 066	C3 067
349	-	-	-	-	-
347	-	-	-	-	-
339	-	-	-	-	-
338	-	-	-	-	-
346	-	-	-	-	-
348	-	-	-	-	-
337	+	-	-	±	0
345	+	-	+	+	+
344	+	-	-	+	±
342	+	-	-	+	+
340	+	-	-	+	+
341	+	-	-	+	+
343	+	-	-	+	+
508	+	-	-	+	±
447	+	-	-	+	+
448	+	-	-	+	+
404	-	-	-	-	-
405	-	-	-	-	-
356	+	-	-	+	+

Anomalous Reactions with Bw4 Sera in Indian Families

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The classical division of B locus specificities into Bw4 associated and Bw6 associated antigens places B13 in the Bw4 group and B40 (Bw60 and Bw61) in the Bw6 group.

Two Indian families studied in this workshop showed exceptions to this classification. The reaction patterns of family 01 are shown in Table 1. The inheritance of the haplotype containing Bw61 and Bw6 can be followed through four generations. (Only the B locus antigens will be discussed for simplification). It is unlikely that the great grandmother (cell 024) is homozygous for Bw61 because of the presence of Bw4. Her daughter (cell 004) inherited Bw61, Bw6 from her mother and B13, - from her father. The existence of a blank instead of being homozygous for Bw6 is proved by two of her children (006 and 007) being negative for Bw6 and only having Bw4 together with either B5 or Bw44 from their father (003). The possibility of cell 004 being homozygous for Bw61 and thus causing false positive reactions with B13 sera can be discounted because her grandson (cell 027) is in fact homozygous for Bw61, Bw6.

The existence of a short Bw4 occurs in family 02 where two HLA identical siblings have inherited Bw61, Bw6 from their father and B13, Bw4X from their mother.

This pattern was also seen in two unrelated individuals (cells 062 and 084) the latter cell also having Bw61 present. The last two cells show a conventional B13, Bw4 pattern for comparison.

These families illustrate the necessity for caution in using the presence or absence of Bw4 and Bw6 as indicators for the presence or absence of various B locus antigens.

TABLE 1

FAMILY	GENERATION	CELL	SERA	B40							B40+13					B13						Bw4							Bw6			HAPLOTYPES
				454	456	453	457	460	459	502	455	458	329	330	501	326	323	324	325	327	328	510	511	514	512	513	515	516	518	519	520	
01	I	024		-	-	-	8	8	-	8	8	8	8	8	8	8	-	4	-	-	-	8	0	8	8	8	8	8	-	6	8	Bw61, Bw6//-, Bw4
	II	003		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	8	8	8	8	-	-	-	-	Bw44, Bw4//B5, Bw4
		004		-	-	-	0	8	8	8	0	8	8	8	4	8	6	6	8	8	-	-	-	-	-	-	-	-	-	-	6	Bw61, Bw6//B13, -
	III	005		-	-	-	0	8	8	8	6	8	8	6	8	-	-	-	-	-	-	8	6	8	4	6	8	-	-	-	8	Bw61, Bw6//Bw44, Bw4
		006		-	-	-	-	-	-	-	-	8	8	8	8	8	6	6	8	-	-	8	8	8	-	8	8	4	-	-	-	B13, - //Bw44, Bw4
		007		-	-	-	-	-	-	-	8	8	-	8	8	8	4	-	6	-	-	8	8	8	8	8	8	8	-	-	-	B13, - //B5, Bw4
		033		-	-	-	6	8	8	8	8	8	-	8	6	-	-	-	-	-	-	8	8	8	8	8	8	4	-	-	8	Bw61, Bw6//Bw44, Bw4
		025		-	4	6	8	8	8	8	8	8	6	8	8	6	6	-	-	-	-	8	6	8	8	8	8	8	8	8	8	Bw61, Bw6//Bw57, Bw4
		026		-	-	-	8	8	8	8	8	8	8	8	8	-	-	-	-	-	-	-	8	-	4	-	-	-	8	-	8	Bw61, Bw6//Bw62, Bw6
	IV	027		-	-	-	8	8	8	8	4	8	6	8	8	4	-	-	-	-	-	-	-	-	-	-	-	-	8	4	8	Bw61, Bw6//Bw61, Bw6
		028		-	-	-	8	8	8	8	8	8	8	8	8	-	4	-	-	-	-	8	8	8	8	8	8	8	-	8	8	Bw61, Bw6//Bw57, Bw4
		052		-	-	-	8	8	8	8	8	8	8	8	8	-	-	-	-	-	-	-	8	8	8	8	6	8	8	-	8	8	Bw61, Bw6//Bw57, Bw4
		053		-	-	-	8	8	8	8	8	8	8	8	8	4	-	-	-	-	-	-	-	-	-	-	-	-	-	6	4	8	Bw61, Bw6//Bw62, Bw6
02	I	013		-	-	8	8	8	8	8	8	8	8	8	8	6	-	-	-	-	-	8	8	8	8	8	8	6	8	8	8	Bw61, Bw6//B5, Bw4
		014		-	-	-	-	-	-	-	8	8	8	8	8	8	8	8	8	8	-	8	8	8	-	8	8	-	-	-	-	B13, Bw4X//B37, Bw4
	II	015		8	8	8	8	8	8	8	8	8	8	8	8	8	8	-	-	-	-	8	8	8	8	8	8	-	-	4	8	Bw61, Bw6//B37, Bw4
		016		-	-	-	-	8	8	8	8	8	8	8	8	8	8	8	8	8	4	4	4	8	-	-	-	-	-	-	8	Bw61, Bw6//B13, Bw4X
		017		-	8	4	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	6	6	8	-	-	-	-	-	-	8	Bw61, Bw6//B13, Bw4X
Unrelated		062		-	-	-	-	-	-	-	8	8	8	8	8	8	8	6	8	6	6	8	6	8	-	-	4	-	-	-	4	Bw35, B13, Bw4X, Bw6
		084		-	-	6	8	8	8	8	8	8	8	8	6	8	6	8	8	4	0	8	8	8	-	-	-	-	4	6	8	B13, Bw61, Bw4X, Bw6
		072		-	-	-	-	-	-	6	8	8	-	8	8	8	8	8	8	8	r	8	8	8	4	6	6	-	-	-	6	B13, -, Bw4, Bw6
		095		-	-	-	-	-	-	-	6	6	8	8	6	8	6	8	8	8	6	8	8	8	8	8	8	8	-	-	-	B13, Bw44, Bw4, (Bw4)

Histocompatibility Testing 1984

Report on the Ninth International Histocompatibility Workshop and Conference
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Edited by
E.D. Albert M.P. Baur W.R. Mayr

With 226 Figures and 432 Tables

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Table 1. Split of A28 into two subtypic specificities (Aw68 and Aw69) with Ninth Workshop reagents

Cells	9WS Sera[a]			9WS Moabs[a]		
	A2	A2+ Aw69	A28	A2 and A28	A2	A2+ Aw69
	0 0 0	0 0 0 0	0 0 0 0 0 0 0	1 1 1 1 1 1 1 1	1 1	1 1
	0 0 0	0 0 0 0	0 0 0 0 0 0 0	1 1 1 1 1 1 1 1	1 1	1 1
	1 1 1	1 1 1 2	2 2 2 2 2 3 3	0 0 0 3 4 4 5 5	3 4	0 3
Bodmer lab data	1 3 1	7 8 9 1	3 4 5 7 9 1 2	2 3 8 0 1 2 1 6	2 4	4 9
A2 Homozygotes	+ + +	+ + + +	? – – ? – – –	+ + + + + + + +	+ +	+ +
A2 Heter (most)	+ + +	+ + + +	– – – – – – –	+ + + + + + + +	+ +	+ +
Aw68(28) (Regular)	– – –	– – – –	+ + + + + + +	+ + + + + + + +	– –	– –
Aw69 (28) (Rare)	– ? –	+ + + +	+ + + + + + –[b]	+ + + + + + + +	– –	+ +
Italy I reg. data (refers to families)						
Aw69(28) (Less common)	– – –	0 + + +	+ + + + ? + ?	+ + + + + + + +	– –	+ +
Aw68(28) (Common type)	– – –	0 – – –	+ + + + ? + ?	+ + + + + + + +	– –	– –

[a] Details of other reactivities not given
[b] Serum 32 appears to lack Aw69 reactivity

References

1. Kennedy LJ, Bodmer JG (1983) A2 variants. Ninth International Histocompatibility Workshop Newsletter 5
2. Richiardi P, Amoroso A, Conighi C, Menicucci A, Savi M, Marsico D, Fina C, Curtoni ES (1984) Further splits of A28. Ninth International Histocompatibility Workshop Newsletter 6

Antigen Report: HLA-A29

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The A29 antigen has been well defined since 1975 [1], and the five sera submitted as monospecific A29 sera for this workshop gave a clear definition in all races.

All five sera have high Q scores (Table 1), and the average strength scores show that nearly all positive reactions with A29 were very strong. Sera 9w104 and 9w108 had some extra reactions with A11 and A1 cells, respectively.

Table 1. A29 serum analysis

Serum	Q score	Ave strength with A29	Extras
9w102	9.9	7.6	
9w103	10.0	7.6	Aw43, Th, (A11)[a]
9w104	9.7	7.9	Aw43
9w106	12.0	7.7	Aw43
9w108	11.0	7.1	(Aw43), (A1)

[a] Antigens in parentheses show that only some cells were positive

The other extra reactions were only seen in the Negroid populations. Three sera, 9w103, 9w104, and 9w106, reacted with Aw43 cells, and serum 9w104 also reacted with cells carrying Th. Several broad Aw19 sera also recognized A29 : 9w149, 9w150, 9w062, and 9w301. There were no discrepancies in the segregation patterns of 45 families.

A29 was not found in Chinese or Japanese cells but was present at low frequency in some southeast Asian populations.

As at previous workshops, A29 and B44 showed a positive linkage disequilibrium in Caucasoid and Negroid populations.

Reference

1. Bodmer T, Curtoni ES, van Leeuwen A, et al (1975) The ABC of HLA. A serological report of the Sixth Histocompatibility Testing Workshop. In: Kissmeyer-Nielsen F (ed) Histocompatibility testing 1975. Munksgaard, Copenhagen, p 21

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This antigen is mainly found in Negroids. Aw36 is closely related to A1 and it is normally defined by a subset of A1 sera. At the Sixth Workshop, the A locus assignment of this antigen was confirmed by segregation patterns in a large Zambian family [4].
Although Aw36 is observed mainly in Negroids, sera with anti-Aw36 activity commonly originate from Caucasoids with no apparent Negroid ancestry. Anti-Aw36 activity is only found in anti-A1 sera and not in combination with any other single A locus specificity.
Three anti-Aw36 plus anti-A1 sera were submitted to the Ninth Workshop: 006, 007 and 009 (Table 1), with which Aw36 can be easily assigned in cells which are negative for A1.

Table 1. Anti-HLA-Aw36 sera

9W serum no.	Average score	% Reactions missed	% Extra reactions	Serum strength	Quality score
006	7.1	11	83	88	7.7
007	6.5	38	89	83	3.1
009	6.4	11	84	80	7.3

Although the majority of cells typed in this workshop as Aw36 reacted with all three anti-Aw36 sera, five cells were positive with 006 and 009 and negative with 007. However, the patterns of reactions were variable and probably do not signify a split of this specificity.
In a recent study of Nigerian cells, Aw36 was found in linkage disequilibrium with Bw53 and Cw4 [3].

References

1. Festenstein H, Adams E, Brown J, Burke J, Lincoln P, Oliver RTD, Rondiak G, Sachs JA, Welch SG, Wolf E (1973) The distribution of HL-A antigens and other polymorphisms in Bantu-speaking Negroids living in Zambia. In: Dausset J, Colombani J (eds) Histocompatibility testing 1972. Munksgaard, Copenhagen, p 359
2. Lawler SD, Klouda PT (1973) West Indian Negroes immigrant in the United Kingdom. In: Dausset J, Colombani J (eds) Histocompatibility testing 1972. Munksgaard, Copenhagen, p 415
3. Okoye R et al (1984) HLA polymorphism in the Nigerian population (submitted)
4. Wolf E, Festenstein H, Pritchard J, Watson B, Sachs J, Traub N (1975) Further HLA heterogeneity in Zambian and Caucasoids populations. In: Kissmeyer-Nielsen F (ed) Histocompatibility testing 1975. Munksgaard, Copenhagen, p 179

Antigen Report: HLA-Aw43

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History

In 1972, the occurrence of apparent triplets associated with HLA-A10 and A29 in the Khoisan populations of Namibia was reported [1]. In 1975 [6], an HLA-A10/A29 allele was defined in terms of the Fifth International Histocompatibility Workshop antisera and designated BK. The Sixth and Seventh Workshops [2, 3] provided further opportunities for studying BK. At both these workshops BK was serologically well defined with reactions involving HLA-A10, A26, and A29 antisera and was shown to segregate clearly within families. During the Eighth Workshop a monospecific HLA-Aw43 antibody was used for the first time, simplifying the assignment of this antigen [4]. Although at the time of the Eighth Workshop, HLA-Aw43 had only been found in Khoisan, Cape Colored, Xhosa, and South African Caucasoid individuals in Cape Town [4], we felt that HLA-Aw43 would eventually be detected in other Southern African groups. The assumption that Aw43 could be present in Southern African Negroes other than the Xhosa was based on the finding of Jenkins et al. [5] that there was a considerable San admixture in most Southern African Negroes.

Serology

The Ninth International Histocompatibility Workshop antisera allow good definition of the allele HLA-Aw43. No splits are apparent, and problems of identification should not arise. In our hands

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Table 1. Serum analysis in South African Negroes

9W Serum	Antigens	r	χ^2	% Missed	% Extras
072	Aw43	0.67	54	0	44
	A26	0.91	86	7	0
074	Aw43	0.65	52	0	46
	A26	0.95	96	0	0
075	Aw43	0.65	51	0	46
	A26	0.95	95	0	0
079	Aw43	0.62	47	0	50
	A26	0.87	82	0	13
081	Aw43	0.62	47	0	50
	A26	0.87	82	0	13
082	Aw43	0.61	44	0	51
	A26	0.79	66	7	20
100	Aw43	0.68	55	6	39
	A26	0.65	45	38	11
101	Aw43	0.92	102	6	0
103	A29	0.70	59	0	37
	Aw43	0.91	81	0	7
106	A29	0.69	57	0	39
	Aw43	0.87	74	0	13

9w101 is an excellent anti-Aw43 serum, with an r value of 0.92, as shown in Table 1. Four families, with 15 individuals positive for HLA-Aw43, were submitted from South Africa. The serum analysis of the ten best antisera for identifying HLA-Aw43 is shown in Table 1.

Gene Frequencies and Linkage Disequilibrium. The gene frequencies of HLA-Aw43 in various Southern African Negroid population groups ranges between 0.02 (Zulu) and 0.10 (Central!Kung) Aw43 is in linkage disequilibrium with B7, Bw70, and Cw4.

Conclusion

The antigen HLA-Aw43 was seen in South Africa during the Ninth Workshop. It was again well defined, particularly with antiserum 9w101.

References

1. Botha MC, du Toit ED, Jenkins T, van Leeuwen A, D'Amaro J, Meera Khan P, van der Steen G, van Rood JJ, van der Does J (1972) The HL-A system in Bushmen (San) and Hottentot (Khoikhoi) populations of South West Africa. In: Dausset J, Colombani J (eds) Histocompatibility testing 1972. Munksgaard, Copenhagen, p 421
2. Botha MC, Campbell E, Briggs BR, du Toit ED (1975) Further observations on BK, an African SD-1 antigen. In: Kissmeyer-Nielsen F (ed) Histocompatibility testing 1975. Munksgaard, Copenhagen, p 261
3. Dick H (1977) Joint Report. HLA-A, B and C serology and antigen reports. In: Bodmer WF, Batchelor JR, Bodmer JG, Festenstein H, Morris PJ (eds) Histocompatibility testing 1977. Munksgaard, Copenhagen, p 164
4. Du Toit ED, Briggs BR, Botha MC, Campbell EM (1980) Joint Report. HLA-Aw43. In: Terasaki P (ed) Histocompatibility testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, p 344
5. Jenkins T, Zoutendyk A, Steinberg AC (1970) Gammaglobulin Groups (Gm and Inv) of various Southern African Populations. Am J Phys Anthropol 32:197–218
6. Nurse GT, Bodmer JG, Bodmer WF, van Leeuwen A, van Rood JJ, du Toit ED, Botha MC (1975) A reassessment of HL-A system in Khoisan populations in South West Africa. Tissue Antigens 5:402

Antigen Report: HLA-Aw66

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History

During the Eighth International Histocompatibility Workshop [3] it was agreed that HLA-A25, A26, and Aw34 were well defined, without evidence for further splits.

Recently, however, a new HLA-A antigen, called LN (= Aw66), was described, which is closely related to A25, A26, and Aw34 [4]. The definition of Aw66 was based on the reaction pattern of "monospecific" A25 and A26 sera and more complex "A10 cross-reacting" sera containing Aw66 reactivity and on segregation in families. Especially important for defining Aw66 were sera reacting with both A11 and Aw66. Linkage disequilibrium of Aw66 with Bw41 was observed [4].

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In conclusion, there were a number of monospecific B51 sera, the most specific being nos. 152, 153, 156, 157, 158, 159. Other useful B51 sera without Bw52 (but with B35 and Bw53) were 171 and 172. There were no monospecific Bw52 sera, the best being nos. 162 and 414. No. 162 was a weak Bw52 serum containing weak B51 and B49 activity. Serum 414 did not react with B51-positive cells but had an anti-B49 reactivity. Despite the lack of monospecific sera, Bw52 could be easily defined with a combination of the B5 and B51 sera (see Table 1 in the Bw53 report, Taylor et al., this volume). There was no evidence of splits or variants of B51 or Bw52 at this Workshop.

Antigen Report: HLA-Bw53

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Only one serum (Workshop no. 163) was submitted as a monospecific anti-Bw53 serum. Other sera submitted as having anti-Bw53 as well as other antigens were: 170 (B5); 171, 172 (B5, B35); and 177 (B5, B35, B18).

For each serum the Q score, number of "correct" positive reactions, and "tail" antigens derived from 2 x 2 comparisons are shown in Table 1.

Serum no. 163 was submitted as a monospecific anti-Bw53 serum but the Workshop data did not confirm this. Bw53 could be differentiated from B51, Bw52, B35, and Bw70 with the Workshop sera, but there was no monospecific serum for Bw53. However, there are still some problems when the above-mentioned antigens are present. The definition of Bw53 is shown in Table 2: sera 174 and 176 reacted with B51, Bw52, and Bw53; sera 312, 181, and 182 with B35, and Bw53; sera 171 and 172 with B51, B35, and Bw53; and sera 199, 315, and 317 with B51, Bw52, B35, Bw70, and Bw53.

Table 1. Q score, percentage of "correct" to reactions with the listed antigen (% Correct), and additional specificities in each serum. The sera are listed in order of quality

9WS no.	Antigen	Q score	% Correct	% 8's	Other specificities
177	Bw53	8.3	100	89	B35, 51, w52, 18
172	Bw53	7.9	98	92	B35, 51
199	Bw53	4.8	98	84	B35, 51, (w52)
171	Bw53	6.7	96	89	B35, 51
176	Bw53	5.5	91	86	B51, w52, 49, (w63), 8w66
181	Bw53	7.0	90	79	B35, (51)
182	Bw53	6.5	91	84	B35 (51, w52)
174	Bw53	5.1	89	78	B51, w52, 49
317	Bw53	4.6	96	73	B35, 51, w52, 18, w62, +
312	Bw53	4.4	92	76	B35, w62, 50, w70 (w57)
315	Bw53	5.8	92	84	B35, 51, w52, w62, w70
316	Bw53	2.6	78	56	Bw62, 35, (w63, 51, w57, w46)

Table 2. Definition of B51, Bw52, Bw53, B35, and Bw70 with the Ninth Workshop sera

	153	156	157	160	202	180	179	316	311	338	199	315	317	312	181	182	171	172	177	174	176	201	164	169	173	175	253	170	167	162	414
B51	+	+	+	+	–	–	–	?	–	–	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Bw52	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	+	+	+	+	+	+	+	+	+	?	?	?	+
Bw53	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
B35	–	–	–	–	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Bw70	–	–	–	–	+	+	+	+	+	+	?	+	+	+	?	?	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

?: weak reaction

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References

1. Hammond MG, Appado B, Brain P (1972) HLA antigens and antibodies in South African Indians. Tissue Antigens 2:389
2. Dick HE, Bodmer WF (1977) Joint report 4: HLA-A, -B and -C serology. In: Bodmer WF, Batchelor JR, Bodmer JG, Festenstein H, Morris PJ (eds) Histocompatibility testing 1977. Munksgaard, Copenhagen, pp 178–180
3. Thompson JP (1980) Joint report: Bw60. In: Terasaki PI (ed) Histocompatibility testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, pp 458–461

Antigen Report: HLA-Bw62 and Other Bw6-Associated Variants of B15

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History

Subdivisions of B15 were suggested at the Fourth International Workshop [18] and were reported at the Sixth Workshop to be found often in Malay and Chinese populations [11]. Sera against these variants were described as early as 1974 [14]. During the Seventh Workshop, one component appeared to be associated with Bw4 and another with Bw6 [9]. At the Eighth Workshop, two splits were defined: Bw62, which is Bw6 associated [17], with one exception recently published [1], and Bw63, which is Bw4 associated [19]; a third split was proposed in Negroids: 8w66, which is Bw4 associated [7]. During the Seventy-second International Cell Exchange, further splits of B15 were suggested: B15.3 in Chinese [12] and a new B15 variant in Vietnamese [13], both of which are Bw6 associated. At the Second Asia-Oceania Histocompatibility Workshop, an antigen report on B15 described a short pattern of reaction within Bw62, called Bw62.1 [15].

Serology

During this Workshop, the complexity of B15 has been described in nine newsletters [2–6, 8, 10, 16, 20], and the different patterns of reactions are shown in Table 1. Bw62 was clearly defined by positive reaction with four monospecific sera: 9w285, 9w286, 9w284, and 9w289, with Q scores of 8.9, 8.5, 7.4, and 5.3 respectively. Bw62.1 or sh (short) was defined by negative reaction with these four key sera for Bw62. Twenty-five families in this Workshop, six from Japan, four from Thailand and China, 11 from South Africa and four from the USA, with 28 informative sibs, showed clear segregation of this split. B15.3 and B15 SL were negative with the above-mentioned sera and also with a number of other sera (see Table 1). B15 G and B15 Sau seem to be rather similar, and further data are needed in order to support a clear difference between the two. B15 S (Siamese), in contrast, seems to have a pattern different from that of other splits mentioned and to react with some Bw45 sera. Separate segregation of B15 S (Siamese) and B15 T (Thai; see report on Bw63) was also seen in one family (FAM ANZ DCH 06; Fig. 1).

Linkage Disequilibrium

The gene frequencies of Bw62 as calculated in the Workshop Central Analysis were 0.06 for Caucasoids and 0.08 for Mongoloids. A strong linkage disequilibrium was noted with Cw3.1 and with Cw3.2, and an association with DR4 in Caucasoids and DRw9 in Mongoloids.

Conclusion

Bw62 was well defined in this Workshop and a number of other Bw6-associated B15 components were defined. Bw62.1 or sh (short) seems to be well defined, but the other splits need further studies.

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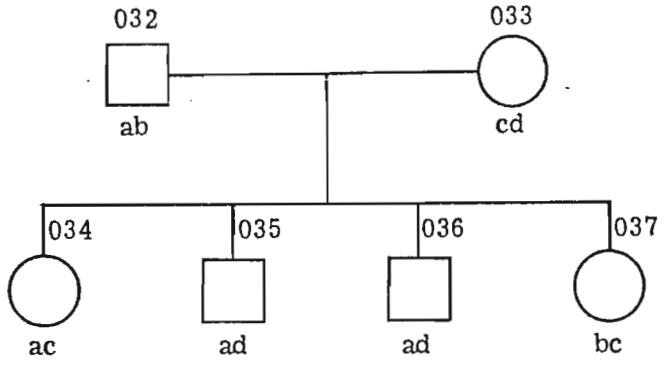
Table 1. Reaction pattern of B15 on Ninth Workshop sera

	1 7 3	1 7 4	1 7 5	1 7 9	1 8 0	2 7 6	2 7 7	2 7 8	2 8 1	2 8 2	2 8 4	2 8 5	2 8 6	2 8 9	2 9 5	3 0 7	3 0 8	3 0 9	3 1 0	3 1 1	3 1 2	3 3 1	4 0 2
Bw62				–	–	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+
Bw62.1 (sh):																							
Ts-1 [16]				–	+	+	+	+	+	+	–	–	–	–	0	–	+	+	+	+	+	+	+
B15 Short Thai [5]				+	+	+	+	+	+	+	–	–	–	–	–	–	+	+	+	+	+	+	+
B15 KEMP [3]				+	0	+	+	+	+	w	–	–	–	–	0	–	+	+	+	+	+	+	+
SH 7 [20]				+	+	+	+	+	+	+	–	–	–	–	0	0	+	+	+	+	+	+	+
Bw62 S [10]				0	0	+	+	+	+	+	–	–	–	–	0	0	+	+	+	+	+	+	+
B15.3				0	0	–	–	–	w	–	–	–	–	–	–	–	+	+	+	+	w	+	+
B15 SL1 [2]				–	–	–	w	–	w	–	–	–	–	0	0	–	+	+	+	+	+	+	+
B15 S (Siamese) [4]				–	–	–	–	+	+	+	0	–	–	+	–	–	+	+	+	–	–	–	–	+	+
B15 Sau [2]				–	+	–	–	–	–	+	–	–	–	–	0	–	–	+	+	+	+	+	+
B15 G [2]				+	+	–	–	–	+	+	w	–	–	–	0	w	+	+	+	+	+	+	+
Bw63	+	+	±	–	–	+	+	+	+	–	–	–	–	w	+	+	+	+	+	+	–	w	w
8w66	+	+	+	–	–	±	±	±	±	–	–	–	–	–	–	+	+	+	+	+	–	+	+
B15 T (Thai) [4]	–	–	–	–	–	+	+	+	–	–	0	/	–	–	–	–	+	+	+	–	+	+	+

(0: not tested; w: weak reaction)

FAM ANZ, DCH 06



a = A2, B15S, Cw3 BW6 b = A2, B15T, Cw-, BW4

c = A11, B51, Cw-, BW4 d = Aw19, Bw44, Cw7, BW4

References

1. Alonso A, Ollier W, Doyle P, Williams E, Festenstein H (1983) Family investigation demonstrating the association of HLA-Bw62 with HLA-Bw4. Tissue Antigens 22:32–36
2. Alonso A, Doyle P, Williams E, Festenstein H (1984) Further splits of HLA-B15. Ninth Workshop Newsletter V
3. Campbell EM, Taljaard DG, Du Toit ED (1984) B15 Kemp/B15 short Thai. Ninth Workshop Newsletter V
4. Chandanayingyong D (1983) A possible new Thai variant of B15. Ninth Workshop Newsletter II
5. Chiewsilp P, Chandanayingyong D, Sujirachato K (1983) B15 short Thai. Ninth Workshop Newsletter II
6. Coates E, Stratton A, Dewar PJ (1983) Definition of Bw62, Bw63 and B15.3. Ninth Workshop Newsletter IV
7. Danilovs J, Pollock C (1980) 8w66. In: Terasaki PI (ed) Histocompatibility testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, pp 477–479
8. Dejour G, Fauchet R, Jalais E, Bouhallier O, Genetet B (1983) B15.3 (Chinese) specificity. Ninth Workshop Newsletter III
9. Dick HB (1978) HLA-A, B and C serology and antigen reports. In: Bodmer W et al (eds) Histocompatibility testing 1977. Munksgaard, Copenhagen, p 157
10. Hammond MG (1984) HLA-B15 complex in South African Indians. Ninth Workshop Newsletter VIII
11. Joysey VC, Roger JH, Bland C, et al (1975) Further studies on a Malay population. In: Kissmeyer-Nielsen F

(ed) Histocompatibility testing 1975. Munksgaard, Copenhagen, p 251
12. Loon J, Bernoco D, Terasaki P, Takemura S (1981) Report of the 72nd International Cell Exchange. UCLA Tissue Typing Laboratory, Los Angeles
13. Loon J, Terasaki PI, Takemura S (1981) Report of the 73rd Cell and 1st Serum Exchange. UCLA Tissue Typing Laboratory, Los Angeles
14. Richiardi P, Castagneto M, D'Amarro J, et al (1974) Four new HLA allelic factors subtypic to HLA-12 and W15. Their correlation with W4 and W6. J Immunogenet 1:323
15. Saueracker G, Christiansen FT, Dawkin RL (1981) Antigen Report ABC B15. In: Simmons MJ, Tait BD (eds) Proceedings of the Second Asia and Oceania Histocompatibility Workshop Conference 1981. Immunopublishing, Victoria, Australia, p 80
16. Shiraki T, Akasa T, Asami M, Ueda Y, Morishima Y, Hasegawa I, Yoshida TO (1983) A study of a new HLA-B locus antigen TS-1 with 9th workshop sera. Ninth Workshop Newsletter III
17. Singal DP, Lung P (1980) Bw62. In: Terasaki PI (ed) Histocompatibility testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, pp 462–464
18. Thorsby E, Kissmeyer-Nielsen F, Svejgaard A (1970) New alleles of the HLA system: serological and genetic studies. In: Terasaki PI (ed) Histocompatibility testing 1970. Munksgaard, Copenhagen, p 137
19. Troup GM (1980) Bw63. In: Terasaki PI (ed) Histocompatibility testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, pp 465–468
20. Zhao TM, Lee TD, Bu KJ, Zhang GL, Rubinstein P (1984) Definition of the HLA-B antigen SH 7, identical to TS-1 in the Chinese population. Ninth Workshop Newsletter VII

Antigen Report: HLA-Bw63 and Other Bw4-Associated Variants of B15

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History

Bw63 was clearly defined at the Eighth International Histocompatibility Workshop as a Bw4-associated subdivision of B15, as had been suggested at previous workshops [3]. At the same workshop another component of B15, also included in Bw4, and found preferentially in Negroids, was also described: 8w66, which had been first suggested in the 31st International Cell Exchange of Terasaki [2].

Serology

At the Ninth Workshop, among the 25 sera reacting strongly with at least one component of B15, none was monospecific for Bw63. This antigen was defined by positive reaction with sera directed against B15 + B17: 9w310, 307, 309, 308, 305, 278, and 299, with Q scores from 9.6 to 5.0, or against B15 alone: 9w276, 281, and 277, with Q scores of 6.3, 4.8, and 4.8, respectively. Among these sera, 9w305 and 299 did not react with Bw62-positive cells. Serum 9w278 was negative on Negroid Bw63-positive cells and positive on Caucasoid Bw63-positive cells. Some sera reacted only with cells from Bw63-homozygous individuals: 9w289, 290, 314. Bw63 was also defined by negative reactions with the Bw62-specific sera (9w282, 284, 285, 286, and 289). The following sera also had some Bw63 activity in the tail analysis: 9w176, 316, 323, 306, 234, 286, 163, and 302. Antigen 8w66 was assigned only to seven cells in the Negroid population. The pattern of reactivity was shorter than that of Bw63: negative reaction with 9w299 and variable pattern with 9w276, 277, 278, and 281. In addition, three sera directed against B5 and B49 reacted with 8w66-positive cells: 9w173, 174, and 175; 9w173 and 174 also contained some anti-Bw63 activity. Another pattern of B15: B15T was described in a Thai family: ANZ DCH 06 [1]: this variant had the same pattern as 8w66, but sera 9w173, 174, and 175 were negative. All the reaction patterns are tabulated in the report on Bw62 in this volume.

Linkage Disequilibrium

The gene frequency of Bw63 is 0.006 in Caucasoid and Mongoloid populations. The most frequent associations were with A24, A32, Cw7, Cw-, DRw6, and BfF in Caucasoids, while Bw63 was associated with A26, Cw3, and DR5 in Mongoloids.

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Conclusion

Bw63 was clearly defined at the Ninth Workshop although no monospecific serum was available. The other variants of B15 associated with Bw4 need further confirmation.

References

1. Chandanayingyong D (1983) A possible new Thai variant of B15. Workshop Newsletter no. 2
2. Danilovs J, Pollock C (1980) Joint report: 8w66. In: Terasaki PI (ed) Histocompatibility testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, pp 477–479
3. Troup GM (1980) Joint report: Bw63. In: Terasaki PI (ed) Histocompatibility testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, pp 465–468

TABLE I REACTION PATTERN OF B15 ON 9TH WORKSHOP SERA

	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	4	4
	7	7	7	7	8	7	7	7	8	8	8	8	8	8	9	0	0	0	0	1	1	1	1	0	0
	3	4	5	9	0	6	7	8	1	2	4	5	6	9	9	5	7	8	9	0	1	2	3	1	2
Bw62	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
TS-1[10]	+	+	+	+	+	+	+	-	-	-	-	0	-	+	+	+	+	+	+	+	+	+	+	+	+
B15 Short Thai[5]	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
B15 Kemp[3]	+	0	+	+	+	+	w	-	-	-	-	0	-	+	+	+	+	+	+	+	+	+	+	+	+
SH 7[11]	+	+	+	+	+	+	+	-	-	-	-	0	0	+	+	+	+	+	+	+	+	+	+	+	+
Bw62 S[9]	0	0	+	+	+	+	+	-	-	-	-	0	0	+	+	+	+	+	+	+	+	+	+	+	+
B15[3]	0	0	-	-	-	w	-	-	-	-	-	-	-	+	+	+	+	+	w	+	+	+	+	+	+
B15 SL[2]	-	-	-	w	-	w	-	-	-	-	0	0	-	+	+	+	+	+	+	+	+	+	+	+	+
B15S (Siamese)	-	-	-	-	+	+	+	0	-	-	+	-	-	+	+	+	-	-	-	-	+	+	+	+	+
B15 SAU[2]	-	+	-	-	-	-	+	-	-	-	-	0	-	-	+	+	+	+	+	+	+	+	+	+	+
B15 G[2]	+	+	-	-	-	+	+	w	-	-	-	0	w	+	+	+	+	+	+	+	+	+	+	+	+
Bw63	+	+	+	-	-	+	+	+	+	-	-	-	-	w	+	+	+	+	+	+	-	w	w	+	+
8w66	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+
B15T (Thai)	-	-	-	-	-	+	+	+	-	-	0	-	-	-	-	+	+	+	+	-	+	+	+	+	+

DEFINITION OF Bw53 IN SOUTH AFRICAN INDIANS

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The antigen Bw53 has always been difficult to define, especially in the presence of other antigens of the B5–35 complex.

The Ninth Workshop sera give a better definition than the Eighth Workshop sera[1] because it is now possible to recognise Bw53 in the presnce of Bw35 without relying on the presence of Bw4. However it is still not possible to distinguish Bw53 in the presence of Bw51.

Bw51 and Bw52 were well defined; serum 441 being exceptionally strong and only giving extra reactions with homozygous Bw51 cells.

Figure 1 shows the reaction patterns of each of these specificities and Figure 2 shows the inheritance of Bw53 through three generations.

REFERENCES

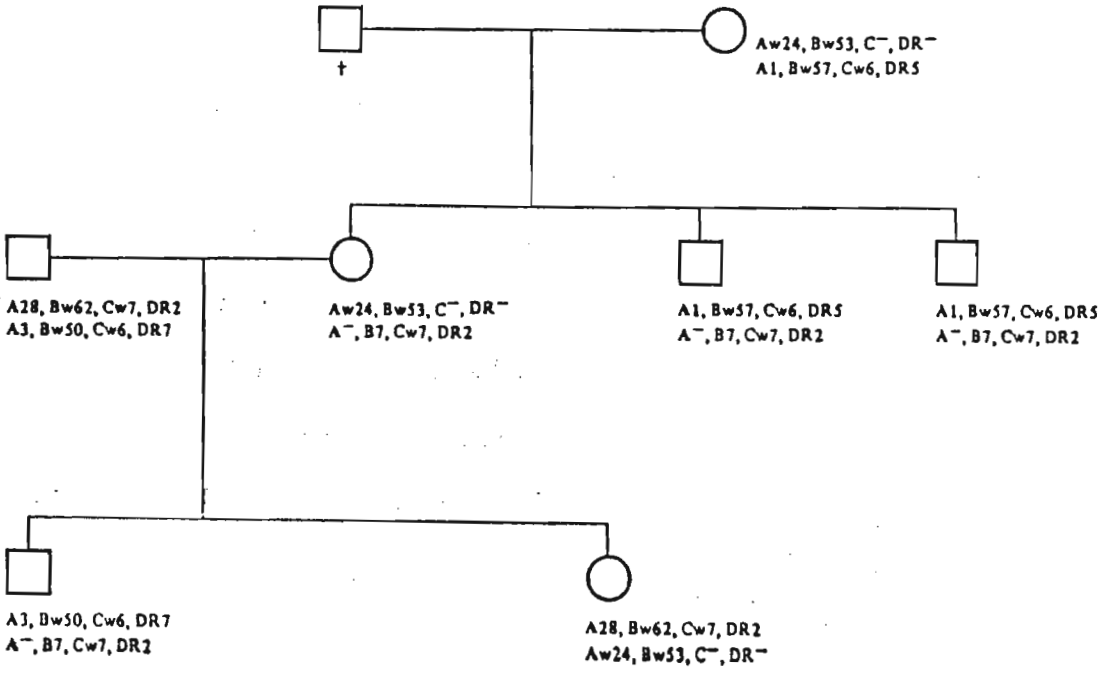
[1] M.G. Hammond. Antigen report Bw53
In: Histocompatibility Testing 1980 pp 429–432.

NINTH WORKSHOP SERA

Antigens	152	153	156	157	158	159	160	167	161	162	414	163	164	166	169	170	173	174	175	176	177	171	172	
Bw51	+	+	+	+	+	+	+	+	–	–	–	w	+	+	+	+	+	+	+	+	+	+	+	30 cells
Bw52	–	–	–	–	–	–	–	–	±	+	+	w	+	+	+	+	+	+	+	+	+	–	–	29 cells
Bw53	–	–	–	–	±	–	–	–	–	–	–	w	+	–	±	w	–	+	+	+	+	+	+	5 cells
Bw35	–	–	–	–	–	–	–	–	–	–	–	w	–	–	–	–	–	–	–	–	+	+	+	28 cells

Figure 1. Reaction pattern of HLA B5–35 complex.

Figure 2. Inheritance of Bw53. Family 23.



SPLITS OF DR4 IN SOUTH AFRICAN INDIANS

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A number of investigators have reported on the heterogeneity of the DR4 antigen both in the 8th International Histocompatibility Workshop (1980) [6-10] and in the 9th Workshop Newsletters, [1-5] but the splits of the antigen was not admitted to DR status at the 8th Workshop.[10]

We support these investigators' observations and report here three splits of DR4 in two families of South African Indians (Table I). We designated these antigens DR4.1, DR4.2 and DR4.3.

DR4.1 is in agreement with other reports in that all the DR4 antisera reacted positively as seen in family 23(a).

DR4.2, as seen in family 23(b) is negative with 5 antisera 9w 591, 592, 593, 594, 582 and is similar to that reported by Borelli *et al.* in Newsletter II, [1] and Gebuhrer *et al.* in Newsletter III.[4]

A very short DR4.3 in family 15 is negative for the 5 sera as in DR4.2 but in addition sera 595, 590, 578 and 587 are also negative.

Two random cells, 92 (DR4.2) and 104 (DR4.1) are also shown.

REFERENCES

1 Borelli, I., Richiardi, P., Curtoni, E.S.
Splits of DR4 with ws alloantisera and monoclonal antibodies and correlation with HLA-D factors.
9th Histocompatibility Workshop Newsletter II.

2 Fauchet, R., Bonhallier, O., Jalais, E., Jejour, G., Genetet, B.
Complexity of DR4 specificity: serological definition.
9th Histocompatibility Workshop Newsletter III.

3 Tait, B. and Boyle, A.
DR4 Serology.
9th Histocompatibility Workshop Newsletter III.

4 Gebuhrer, L., Betuel, H., Lambert, J., Fredel, A.C. and Farre, A.
Subtypes of HLA-DR4.
9th Histocompatibility Workshop Newsletter III.

5 Schreuder, I. and Parlevleit, J.
HLA-DR4 on HTC and in Families.
9th Histocompatibility Wrokshop Newsletter IV.

6 Betuel, H., Gebuhrer, L., Bertrand, J.
Division of HLA-DRw2 and of DRw4.
In Histocompatibility Testing (1980) p 800–801, ed. P.I. Terasaki.

7 Colombe, B., Pask, S., and Payne, R.
Further complexity associated with DR4 typing.
In Histocompatibility Testing (1980), p 802, ed. P.I. Terasaki.

8 Mizrachi, Y., Orgad, S., Jonash, A., Arigad, S., Yaron, M., Schiff, B. and Gazit, E.
Heterogeneity of DRw4 in an Israeli population.
Histocompatibility Testing (1980), p 803, ed. P.I. Terasaki.

9 Walker, M., and Rubinstein, P.
On splits of DRw4: genetics vs serology.
In Histocompatibility Testing (1980) p 804, ed. P.I. Terasaki.

10 Festenstein, H.
DR4 Histocompatibility Testing (1980) p 515, ed. P.I. Terasaki.

TABLE 1. SEROLOGICAL PATTERNS OF SPLIT DR4 IN SOUTH AFRICAN INDIANS

			Ninth Workshop Sera																	
			5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
			8	7	7	8	8	8	8	9	8	9	7	9	9	9	8	9
			9	5	6	0	4	5	1	5	7	0	8	1	2	3	2	4
FAM	23(a)	DR GENOTYPE																
129	MOTHER	4.1, 7	6	6	6	4	6	6	4	6	6	6	6	4	4	4	6	4
128	FATHER	W10, 7	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1
130	SIB 1	4.1, 7	8	8	6	8	8	8	8	8	8	8	8	8	8	8	8	6
147	SIB 2	4.1, 7	1	6	6	6	4	4	6	6	4	4	6	4	4	1	4	4
FAM	23(b)																	
145	MOTHER	4.2, 2	6	8	6	6	6	6	6	6	1	6	6	1	1	1	1	1
136	FATHER	4.2, 2	6	8	8	6	8	6	8	8	6	6	6	1	1	1	1	1
138	SIB 1	4.2, 2	6	8	6	4	6	6	4	6	4	4	6	1	1	1	1	1
137	SIB 2	4.2, 4.2	4	8	6	4	6	6	1	6	6	6	6	1	1	1	1	1
146	SIB 3	4.2, 4.2	4	6	6	1	4	6	1	6	4	1	6	1	1	1	1	1
FAM	15																	
119	MOTHER	4.3, w6	6	6	6	6	6	6	1	1	1	1	1	1	1	1	1	1
915	FATHER	– –	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
110	SIB 1	4.3, 1	6	6	6	4	6	6	4	6	1	1	1	1	1	1	1	1
120	SIB 2	4.3, w6	4	6	6	6	6	4	1	1	1	1	1	1	1	1	1	1
121	SIB 3	4.3, 1	6	6	6	6	8	8	4	1	1	1	1	1	1	1	1	1
122	SIB 4	4.3, 1	6	6	6	8	8	8	8	1	1	1	1	1	1	1	1	1
123	SIB 5	4.3, 6	6	6	6	8	8	8	8	1	1	1	1	1	1	1	1	1
92	RANDOM		6	6	6	4	4	4	4	4	4	6	6	1	1	1	1	1
104	RANDOM		1	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6

THE HLA A10 AND Aw19 COMPLEX IN SOUTH AFRICAN INDIANS AND NEGROES

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Figure 1 shows the reaction pattern of sera recognising antigens of the HLA A10 complex. The Aw34 was only found in one Negro family and in a Coloured family. The reaction pattern of LN[1] is similar to that given by Moreno and Kreisler[2] and Gebuhrer et al.[3] except that sera 071 and 151 were positive. This antigen was only found in one South African Indian family.

The Aw19 complex is illustrated in Figure 2. Aw33 was seen in five Indian families with the same reaction pattern except that sera 135 and 144 were occasionally negative. None of the variations described by Chandanayingyong[4] were found. Campbell et al.[5] described an antigen 19BAC similar to TH.[6] The antigen 19NEW in Figure 2 is positive with serum 106 as well. It was found in two grandchildren of a large Coloured family but unfortunately the father was not available for testing.

REFERENCES

1 Mesman, B., De Lange, G. and Engelfriet, C.P. (1983). A new HLA-A antigen, called LN, closely related to A25, A26 and Aw34. Tissue Antigens 21, 192.

2 Moreno, M.E. and Kreisler, J.M. (1983). Definition of antigen LN with 9th Workshop sera in two informative families. Ninth IHW Newsletter V p4.

3 Gebuhrer, L., Primard, Y., Labonne, M.P. and Betuel, H. (1983). Recognition of antigen LN (Locus A) in an informative family. Ninth IHW Newsletter III p14.

4 Chandanayingyong, D. (1983). Further splits of antigen Malay. Ninth IHW Newsletter III P18.

5 Campbell, E.M., Taljaard, D.G. and du Toit, E.D. (1983). 19BAC – A possible new Negroid HLA-A locus specificity. Ninth IHW Newsletter V P8.

6 Wolf, E., Pritchard, J., Watson, B. and Festenstein, H. (1981). Characterisation of a new Negroid A locus specificity TH In: Histocompatibility Testing 1980. Ed. P. Terasaki UCLA Los Angeles p744.

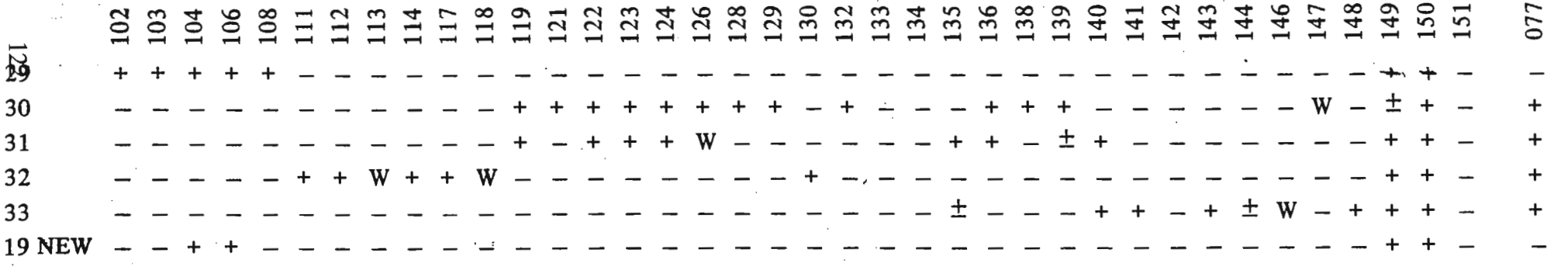
Figure 1. HLA A10 in South African Indians and Negroes

Ninth Workshop Sera

	25	26	34	LN
071	+	+	-	+
072	+	+	-	+
074	+	+	-	+
075	+	+	-	+
076	+	+	+	+
077	+	+	+	+
078	+	+	+	+
079	+	+	+	+
081	+	+	+	+
082	+	+	+	+
083	-	-	+	-
084	-	-	+	-
085	+	-	-	-
086	+	-	-	-
087	+	-	-	-
090	+	-	-	-
091	+	+	-	-
093	-	+	-	+
094	-	+	-	W
095	-	+	-	+
098	-	+	-	-
099	-	+	-	-
140	-	-	-	-
141	-	-	-	-
142	-	-	-	-
143	-	-	+	-
144	-	-	+	-
146	-	-	-	-
147	+	+	+	+
148	+	+	+	+
149	+	+	+	+
150	+	+	+	+
151	+	+	+	+

Figure 2. HLA Aw19 in South African Indians and Negroes

Ninth Workshop Sera



HLA B15 COMPLEX IN SOUTH AFRICAN INDIANS

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The cross-reacting group of antigens comprising Bw62, Bw63, B15.3, BU and SV have been defined in many different ways with many variations such as B15 KEMP, B15 THAI, B15 G and others.[1-10]

Table 1 shows the reaction pattern found in South African Indians. Bw62 was clearly defined. Bw62S was also clear and also associated with Bw6 and shows a close similarity with B15 THAI and B15 KEMP. Bw63 was associated with Bw4 and easily confirmed by sera 176, 305 and 299 in the absence of Bw52.

Only serum 314 defined SV; serum 180 also reacted with many of the Bw62S cells.

REFERENCES

1 Raffoux, C., Lepage, V., Dehay, C., Degos, L., Busson, M., Colombani, J. and Dausset, J.
Da(6) and BU, SV or 8W59 antigen.
Ninth IHW Newsletter I 1983.

2 Chiewsilp, P., Chandanayingyong, D. and Sujirachato, K.
B15 short Thai.
Ninth IHW Newsletter II 1983.

3 Chandanayingyong, D.
A possible new Thai variant of B15.
Ninth IHW Newsletter II 1983.

4 Graugaard, B., Junge, K., Jorgensen, H. and Kissmeyer-Nielsen, F.
The BU, SV or 8w59 antigen.
Ninth IHW Newsletter II 1983.

5 Dejour, G., Fauchet, R., Jalais, E., Bouhallier, O. and Genetet, B.
B15.3 (Chinese) specificity.
Ninth IHW Newsletter III p 21 1983.

6

[6] Coates, E., Stratton, A. and Dewar, P.J.
Definition of Bw62, Bw63 and B15.3.
Ninth IHW Newsletter IV p5 1983.

[7] Laundy, G.J., Lewis, L., Hardiman, P., Roberts, I., and Bradley, B.A.
Partition of Bw59 into BU and SV by polyclonal and monoclonal reagents.
Ninth IHW Newsletter IV p 7 1983.

[8] Campbell, E.M. Taljaard, D.G. and du Toit, E.D.
B15 Kemp/B15 short Thai.
Ninth IHW Newsletter V p12 1983.

[9] Alonso, A., Doyle, P., Williams, E. and Festenstein, H.
Further splits of HLA B15.
Ninth IHW Newsletter V p 13 1983.

[10] Andrien, M. and du Pont, E.
Further characterization of BU-SV antigens.
Ninth IHW Newsletter V p 16 1983.

FIG. 1 HLA B15 COMPLEX IN SOUTH AFRICAN INDIANS

	Ninth Workshop Sera																												
	284	285	286	289	282	276	277	278	281	307	308	309	310	311	312	313	315	316	317	338	314	180	186	176	305	299		
Bw62	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	–	–	–	–	–	–	–	Bw6	21 cells
Bw625	–	–	–	–	w	w	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	±	–	–	–	–	Bw6	21 cells
Bw63	–	–	–	–	–	–	+	+	±	+	+	+	+	–	–	–	–	±	–	–	–	–	–	+	+	+	Bw4	7 cells
BU	–	–	–	–	–	–	–	–	–	±	–	–	–	+	+	+	+	+	+	±	+	+	±	–	±	–	Bw6	4 cells
SV	–	–	–	–	–	–	–	–	–	±	–	–	–	+	+	+	+	±	+	+	–	–	±	–	±	–	Bw6	16 cells

SHORT Bw41

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Tibensky *et al.*[1] reported a variant of Bw41 in a family of East European ancestry. We report here a similar reaction pattern in an Asian Indian. The key serum (9w241) was negative as well as sera 9w380, 381 and 386 as shown in Figure 1. The other Bw41 cells were from a Negro family.

The split of Bw60 reported by Chiewsilp and Sujirachato[2] was not seen in the Asian Indians we tested and the difference between Bw61 and Bw47 was clear.

REFERENCES

1 Tibensky, D., Morochove, L. and Mervart, H. Possible variant of Bw41. Ninth IHW Newsletter III p26 1983.

2 Chiewsilp, P. and Sujirachato, K. B40 (Bw60, 61), 48, 41, 47. Ninth IHW Newsletter II 1983.

Figure 1. Reaction pattern with B40 and related antigens

Ninth Workshop Sera

	360	361	362	363	365	370	353	355	356	357	359	368	369	372	373	374	376	377	378	380	381	384	386	346	348	350	352	241	243	244		
Bw60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	Bw6	14 cells
Bw61	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	Bw6	33 cells
Bw41	+	–	–	–	–	–	–	–	–	–	±	–	–	–	–	–	+	–	±	+	+	+	+	–	–	–	–	+	–	–	Bw6	3 cells
Bw41S	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	+	–	–	–	–	–	–	–	–	Bw6	1 cell
Bw47	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–	Bw4	5 cells
B13	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	+	+	+	+	–	+	–	Bw4	6 cells
B27	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	+	Bw4	9 cells

HLA IN ASIA - OCEANIA

1986

PROCEEDINGS OF THE THIRD ASIA-OCEANIA HISTOCOMPATIBILITY WORKSHOP AND CONFERENCE

Held in Sapporo, Japan
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ANTIGEN REPORT : HLA A3

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The HLA A3 antigen was well defined with the four sera listed in Table 1.

The pattern analysis shows that there were very few extra reactions with any of these sera and the lower Q-scores for sera 100 and 803 were caused by missed reactions. Serum 100 was a weak serum with only 60% 8+ reactions but serum 803 (a monoclonal antibody) had 88% strong reactions.

The frequency of A3 ranges from 25.0% in the West (Caucasians) to about 2% in the East (Chinese and Japanese). The exceptions to this trend are the African Blacks (13%) and the New Zealand Maoris (12%). The distribution is shown in the map.

There was linkage disequilibrium between A3 and B7 in Northern Chinese, Malays and Maoris as well as in Caucasian populations.

The Japanese and Koreans had A3,B44 while the A3,B8 haplotype was found in Koreans, Chinese in Thailand and African Blacks.

TABLE 1 HLA-A3 antisera

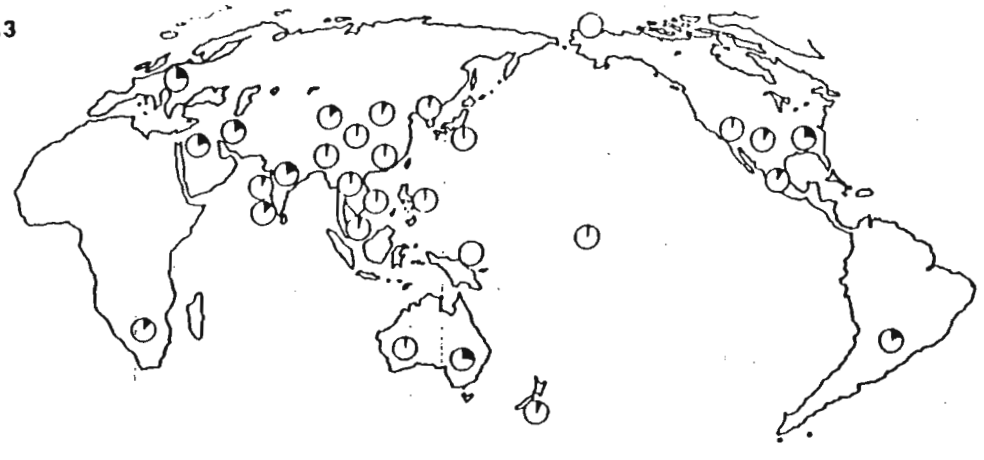
Key sera	Strength	r	Q-score
3AO 103	0.94	0.93	8.0
3AO 102	0.93	0.90	7.5
3AO 803	0.88	0.75	4.2
3AO 100	0.61	0.72	4.2

A3

HLA	SERUM	A R +/+	A R +/-	A R -/+	A R -/-	QS	R	SI	INCLUDING
A3	AOH102	76	2	9	1239	8.326	0.929	0.929	
	AOH103	78	0	10	1237	10.036	0.938	0.943	
	AOH803	54	21	4	1233	4.736	0.810	0.879	
	AOH100	49	26	12	1183	4.093	0.709	0.672	

A3	
AOH102	
AOH103	
AOH803	
AOH100	

A3



Antigen Report : HLA A11

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HLA A11 was very well defined by three key sera, 025, 028 and 022 and several other sera also reacted with A11 cells (see Table 1).

One of the monoclonal sera (809) together with serum 038 was reported by Chandanayingyong and Bejrachandra in a pre-workshop newsletter (Minipaper No. 5) as a short A11 in Thais. The same reaction pattern in southern Chinese was described by Hawkins at a symposium on HLA typing in Chinese. Although about 50% of positive reactions with serum 414 were with HLA A11 cells, Zhao (this volume) reported that absorption studies showed that this serum did not contain A11 antibodies but recognised an antigen CSH2 which was in strong linkage disequilibrium with A11.

The distribution of A11 is shown in the map. The highest frequencies (40-58%) are found in southern China, Phillipines, Thailand, Malaya and Nepal. The frequencies decrease westward to European Caucasians (12%), and eastward to Japan (16%). Australian aborigines have a frequency of 18% but A11 is absent from African blacks so that the frequency of A11 in American blacks can be used to measure the amount of admixture with North American Caucasians.

Linkage disequilibrium between A11 and B5 was significant in all Caucasoid populations as far as Nepal and also in Phillipinos. The southern Chinese, Koreans and Thais had the A11,B15 haplotype.

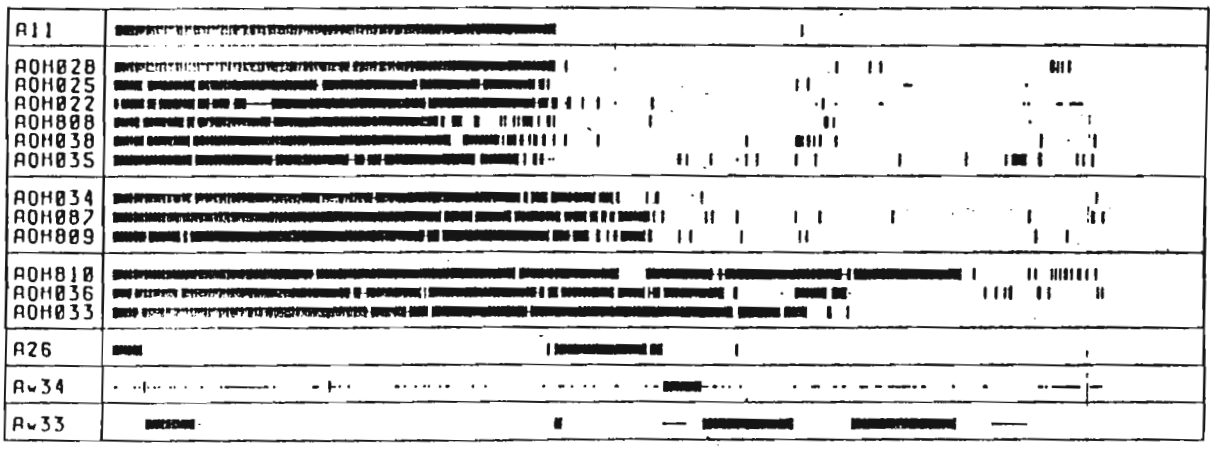
TABLE 1 HLA A11 antisera

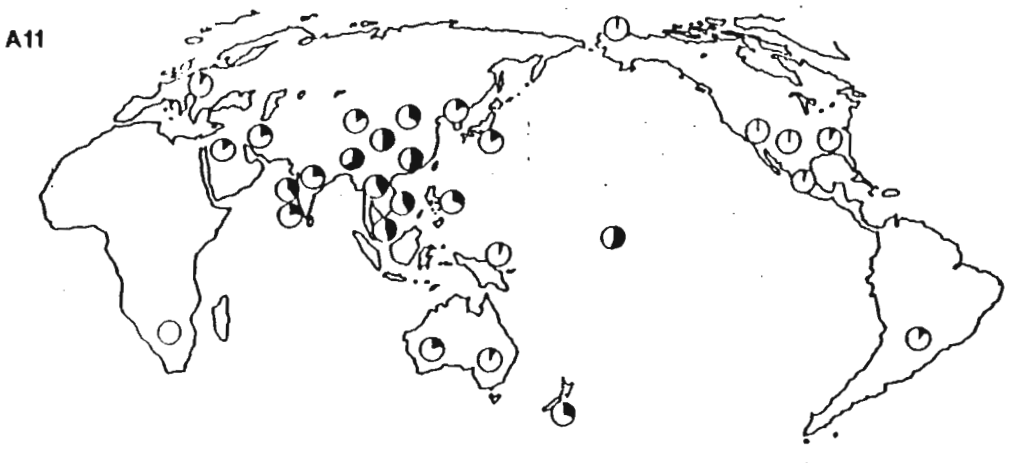
Key sera	Strength	r	Q-score	Remarks
3AO 025	0.93	0.95	7.7	
3AO 028	0.97	0.94	7.3	
3AO 022	0.94	0.93	7.0	
Other sera				
3AO 808	0.89	0.84	4.8	Monoclonal
3AO 035	0.84	0.84	4.6	
3AO 034	0.88	0.79	4.3	A26
3AO 087	0.88	0.78	4.2	A10
Possible split				
3AO 809	0.87	0.80	4.4	A26 Monoclonal
3AO 038	0.90	0.87	5.2	Weak A1

Multispecific sera 033, 036, 117, 810 also reacted with A11.

A11

HLA	SERUM	A R +/+	A R +/-	A R -/+	A R -/-	QS	R	SI	INCLUDING
A11	AOH028	332	4	26	962	8.046	0.942	0.961	
	AOH025	317	15	7	973	8.916	0.955	0.923	
	AOH022	310	6	28	933	7.439	0.931	0.929	
	AOH808	280	54	18	967	4.772	0.853	0.886	
	AOH038	310	26	54	935	5.249	0.846	0.863	
	AOH035	300	26	58	911	5.043	0.835	0.804	
A11,A26	AOH034	426	41	13	844	5.865	0.910	0.875	
	AOH087	441	27	36	821	6.007	0.896	0.862	
	AOH809	420	45	18	839	5.995	0.895	0.858	
A11,A10	AOH810	649	74	88	478	3.552	0.744	0.897	AW33,A28,BW57
	AOH036	577	74	49	597	4.003	0.811	0.872	A1, A3, B8
	AOH033	475	19	90	711	5.717	0.832	0.874	





NORTH INDIANS - ETHNIC STUDY

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INTRODUCTION:

India was predestined by its geographical structure to be one of the great breeding grounds of humanity. In the diversity of its natural conditions, it constitutes a whole world in itself. The people of India are largely the product of successive invasions that swept into this continent from times immemorial. Though the Indian population can be divided into various groups with different castes, languages, religion and tribes; broadly it could be classified as Dravidians and Aryans. The former were considered the original inhabitants of India who were driven Southwards following invasion by Aryans who crossed the Hindu Kush Mountains from the Northwest during second and third millenium B.C. This was followed by periodic intrusions by Moguls and Arabs between 12th and 16 Century A.D. and the Mongols thereafter. Historically, therefore, the Indian sub-continent constituted a cul-de-sac for different migratory racial groups who largely halted here and led to a considerable intermingling of culture and races,. Presently language rather than ethnic origin is the primary distinction between diverse Indian peoples, and terms such as Aryans or Dravidian have no significance when attached indiscriminately.

The Aryans who were mostly descendants of the Bronze age invaders introduced the major features of the Hindu religion to India and the framework of an elaborate caste system with its basic fourfold division into priests (Brahmins), warriors (Ksatriyas), tradesmen and cultivators (vaisya) and inferior craftsmen (Ksudras). By practising endogamy and observing strict dietry restrictions, they have preserved genetic continuity with their Aryan ancestors to a considerable extent, particulary in the upper castes; while the lower castes physically suggest in varying extents, the absorption of earlier Dravidians, particularly in skin colour. Thus, fairest skin is found in the Northwest India and Pakistan; the black element predominates in the Deccan (but does not present the hair and lips of the Negroid), yellow skins with high cheek bones live in the neighbourhood of Tibet, upper Burma and Eastern India.

The North Indians studied here are the descendants of Aryans. They are light to dark skinned people with dark hair and light eyes. In the present workshop. HLA data on North Indians was compiled from those of the native Indian inhabitants as well as from those settled abroad.

MATERIALS AND METHODS:

A total of 156 unrelated healthy individuals representing the North Indian Hindus were studied for the 3 AOH workshop. (Table 1). Care was taken to exclude any blood relatives and individuals belonging to South India.

Table 1: Source and composition of the data contributed

Lab/contributor	Number studied	Origin
BAL/Balakrishnan	44	North Indians settled in USA
Con/Contractor	18	Native inhabitants
HAM/Hammond	41	Noth Indians settled in South Africa.
VAI/Mehra	33	Native inhabitants
UND/Undevia	20	Native inhabitants

Data on 400 North Indians previously studied by us (Mehra et al, 1986) was also combined for analysis so as to have a reasonably larger sample size. This report, therefore, it based on 556 individuals (156+400) studied for HLA class I antigens and 275 individuals (141+134) for class II (DR and DQ) antigens.

RESULTS AND DISCUSSION:

The percent antigen and gene frequencies for HLA-A,B,C, DR and DQ alleles is represented in table II, III and IV. Most of the antigens detected in the north Indians are found in the European and North American caucasoids suggesting a close kinship of the two population groups. The antigens appearing with highest frequencies in the A locus are A1 (25.7%), A2(23.9%), A9(27.5%), A11(25.2%) and Aw19 (32.5%). In the A9 specificity, A23 was low while most of the split was that of A24. Similarly, the A10 antigen was represented almost exclusively by the A26 split. The genes for Aw34, Aw66 and Aw43 could not be detected in this population.

In the B locus, the most frequent antigens are B5 (28.2%), B35(27.1%) and B40 (22.3%) in that order. These frequencies are comparable to those reported amongst the western caucasoids. However, the most remarkable difference was concerning antigens B14 and B16. While the former was almost absent, the latter appeared with a significantly decresed frequency amongst the North Indians as compared to the European and North American caucasians. The only two individuals positive for B14 originated from South Africa and were ethnically muslims. It is interesting to note that B14 occurs with a significantly high frequency amongst the Parsis living in and aroung Bombay (reported in this workshop). A comparison of the frequencies of thise two antigens amongst various population groups around the world yields important results. Whereas B14 is absent or rare amongst the Mongoloids,Australian aborigines and most Asiatic populations, it occurs with a frequency of 3-19 % amongst the western caucasoids and Negroids. An almost reverse trend is seen for antigen B16. This antigen is absent or rare in negroes, Australian aborigines and scots whereas the caucasords and the Japanese present it with a frequency of 4-9%.

Another important point in this population is that concerning the split of 'broad' B locus specificities. Most of the split antigens detected in the North Indians are B44 for B12, B62 for B15, Bw50 for B21 and Bw61 for B40. Genes for Bw42, Bw46, Bw59, Bw70 and Bw71 were not detected. The HLA-C locus antigens in the present study showed an almost similar distribution as in European caucasoids except for Cw7 which was significantly more frequent amongst the North Indians.

In the DR locus, HLA-DR2 appears to be the most frequent allele in this population occuring with a frequency of 46.2% which is significantly much

higher than the value of 25.1 % reported amongst the European Caucasoids (Baur and Danilovs, 1980) Similarly DRw6 occurs much more significantly amongst the North Indians.

The most common haplotypes with significant positive and negative linkage disequilibria are given in tables V and VI respectively. Amongst these, A10-B8 and A26-B8 appear to be the characteristic haplotypes in the North Indians. The characteristic caucasion haplotype A1-B8 was not present amongst North Indians. Other more frequent haplotypes observed in this population are A1-B17, AW33-B44, A3-B7, A1-B37, B35-CW4, B27-CW2, B17-CW2, B18-DR5, B8-DR3, B17-DR7, DR3-DQw2, DR2-DQW1 most of which are common with the European and North American caucasions.

CONCLUSIONS: The populations of the Indian subcontinent are essentially caucasions. There is a complete lack of B14 and low prevalence of B16 antigens amongst North Indians. The most characteristic North Indian haplotypes are A10-B8 and A26-B8.

REFERENCES

Baur, MP and Danilovs, JA. Population analysis of HLA-A,B,C, DR and other genetic markers. In: Histocompatibility Testing 1980. ed Terasaki, PI. UCLA Tissue Typing Laboratory, Los Angeles, California, P955-993, 1980.

Mehra, NK, Taneja V, Kailash, S., Raizada N and Vaidya, MC. Distribution of HLA antigens in a sample of the North Indian Hindu population. Tissue Antigens. 27:64-76, 1986.

TABLE II : Percent Gene and antigen frequencies for HLA-A,B,C antigens in North Indians

HLA Antigens	3 AOHUC Study N=156		Published Data * N=400		Total North Indians N=556	
	AF	GF	AF	GF	AF	GF
HLA - A1	28.4	11.9	27.0	14.5	25.7	13.8
A2	27.5	14.8	22.5	11.9	23.9	12.8
A3	19.2	10.1	17.0	8.7	17.6	9.2
A11	23.7	12.6	25.7	13.8	25.2	13.5
A9	28.8	15.6	27.0	14.5	27.5	14.8
A23	1.9	0.9	-	-	-	-
A24	26.4	14.2	-	-	-	-
A10	7.6	3.9	10.5	5.4	9.7	4.9
A25	0.0	0.0	-	-	-	-
A26	7.0	3.5	-	-	-	-
AW34	0.8	0.4	-	-	-	-
AW66	0.0	0.0	-	-	-	-
A28	16.0	8.3	15.2	7.9	15.5	8.0
AW68	4.7	2.4	-	-	-	-
AW69	0.0	0.0	-	-	-	-
AW19	30.1	16.4	33.5	18.4	32.5	17.8
A29	3.2	1.6	4.2	2.1	3.9	1.9
A30	0.0	0.0	9.7	3.5	7.0	3.5
A31	4.4	2.2	4.2	2.1	4.3	2.1
A32	7.6	3.9	9.7	5.0	9.2	4.7
AW33	16.0	8.3	4.0	2.0	7.4	3.8
AW36	1.3	0.6	-	-	-	-
AW43	0.0	0.0	-	-	-	-
AX	-	5.4		4.7		5.2
HLA - B5	25.0	13.4	29.5	16.0	28.2	15.2
B51	8.9	4.5	-	-	-	-
B52	16.6	9.7	-	-	-	-
B7	15.3	8.0	12.7	6.5	13.4	6.9
B8	8.9	4.5	8.7	4.4	8.8	4.5
B12	12.8	6.6	17.2	9.0	16.0	8.3
B44	12.8	6.6	14.5	7.5	14.0	7.2
B45	0.0	0.0	2.7	1.3	1.9	0.9
B13	1.2	0.6	8.0	4.0	6.1	3.1
B14	1.2	0.6	0.0	0.0	0.3	0.1
BW64	0.0	0.0	-	-	-	-
BW65	0.0	0.0	-	-	-	-
B15	17.3	9.6	13.5	6.9	14.5	7.5
BW62	13.4	6.9	10.5	5.4	11.3	5.8
BW63	3.6	1.8	3.0	1.5	3.0	1.5
B16	2.5	1.2	2.2	1.1	2.3	1.1
B38	0.7	0.3	1.2	0.6	1.0	0.5
B39	1.7	0.9	1.0	0.5	1.2	0.6
B17	16.0	8.3	15.0	7.8	15.2	7.9
BW57	7.7	3.9	9.7	5.0	9.2	4.7
BW58	6.5	3.3	4.0	2.0	7.4	3.8
B18	4.4	2.2	4.5	2.2	4.4	2.2
B21	8.3	4.2	6.5	3.3	7.0	3.5
B49	2.1	1.0	1.5	0.7	1.6	0.8
BW50	7.3	3.7	-	-	-	-
BW22	6.4	3.2	-	-	-	-
BW54	0.0	0.0	-	-	-	-
BW55	5.1	2.6	-	-	-	-
BW56	1.2	0.6	-	-	-	-
B27	5.1	2.6	6.0	3.0	5.7	2.9
B35	27.5	14.8	27.0	14.5	27.1	14.6
B37	1.9	0.9	4.7	2.4	3.9	1.9
B40	20.5	10.8	23.0	12.2	22.3	11.8
BW60	4.4	2.2	-	-	-	-
BW61	16.0	8.3	-	-	-	-
BW41	0.0	0.0	0.2	0.1	0.17	0.08
BW42	0.0	0.0	0.2	0.1	0.17	0.08
BW46	0.0	0.0	-	-	-	-
BW47	1.4	0.7	-	-	-	-
BW48	0.7	0.3	-	-	-	-
BW53	1.2	0.6	-	-	-	-
BW59	0.0	0.0	-	-	-	-
BW67	0.0	0.0	-	-	-	-
BW70	1.6	0.8	-	-	-	-
BW71	0.0	0.0	-	-	-	-
BW72	0.0	0.0	-	-	-	-
BW73	0.0	0.0	-	-	-	-
BX		5.7		3.6		5.3
HLA - CW1	6.4	3.2	4.0	2.0	4.6	2.3
CW2	14.1	7.3	4.0	2.0	6.8	3.4
CW3	16.0	8.3	16.2	8.4	16.1	8.4
CW4	25.0	13.4	17.0	8.9	19.2	10.1
CW5	3.2	1.6	0.5	0.2	1.2	0.6
CW6	8.9	4.5	3.7	1.8	5.2	2.6
CW7	22.4	11.9	-	-	-	-
CW8	0.8	0.4	-	-	-	-
CX		49.1		76.7		72.6

AF = Antigen Frequency GF = Gene Frequency * Mehra etal, 1986

Table III: Percent gene and antigen frequencies in North Indians for HLA - DR Locus antigens.

Antigens	3 AOHWC Study N=141		Published Data * N=134		Total North Indians N=275	
	AF	GF	AF	GF	AF	GF
HLA - DR1	7.9	3.9	14.1	7.3	10.9	5.6
DR2	45.3	26.1	47.0	27.2	46.2	26.6
DR3	27.6	14.9	26.1	14.0	26.9	14.5
DR4	9.9	5.0	26.1	14.0	17.8	9.3
DR5	22.7	12.0	23.1	12.3	22.9	12.2
DRW11	0.0	0.0	-	-	-	-
DRW12	1.0	0.5	-	-		-
DRW6	18.4	9.6	17.9	9.4	18.2	9.5
DRW13	5.4	2.7	-	-	-	-
DRW14	0.7	0.3	-	-	-	-
DR7	23.4	12.4	22.3	11.8	22.9	12.2
DRW8	10.6	5.4	0.7	0.3	5.8	2.9
DRW9	1.4	0.7	2.9	1.5	2.2	1.1
DRW10	7.0	3.6	2.2	1.1	4.7	2.4
DRX		5.8		1.1		4.6

AF=Antigen Frequency GF=Gene Frequency * Mehra et al , 1986

Table IV: Percent Gene and antigen frequencies in North Indians for HLA - DQ Locus antigens.

Antigens	Number	Number Positive	AF	GF
HLA -DRW52	141	93	65.9	41.6 ± 2.1
DRW533	141	51	36.1	20.1 ± 1.7
DQW1	141	101	71.6	46.7 ± 2.1
DQW2	141	32	22.7	12.0 ± 1.4
DQW3	141	56	39.7	22.3 ± 1.7
DQWA	73	0	0.0	0.0
TA10	91	25	27.4	14.8 ± 1.8

AF=Antigen frequencies GF=Gene frequencies

Table V: Positive linkage disequilibrium (Δ) between HLA-loci A,b,c and DR in North Indians (per 10^4).

Haplotype (556)	Δ	X^2	H.F.	Haplotype (275)	Δ	X^2	H.F.
Loci A and B				Loci A and DR			
A10-B8	179	80.6	202	Aw32-DRw10	72	9.1	85
A26-B8	149	69.8	166	A24-DRw9	60	6.8	71
A19-B12	229	22.2	375	Aw33-DRw53	136	8.7	191
A19-B44	209	21.1	337	Loci B and DR			
A30-B13	50	14.8	58	B18-DR5	143	16.8	175
A23-Bw57	17	13.8	17	B17-DR7	225	16.1	313
Aw33-B44	17	13.8	17	Bw63-DRw10	50	14.3	53
A1-B17	140	10.7	248	B8-DR3	146	10.6	199
A1-B37	75	10.5	104	B44-DR7	160	8.9	237
A3-B7	105	9.5	169	B52-DRw53	124	13.5	153
Aw33-B12	76	9.4	107	B52-DRw52	171	14.3	227
A28-Bw63	45	8.0	56	Bw57-DRw53	85	9.1	103
A23-B17	29	6.7	34	B17-DRw53	146	7.8	218
A1-Bw63	52	6.5	72	Bw58-DRw53	72	7.8	86
Loci B and C				Bw61-DRw53	116	7.7	160
B35-Cw4	850	82.2	1183	Loci B and DQ			
Bw57-Cw2	399	71.4	431	Bw61-DQw1	222	13.6	308
B52-Cw8	839	67.2	934	Bw52-DQw2	120	11.4	139
Bw58-Cw3.1	563	61.6	603	Bw57-DQw3	357	11.1	403
Bw61-Cw5	721	54.7	796	Bw52-DQw2	95	9.5	106
Bw55-Cw1	344	51.7	366	Loci C and DQ			
Bw22-Cw1	376	49.6	410	Cw8-DQw3	110	8.1	235
B51-Cw7	824	28.1	934	Cw7-DQw3	107	5.9	258
Bw60-Cw8	813	21.6	904	Loci DR and DQ			
B52-Cw5	709	20.2	786	DR3-DQw2	46.21	20.68	618.23
B17-Cw3	438	19.2	575	DR2-DQw1	948.02	19.83	1988.3
B27-Cw2	381	16.8	426	DRw53-DQw3	381.04	9.51	615.76
B17-Cw2	394	15.9	473	DR5-TA10	183.04	5.02	276.60
Bw60-Cw5	690	14.9	757				
B5-Cw4	267	8.3	592				
Bw61-Cw2	357	7.0	391				
B35-Cw2	203	6.5	329				
Bw60-Cw6	678	6.1	757				

*Only haplotypes with X^2 more than 5 have been considered.

Table VI: Negative linkage disequilibrium (Δ) between HLA-loci A,B,C, and DR in North Indians (per 10^4)

Haplotype	Δ	X^2	H.F.	Haplotype	Δ	X^2	H.F.
Loci A and B				Loci A and DR			
A1-B7	-103	6.1	-7	Aw36-DRw9	-0.3	13.1	-0.2
A11-B17	-93	4.4	14	A11-DRw7	-88	5.8	-25
				A3-DR2	-177	4.5	14
				Loci B and DR			
				B13-DRw52	-112	6.2	-63
				B45-DRw9	-0.7	6.0	-0.39
				Loci A and DQ			
				A2-DQw3	-139	4.0	-17
				B13-DQw1	-77	5.8	-23

HLA ANTIGENS IN AFRICAN BLACKS

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We tested 160 African Blacks with the 3AOH workshop serum set. They were randomly selected blood donors and staff and were all Zulus. Only 134 were tested for B lymphocyte antigens.

Table 1 shows the frequencies at the A locus for this workshop and compares them with the frequencies obtained from previous workshops and with local assignments. The frequencies were similar except for Aw30 and 31 which were difficult to define with 3AO sera. HLA A11 is virtually absent from African Black populations. We have only found one cell with this antigen and thus the frequency is 0.05%.

At the B locus the frequencies found for this workshop were similar to previous workshops. The antigens Bw22, B37 and Bw52 are absent or extremely rare in African Blacks (Table 2). We were surprised to find three random cells positive for HLA B27 and analysed all B27 Black cells as shown in Table 3. This shows that the frequency of 1.9% for this workshop was a chance event and the true frequency of HLA B27 in African Blacks is 0.4%.

Cw2 could not be defined with 3AO sera but Cw1, Cw3 and Cw4 were as expected (Table 4). The other C-locus antigens were difficult to define because of the variations between antisera.

The frequencies of DR1 and DR7 were increased for this workshop but these antigens are well defined in both this and previous workshops. Unfortunately, DQ2 could not be defined with the workshop serum set but the frequencies of DQ antigens in African Blacks are shown in Table 5.

Haplotypes showing significant linkage disequilibrium are shown in Table 6. The A and B locus haplotypes are distinctive and typical of African Blacks, e.g. Aw30, Bw42; A1, B7; A3, B8 but the associations of B locus and DR locus antigens are also found in other races, e.g. B7, DR2 and B8, DR3.

TABLE 1 SOUTH AFRICAN BLACKS

HLA	3AO N = 160	Other WS + Local 1707	Total 1867
A1	10,6	6,9	7,2
A2	23,1	21,9	22,0
A3	13,8	12,7	12,8
A11	0	0,06	0,05
A23	20,6	18,5	18,6
A24	3,8	5,0	4,9
A25	0	0,5	0,4
A26	13,1	11,0	11,7
A28	21,3	21,0	21,0
A29	12,5	16,5	16,1
Aw30	23,1	36,0	34,9
Aw31	10,6	5,4	5,8
Aw32	0,6	2,1	2,0
Aw33	4,4	2,3	2,5
Aw34	11,3	13,6	13,4
One ant.	31,3	26,7	26,8

TABLE 2 SOUTH AFRICAN BLACKS

HLA	3AO N = 160	Other WS + Local 1707	Total 1867
B7	27,5	21,4	21,9
B8	13,1	13,0	13,0
B13	2,5	3,7	3,6
B14	4,4	5,7	5,6
B15	3,8	3,7	3,7
B16	6,3	3,3	3,6
B17	38,8	38,4	38,5
B18	5,6	5,4	5,4
B21	2,5	1,8	1,8
Bw22	0	0,06	0,05
B27	1,9	0,3	0,4
B35	5,0	7,0	7,0
B37	0,6	0,06	0,1
B40	0	0,5	0,5
Bw41	0	1,6	1,4
Bw42	13,8	22,1	21,2
B44	13,1	15,8	15,6
B45	6,9	8,6	8,4
Bw47/48	0,6	0,06	0,1
Bw51	2,5	1,1	1,2
Bw52	0	0	0
Bw53	1,9	1,4	1,4
Bw70	25,6	17,1	17,8
One ant.	23,8	28,0	27,2

TABLE 3 HLA B27 IN SOUTH AFRICAN BLACKS

	Pos	N	Percentage
Random	8	1867	0,43
Rheum. arthritis	1	172	0,58
Cancer	2	732	0,27
Heart disease	2	264	0,76
Choriocarcinoma	1	90	1,11
Hyperimmune	2	153	1,31
Renal disease	1	186	0,54
Tuberculosis	3	509	0,59
Schistosomiasis	1	194	0,52
Thyroid disease	1	112	0,89
Diabetes	0	176	0,0
Other	1	631	0,16
	15	3219	0,47
Ankylosing spondylitis	7	27	25,9

TABLE 4 SOUTH AFRICAN BLACKS

HLA-C	3AO N = 160	Other WS + Local 1707	Total 1867
Cw1	0,6	0,3	0,3
Cw2	NT	17,6	-
Cw3	8,1	11,3	11,0
Cw4	15,6	11,7	12,0

TABLE 5 SOUTH AFRICAN BLACKS

HLA DR	3AO 134	Other WS + Local 275	Total 409
DR1	10,4	2,2	4,4
DR2	21,6	25,5	24,0
DR3	38,8	34,6	35,8
DR4	6,7	11,6	9,9
DR5	29,1	30,9	31,0
DRw6	11,2	17,1	16,6
DR7	23,9	12,4	17,4
DR8	7,5	2,2	5,4
DR9	1,5	0,4	0,4
DR10	1,5	2,6	2,7
	134	128	262
DRw52	75,4	73,4	74,4
DRw53	25,4	35,2	30,2
	134	64	198
DQ1	53,0	68,8	58,1
DQ2	NT	23,4	-
DQ3	29,1	31,3	29,8

TABLE 6 SOUTH AFRICAN BLACKS

HAPLOTYPES WITH SIGNIFICANT DELTA VALUES

N = 1867

	Freq./1000	Δ/SE
Aw30 - Bw42	62	7,9
A1 - B7	24	6,8
A24 - B7	18	6,3
A29 - B44	26	6,0
A25 - B44	24	5,9
A29 - B13	9	4,1
A2 - B45	16	4,0
A3 - B8	13	3,3
A28 - B14	10	3,1

N - 413

B7 - DR2	58	4,2
B8 - DR3	32	2,2
B17 - DR7	38	1,9
Bw42 - DR3	59	4,1
Bw70 - DR5	38	1,5

Bo Dupont
Editor

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2. Schreuder I, Bos A, van de Berg-Loonen E. SV=PR: A new B-locus antigen discovered. In: Bodmer WF, Batchelor JR, Bodmer JG, Festenstein H, Morris PJ (eds): Histocompatibility Testing 1977. Munksgaard, Copenhagen, 1977, p 408.
3. Schreuder I, Nieman HG, Laundy GJ, Entwistle CC. Bu and SV: Two closely related B-locus specificities. Tissue Antigens 1980;16:169.
4. Hall PJ, Levin AG, Entwistle CC, Knight SC, Wasunna A, Brubacker G. B15 heterogeneity in East African blacks. Tissue Antigens 1980;16:326.
5. Hall PJ, Levin AG, Entwistle CC, Knight SC, Wasunna A, Kung'n A, Brubacker G. HLA antigens in African black patients with Burkitt's lymphoma or nasopharyngeal carcinoma and in controls. A pilot study. Hum Immunol 1982;5:91.
6. Entwistle CC, Laundy GJ. 8w58: Joint report. In: Terasaki PI (ed): Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, 1980, p 475.
7. Laundy GJ, Raffoux C, Schreuder GMT, Klouda PT. Antigen Report: HLA-Bw70, HLA-Bw71 and HLA-Bw72. In: Albert ED, Baur MP, Mayr WR (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, 1984, p 173.
8. Guttridge MG, Laundy GJ, Klouda PT. 1988. Biochemical variants of the Bw70 antigen (Bw71, Bw72). In: Dupont B (ed): Immunobiology of HLA, Volume I: Histocompatibility Testing 1987. New York: Springer-Verlag, 1989.

Antigen Society #9 Report (Bw46 and the Subgroups of B15)

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History

The antigen HLA-B15, first described as LND (Thorsby et al. 1970 (29)) or TE15 (Albert et al. 1970 (1)), was first recognized to be heterogeneous at the Fourth International Workshop (Thorsby et al. 1970b (30)). In the following Workshops, a growing number of reports documented the heterogeneity of B15 (Richiardi et al. 1974 (23), Joysey et al. 1975 (18), Dick et al. 1978 (15), Singal et al. 1980 (27), Danilovs and Pollock 1980 (13), Saueracker et al. 1981 (24), Alonso et al. 1983 (2), Zhao and Shiraki 1986 (33)). It was becoming clear that most of the variants of B15 are found in Southeast Asian populations. During the Ninth International Histocompatibility Workshop, the complexity of B15 was discussed in nine different Newsletter contributions (3,6,9,11,12, 14,17,25,33) and summarized by Chandanayingyong et al. (10) and Cambon-Thomsen et al. (5). From this summary it appears that next to the classical Bw62 antigen there exists a Bw6-associated short Bw62 antigen, which has been observed by several different authors (6,9,11 17,25,34) mostly in Asian populations, and named Bw62.1, TS1, B15short Thai, B15 Kemp, SH7, Bw62S. This antigen is characterized by cross-reactions with B35 sera. There was evidence for an even shorter split of B15, which is also Bw6-associated and which was named B15.3 or B15SL1. Two further antigens described B15SAU and B15G (4) may or may not be equivalent with B15.3. In the Thai population, there exists one further split of B15, named B15S, with a characteristic cross-reaction with anti-B45 sera (9). Among the Bw4-associated splits of B15, there is, in addition to the classical Bw63, an antigen found in Negroid populations and named 8w66 (31), which has a shorter reaction pattern than Bw63. One further split of B15 with a clearly shorter reaction pattern than Bw63 occurs in the Thai population and is named B15T (9).

B15 Serology X. International Histocompatibility Workshop

Using Core sera and Antigen Society sera, seven different subtypes of B15 could be identified. The reaction patterns and the key antisera for the definition of the various subtypes are given in Tables 1 and 2. It is quite likely that several more subtypes of B15 do exist; however, in the absence of family segregation data it was difficult to assess slight differences in reactivity.

Antigen Bw62. This antigen is by far the most common subtype of B15. It is characterized by association with Bw6 and it can be very well defined using a large number of antisera, of which only the best examples were chosen for the reaction patterns given in Tables 1 and 2. There are many antisera reacting with all B15 subtypes, but only two antisera (nos. 250 and 252) distinguish Bw62 and Bw76 from the rest of the B15 group.

Antigen Bw75 (Equivalents: Bw62.1, TS1, B15short Thai, B15 Kemp, SH7, Bw62S). This antigen was already clearly defined in the Ninth International Histocompatibility Workshop (10) as a short, Bw6-associated variant of Bw62, which is characterized by a cross-reactivity with B35 (i.e., in the presence of Bw75 and the absence of B35 and Bw53 some of the anti-B35, Bw53 antisera react positively). Among the cells, whose reaction pattern corresponds closely to the Bw75 pattern given in Table 1, we have found the following codings:

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Participating Laboratories: ANZCRS, ANZTAT, BENBER, SAFDUT, UKIAST, UKIBRS, UKIFES, US7POL, NCYMRV

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Table 1. Reaction Pattern of B15 Antisera from the Core Serum Set

Specificities	All B15	Bw62	Bw62 Bw75 Bw76	Bw76 B45	B35 Bw63 Bw75	B35 Bw75 Bw70	B51 Bw53 B49 Bw77	Bw63 Bw57 Bw58			
WS-Sera No.	2 2 2 3 4 1 8 2 6	2 2 5 5 2 0	2 2 2 4 3 5 8 0 6	4 4 2 3 8 2	2 2 2 1 2 1 0 5 9 0 2 8 5 2 5	2 2 2 3 3 2 3 4 8	2 2 2 1 2 2 2 6 2 3 5 6	2 2 4 6 6 4 6 7 8	2 1 0 5	Bw4	Bw6
Bw62	+ + +	+ +	+ + −	− −	− − − − −	+ + +	− − − −	− − −	−	−	+
Bw75	+ + +	− −	+ + −	− −	+ + + + +	+ + +	− − − −	− − −	+	−	+
Bw76	+ + +	+ +	+ + +	+ +	− − − − −	− − −	− − − −	− − −	+	−	+
B15.3	+ + +	− −	− − −	− −	− − − − −	+ + +	− − − −	− − −	−	−	+
Bw77	+ + +	− −	− − −	− −	+ + + − −	− − −	+ + + +	− − −	+	+	−
Bw63	+ + +	− −	− − −	− −	− − − − −	− − −	− − − −	+ + +	−	+	−
Bw63.1	+ + +	− −	− − −	− −	− − − − −	− − −	+ + + +	+ + +	−	+	−

Table 2. Reaction Patterns of B15 on Tenth Workshop Antigen Society No. 9 Sera Set

	Specificities of Antisera:																		
Antigen	w77 w53 w63 9275	w77 w63 15.3 9303	w77 9289	w77 w53 15.3 w75 B35 9288	w77 w53 9293	w62 w63 w57 9310	w46 A31 B15 4508	w75 w77 9314	w76 15.3 w75 4507	all B15 9306	w62 w76 4493	w62 w76 9313	w62 w76 4487	w71 B35 w75 9241	w76 w75 9235	w76 9245	w76 B44 9274	Bw4	Bw6
Bw62	−	−	−	−	−	+	+	+	+	+	−	+	w	−	−	−	−	−	+
Bw75	−	−	−	+	−	+	+	+	+	+	−	−	−	+	+	−	−	−	+
Bw76	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Bw63	+	+	−	−	+	+	+	+	+	+	−	−	−	−	−	−	+	+	−
Bw77	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−

Bw62, Bw62.1, B15Sw6, B15, TE79, B15K. As all four cells coded TE79 and all 13 cells coded B15K show a reaction pattern that is very similar to that of Bw75, it is possible that one should add TE79 and B15K to the list of equivalents for Bw75. For Te79 this is also borne out by the fact that the three antisera (nos. 215, 216, and 221) submitted as anti-Te79 react with all cells positive for Bw75. For the specificity called B15SL1, there is different coding in different laboratories. The cells from Dr. Zhao in Shanghai, which have been called B15SL1, correspond exactly to the Bw75 reaction pattern, while one cell from Dr. Festenstein's laboratory has a different reaction pattern. Also in the Ninth International Histocompatibility Workshop, B15SL1 showed a reaction pattern clearly different from that of Bw75 (then Bw62.1) (10). Bw75 is found mostly in Chinese, Thais, and other Southeast Asian populations and only very occasionally in cells coded as Caucasian. The relatively frequent occurrence of this antigen in the Cape colored population of South Africa probably reflects the contribution of Southeast Asian genes to the gene pool in this population.

Antigen Bw76 (Equivalent: B15S (Siamese)). This Bw6-associated split of B15 was first described during the Ninth International Histocompatibility Workshop (9). It is characterized by a strong cross-reactivity with anti-B45 sera. This antigen seems to be restricted to the Southeast Asian populations. The characteristic reaction pattern for Bw76 is given in Tables 1 and 2.

Antigen B15.3. This designation characterizes a Bw6-associated antigen with a short Bw62 reaction pattern. It has been noted that in this workshop there was an inconsistent use of this designation, as cells belonging to clearly distinct subtypes of B15 were called B15.3. Among the cells coded as B15.3, there is a group characterized by a short Bw62 reaction pattern (see Tables 1 and 2), with reactivity with long B35/Bw62/Bw70 antisera. It must be stressed however, that this specificity is ill-defined and there is considerable indication for the existence of several more Bw6-associated short variants of B15.

Antigen Bw63. This antigen is characterized by its association with Bw4 and by a short reaction pattern (see Tables 1 and 2). Reactivity with Bw63 cells is frequently found in anti-Bw57 and anti-Bw58 sera. There appears to be a variant of Bw63, tentatively called Bw63.1, found in Negroid populations which reacts, in addition to the Bw63 typical pattern, with antisera directed against B51, Bw53, and B49. This antigen may be identical to the specificity 8w66 (20,21). Unfortunately, there were too few cells coded for this specificity in order to determine identity or non-identity with what has been called Bw63.1.

Antigen Bw77 (Equivalent B15T). This antigen was first described in the Ninth International Histocompatibility Workshop in the Thai population (9). It is associated with Bw4 and characterized by a short B15 reaction pattern and cross-reactivity with antisera containing anti-Bw53 activity (see Tables 1 and 2).

Table 3. Reaction Pattern of Bw46 on the Tenth Workshop Core Sera Set

Specificities	Bw46								Bw6					
WS Sera No	263	257	258	261	262	9246	9250	9251	500	507	501	499	504	216
Bw46	+	+	+	+	+	+	+	+	+	+	+	−	−	−

Antigen Bw46. This antigen does not belong to the B15 complex even though in almost all long B15 antisera, anti-Bw46 activity can be detected. The antigen occurs almost exclusively in Chinese, Japanese, Thais, or other Southeast Asians. Bw46 is strongly associated with Cw11 and DRw8 among Japanese and DR9 among Chinese. At the Ninth International Histocompatibility Workshop, there was a suspicion of a split of Bw46 (7) that could not be substantiated in the Third Asia-Oceania Histocompatibility Workshop (8).

In this Workshop, Bw46 was very well defined with three narrow antisera (263, 257, and 258) and two broad sera (261 and 262) as well as with three broad sera from the Antigen Society set (nos. 9246, 9250, and 9251). With anti-serum 258, there was some suspicion of a mixup, as apparently this serum was a perfect anti-Bw46 in some laboratories and completely negative in other laboratories (Kennedy et al., Newsletter No. 1 (19)). In cells that possess in addition to Bw46 a Bw4-associated B-locus antigen, it has been observed that there is a short reaction pattern for Bw6 as given in Table 3. This may be in accordance with the finding from DNA sequencing of the Bw47 gene that the Bw46 gene carries in the position covering aminoacid 79-83, which is responsible for the Bw6/Bw4 variability—a sequence coming from a Cw3 gene (Parham et al., this volume (22)).

Computer Cell Typing

Using the computer cell-typing procedure developed for the Ninth International Histocompatibility Workshop (26), HLA-A,B,C computer cell-typing was performed on the basis of local assignments. For the antigen Bw75, cells that were coded as B15SL1 or SH7 and corresponded in their reaction pattern to the reaction pattern of Bw75 were recoded as Bw75. As is shown in Table 4 for the antigens discussed in this report, there is a very high percentage of cells for which the local assignment and the computer assignment is identical. It can be seen that the two least well defined and probably heterogeneous antigen groups, Bw63 and B15.3, show the lowest R value between local and computer assignment.

Table 4. Computer-Cell Typing and Correlation with Local Assignment

Antigen	No. Cells Tested	Lab + Prog. +	Lab + Prog. −	Lab − Prog. +	R value
Bw46	738	110	10	8	0.91
Bw62	738	169	17	11	0.90
Bw63	738	16	5	14	0.63
Bw75	562	55	2	6	0.93
Bw76	562	18	0	0	1.00
Bw77	717	10	1	1	0.91
B15.3	562	9	2	2	0.82

Listing of Sera Typing Information of All Sera Relevant to the Antigens of the Antigen Society and of all Antigen Society Sera

Sera typing was performed using the procedure and the format developed for the Ninth International Histocompatibility Workshop. The definition of most of the B15 subgroups is dependent on the reactions of many broad antisera and therefore it is important to investigate the inclusion of narrow specificities into the broad ones. For a restricted analysis, this is only possible if the narrow specificities are analyzed first. The sera typing of the relevant Core sera is given in Table 5 and those of the Antigen Society sera in Table 6.

T-Cell Defined Bw62 Variants

Information obtained from Dr. Beatty, Seattle, indicates that a cytolytic T-cell clone (HAN 4) and a proliferative T-cell clone LAY-1 recognize all core cell lines expressing Bw62. In addition, the T-cell clone HAN 4, when tested with Bw62 variants, appears to be recognizing subtypes, which are positive for the Workshop sera 250 and 252 (Bw62 and Bw76).

Correlation Between Biochemical Subtypes and Serology

Information from Dr. Choo, Seattle, shows that the biochemical variant B15.1 corresponds to Bw75, B15.2 corresponds to Bw76, and B15.3 corresponds to Bw62.

Table 5. Seratyping of Core Serology Sera Defining B15 and Its Subgroups

SERUM	ANTIGEN	NO. REAC	AVE	++	MISS +-	EXTR -+	--	PERCENTAGE STR	MISS	EXTR	INCL	R	CHI	QSCORE	QNORM	ANTIGEN
10W165	TRAY:	6 POS: 6			LOCAL SPECIFICITY: B5											
10W165	B51	530	7.9	39	0	73	418	76	0	65	100	0.54	157.1	7.73	0.85	B51
10W165	BW52	491	8.0	15	0	58	418	65	0	79	100	0.42	88.6	6.52	0.84	BW52
10W165	BW53	476	7.7	6	0	52	418	56	0	89	100	0.30	43.8	5.30	0.81	BW53
10W165	BW77	470	7.7	7	1	45	417	53	12	86	88	0.32	48.3	4.48	0.65	BW77
10W165	BW63	462	7.2	5	4	40	413	48	44	88	56	0.22	21.9	3.03	0.43	BW63
10W165	B15.3	453	6.0	4	3	36	410	47	42	90	58	0.21	20.6	2.75	0.41	B15.3
10W165	B18	446	6.7	17	14	19	396	50	45	52	55	0.47	98.2	2.47	0.29	B18
10W165	B35	415	6.4	11	30	8	366	42	73	42	27	0.35	51.6	0.74	0.08	B35
10W166	TRAY:	4 POS: 35			LOCAL SPECIFICITY: B5											
10W166	B51	524	8.0	40	0	59	425	84	0	59	100	0.60	185.9	7.35	0.81	B51
10W166	BW52	484	7.7	15	0	44	425	74	0	74	100	0.48	111.5	5.95	0.77	BW52
10W166	BW77	469	8.0	8	1	36	424	70	11	81	89	0.38	68.2	4.37	0.62	BW77
10W166	BW63	460	8.0	4	5	32	419	63	55	88	45	0.19	17.1	3.36	0.48	BW63
10W166	BW53	451	6.0	4	2	28	417	59	33	87	67	0.27	32.7	2.93	0.45	BW53
10W166	BW57	445	7.1	7	16	21	401	60	69	75	31	0.23	24.0	0.85	0.11	BW57
10W166	BW58	422	7.2	10	34	11	367	57	77	52	23	0.28	32.7	0.61	0.07	BW58
10W166	BW4	378	6.4	11	141	0	226	45	92	0	8	0.21	16.8	1.43	0.13	BW4
10W185	TRAY:	6 POS: 5			LOCAL SPECIFICITY: B51,BW52,BW53											
10W185	B51	529	8.0	40	0	33	456	83	0	45	100	0.71	270.3	9.15	0.98	B51
10W185	BW52	489	7.7	15	0	18	456	63	0	54	100	0.66	213.8	7.40	0.93	BW52
10W185	BW53	474	7.0	4	1	14	455	44	20	77	80	0.41	80.3	5.59	0.86	BW53
10W185	BW77	469	6.7	6	1	8	454	35	14	57	86	0.60	167.9	4.82	0.69	BW77
10W185	BW4	462	5.6	5	229	3	225	12	97	37	3	0.03	0.5	0.83	0.08	BW4
10W189	TRAY:	6 POS: 9			LOCAL SPECIFICITY: BW53,BW52,B51,B35											
10W189	BW77	532	7.5	11	1	112	408	52	8	91	92	0.25	32.5	5.18	0.72	BW77
10W189	BW75	520	6.8	42	18	70	390	49	30	62	70	0.43	94.3	3.44	0.37	BW75
10W189	B15.3	460	6.8	5	4	65	386	38	44	92	56	0.16	11.6	2.44	0.36	B15.3
10W189	B35	451	6.6	28	14	37	372	36	33	56	67	0.48	102.5	3.06	0.35	B35
10W189	B51	409	6.7	17	16	20	356	32	48	54	52	0.44	78.7	2.65	0.32	B51
10W192	TRAY:	6 POS: 11			LOCAL SPECIFICITY: BW53,B35											
10W192	B35	530	7.6	39	8	57	426	66	17	59	83	0.53	146.3	5.61	0.61	B35
10W192	BW75	483	7.0	42	13	15	413	57	23	26	77	0.72	248.6	4.36	0.47	BW75
10W198	TRAY:	6 POS: 23			LOCAL SPECIFICITY: BW71,B35,BW53,B15											
10W198	B35	531	7.6	47	0	125	359	63	0	72	100	0.45	107.6	8.15	0.92	B35
10W198	BW75	484	7.3	56	0	69	359	56	0	55	100	0.61	181.9	8.11	0.89	BW75
10W198	BW62	428	6.6	44	62	25	297	47	58	36	42	0.40	67.2	1.70	0.17	BW62
10W198	B51	322	6.7	6	24	19	273	44	80	76	20	0.15	6.9	1.22	0.15	B51
10W198	BW70	292	6.3	13	33	6	240	36	71	31	29	0.38	42.5	0.95	0.11	BW70
10W198	BW6	246	5.2	5	150	1	90	16	96	16	4	0.07	1.1	0.91	0.09	BW6

Table 5. *Continued*

10W200	TRAY:	6	POS: 22			LOCAL SPECIFICITY: B35,BW62,BW71,BW72									
10W200	BW75	532	7.9	60	0	166	306	69	0	73	100	0.41	91.6	6.96	0.78 BW75
10W200	B35	472	8.0	39	4	127	302	60	9	76	91	0.37	64.0	4.71	0.55 B35
10W200	BW62	429	6.6	60	47	67	255	48	43	52	57	0.33	47.9	2.23	0.23 BW62
10W200	BW58	322	6.5	23	20	44	235	47	46	65	54	0.32	32.2	1.74	0.21 BW58
10W200	BW70	279	6.7	22	18	22	217	50	45	50	55	0.44	54.1	1.58	0.19 BW70
10W200	BW57	239	6.4	5	11	17	206	54	68	77	32	0.20	10.0	1.09	0.15 BW57
10W200	B51	223	6.9	7	21	10	185	52	75	58	25	0.25	13.7	0.45	0.06 B51
10W200	BW6	195	6.8	10	138	0	47	50	93	0	7	0.13	3.3	0.51	0.10 BW6
10W202	TRAY:	6	POS: 24			LOCAL SPECIFICITY: BW72,BW62,B5,B35									
10W202	BW75	521	7.9	60	0	254	207	80	0	80	100	0.29	44.7	6.93	0.78 BW75
10W202	B35	461	7.9	43	0	211	207	77	0	83	100	0.29	38.6	6.58	0.78 B35
10W202	BW76	418	8.0	14	0	197	207	73	0	93	100	0.18	14.2	5.34	0.77 BW76
10W202	BW52	404	8.0	13	0	184	207	72	0	93	100	0.19	14.1	5.25	0.77 BW52
10W202	B51	391	8.0	34	1	150	206	70	2	81	98	0.31	38.7	5.69	0.70 B51
10W202	B18	356	7.5	24	1	126	205	63	4	84	96	0.30	32.0	4.80	0.62 B18
10W202	B15.3	331	8.0	6	1	120	204	60	14	95	86	0.14	6.9	3.42	0.57 B15.3
10W202	BW77	324	8.0	5	1	115	203	58	16	95	84	0.13	5.6	3.23	0.55 BW77
10W202	BW62	318	6.9	70	12	45	191	56	14	39	86	0.60	115.9	3.88	0.42 BW62
10W202	BW70	236	7.1	28	15	17	176	53	34	37	66	0.55	72.2	3.34	0.40 BW70
10W202	B37	193	7.0	4	5	13	171	35	55	76	45	0.28	14.9	1.41	0.22 B37
10W205	TRAY:	6	POS: 10			LOCAL SPECIFICITY: B35									
10W205	BW75	531	7.6	60	0	124	347	63	0	67	100	0.49	127.6	6.59	0.77 BW75
10W205	B35	471	7.7	42	1	82	346	54	2	66	98	0.51	124.2	5.52	0.68 B35
10W205	BW41	428	6.0	4	3	78	343	40	42	95	58	0.12	6.6	1.93	0.33 BW41
10W205	BW70	421	6.3	23	23	55	320	39	50	70	50	0.28	33.9	1.59	0.20 BW70
10W205	BW42	375	6.7	6	10	49	310	38	62	89	38	0.14	7.0	1.01	0.15 BW42
10W205	BW76	359	6.5	4	10	45	300	36	71	91	29	0.09	2.8	0.89	0.13 BW76
10W205	B39	345	6.8	5	15	40	285	37	75	88	25	0.09	2.7	0.57	0.08 B39
10W205	BW6	325	5.9	32	189	8	96	35	85	20	15	0.10	3.0	0.81	0.08 BW6
10W206	TRAY:	6	POS: 7			LOCAL SPECIFICITY: B35									
10W206	BW53	532	6.0	5	2	57	468	58	28	91	72	0.22	24.6	4.41	0.80 BW53
10W206	B35	525	7.2	36	10	21	458	61	21	36	79	0.67	236.7	4.34	0.47 B35
10W206	BW75	479	7.0	12	44	9	414	52	78	42	22	0.30	43.9	0.73	0.08 BW75
10W208	TRAY:	6	POS: 21			LOCAL SPECIFICITY: B35									
10W208	B35	532	8.0	47	0	118	367	80	0	71	100	0.46	114.7	8.16	0.91 B35
10W208	BW53	485	8.0	6	0	112	367	72	0	94	100	0.20	18.9	5.59	0.89 BW53
10W208	BW75	479	7.3	55	1	57	366	71	1	50	99	0.64	198.2	6.96	0.75 BW75
10W208	BW77	423	8.0	10	1	47	365	73	9	82	91	0.37	58.1	5.23	0.74 BW77
10W208	B51	412	7.9	31	3	16	362	68	8	34	92	0.75	233.3	5.73	0.67 B51
10W212	TRAY:	6	POS: 16			LOCAL SPECIFICITY: B51,B35									
10W212	BW75	517	7.1	58	1	80	378	79	1	57	99	0.58	174.5	6.78	0.73 BW75
10W212	BW77	458	8.0	10	1	70	377	88	9	87	91	0.30	42.2	5.00	0.71 BW77
10W212	B35	447	7.9	38	3	32	374	87	7	45	93	0.67	202.8	5.75	0.65 B35
10W212	B51	406	7.9	21	12	11	362	78	36	34	64	0.62	153.8	3.13	0.37 B51

10W213	TRAY:	6	POS:	8		LOCAL	SPECIFICITY:	B35,B51,BW52,BW53								
10W213	B35	531	7.4	46	1	115	369	68	2	71	98	0.46	111.4	7.59	0.84	B35
10W213	BW77	484	8.0	10	1	105	368	65	9	91	91	0.24	28.0	5.08	0.72	BW77
10W213	BW53	473	6.4	5	1	100	367	61	16	95	84	0.17	13.2	4.34	0.69	BW53
10W213	BW52	467	6.6	10	3	90	364	63	23	90	77	0.23	24.5	4.00	0.55	BW52
10W213	B51	454	7.0	24	11	66	353	65	31	73	69	0.35	56.7	3.79	0.45	B51
10W213	BW75	419	7.7	33	21	33	332	65	38	50	62	0.48	96.1	2.80	0.31	BW75
10W213	BW62	365	6.3	31	60	2	272	39	65	6	35	0.50	92.3	1.55	0.16	BW62
10W214	TRAY:	8	POS:	29		LOCAL	SPECIFICITY:	B35,B51,BW52,BW53								
10W214	BW75	533	7.6	60	0	143	330	81	0	70	100	0.45	109.9	5.59	0.60	BW75
10W214	B35	473	8.0	42	1	101	329	80	2	70	98	0.46	102.0	4.67	0.53	B35
10W214	BW77	430	8.0	10	2	91	327	73	16	90	84	0.24	24.6	3.22	0.45	BW77
10W214	B51	418	7.3	25	9	66	318	70	26	72	74	0.37	58.2	2.77	0.33	B51
10W214	CW4	384	7.6	43	4	23	314	66	8	34	92	0.74	207.7	2.79	0.28	CW4
10W214	BW52	337	5.0	4	6	19	308	30	60	82	40	0.23	17.8	1.66	0.23	BW52
10W214	BW62	327	6.0	12	60	7	248	31	83	36	17	0.25	19.9	0.59	0.06	BW62
10W214	BW6	255	6.7	6	172	1	76	42	96	14	4	0.06	0.9	0.72	0.14	BW6
10W215	TRAY:	6	POS:	12		LOCAL	SPECIFICITY:	B35,BW62,BW50,TE79								
10W215	B35	529	7.5	45	2	99	383	66	4	68	96	0.48	122.3	5.73	0.64	B35
10W215	BW75	482	7.4	53	3	46	380	61	5	46	95	0.67	213.2	5.62	0.61	BW75
10W215	BW70	426	5.8	13	33	33	347	36	71	71	29	0.20	16.3	0.66	0.07	BW70
10W215	BW62	380	5.8	26	79	7	268	36	75	21	25	0.35	47.3	0.66	0.07	BW62
10W215	BW6	275	6.0	5	160	2	108	42	96	28	4	0.04	0.4	0.82	0.08	BW6
10W216	TRAY:	6	POS:	51		LOCAL	SPECIFICITY:	BW62,BW63,BW57,TE79								
10W216	BW75	530	8.0	60	0	185	285	91	0	75	100	0.39	78.7	9.06	0.98	BW75
10W216	BW62	470	7.9	115	0	70	285	89	0	37	100	0.71	234.6	9.78	0.98	BW62
10W216	BW76	355	8.0	13	0	57	285	77	0	81	100	0.39	54.9	7.02	0.97	BW76
10W216	BW77	342	8.0	11	0	46	285	71	0	80	100	0.41	56.8	6.78	0.97	BW77
10W216	BW63	331	7.3	9	1	37	284	65	10	80	90	0.39	49.9	6.11	0.89	BW63
10W216	B15.3	321	8.0	5	2	32	282	62	28	86	72	0.28	25.2	4.69	0.73	B15.3
10W216	BW57	314	6.7	9	8	23	274	56	47	71	53	0.34	35.9	2.44	0.32	BW57
10W216	BW46	297	7.2	20	19	3	255	56	48	13	52	0.63	119.1	2.41	0.28	BW46
10W220	TRAY:	6	POS:	55		LOCAL	SPECIFICITY:	BW57,BW46,B15,TE77								
10W220	BW75	523	8.0	59	0	236	228	87	0	80	100	0.31	51.4	8.10	0.92	BW75
10W220	B15.3	464	8.0	9	0	227	228	84	0	96	100	0.14	8.9	5.80	0.92	B15.3
10W220	BW77	455	8.0	12	0	215	228	83	0	94	100	0.16	12.4	6.14	0.92	BW77
10W220	BW76	443	8.0	14	0	201	228	82	0	93	100	0.19	15.3	6.33	0.91	BW76
10W220	BW62	429	8.0	104	1	97	227	81	0	48	100	0.60	152.1	7.95	0.84	BW62
10W220	BW57	324	7.8	18	1	79	226	63	5	81	95	0.35	40.4	5.61	0.77	BW57
10W220	BW63	305	8.0	7	1	72	225	58	12	91	88	0.23	16.2	4.51	0.73	BW63
10W220	BW46	297	7.1	34	5	38	220	54	12	52	88	0.57	96.8	4.71	0.57	BW46
10W220	BW70	258	6.0	28	18	10	202	39	39	26	61	0.61	94.9	2.98	0.36	BW70
10W220	B35	212	6.7	6	24	4	178	50	80	40	20	0.29	18.2	0.70	0.09	B35

Table 5. *Continued*

10W221	TRAY:	6	POS: 52			LOCAL SPECIFICITY: BW62,BW63,TE77,TE79									
10W221	BW75	529	7.9	60	0	192	277	85	0	76	100	0.37	74.4	7.91	0.89 BW75
10W221	BW76	469	8.0	14	0	178	277	82	0	92	100	0.21	20.8	6.19	0.89 BW76
10W221	BW77	455	8.0	12	0	166	277	80	0	93	100	0.21	19.2	5.99	0.89 BW77
10W221	BW62	443	7.9	110	3	56	274	79	2	33	98	0.72	232.1	8.46	0.88 BW62
10W221	B15.3	330	7.7	6	1	50	273	50	14	89	86	0.27	24.0	5.07	0.84 B15.3
10W221	BW63	323	6.5	8	2	42	271	46	20	84	80	0.32	32.8	3.46	0.53 BW63
10W221	BW57	313	6.9	7	10	35	261	45	58	83	42	0.20	11.9	1.47	0.20 BW57
10W221	B35	296	6.9	9	23	26	238	42	71	74	29	0.18	9.1	1.19	0.15 B35
10W221	BW70	264	5.8	13	32	13	206	34	71	50	29	0.29	22.2	1.17	0.14 BW70
10W221	BW46	219	6.9	9	29	4	177	46	76	30	24	0.34	25.9	0.63	0.08 BW46
10W222	TRAY:	4	POS: 31			LOCAL SPECIFICITY: BW53,B49,BW78,B51									
10W222	BW52	531	8.0	15	0	72	444	87	0	82	100	0.39	78.8	6.02	0.76 BW52
10W222	BW63	516	6.6	7	2	65	442	86	22	90	78	0.25	31.1	4.70	0.75 BW63
10W222	BW53	507	7.7	7	0	58	442	87	0	89	100	0.31	48.3	5.15	0.74 BW53
10W222	B51	500	7.9	37	2	21	440	87	5	36	95	0.76	286.0	5.70	0.62 B51
10W222	BW77	461	8.0	7	2	14	438	87	22	66	78	0.50	113.2	3.61	0.50 BW77
10W222	BW4	452	6.8	13	213	1	225	57	94	7	6	0.15	10.6	1.10	0.10 BW4
10W223	TRAY:	4	POS: 32			LOCAL SPECIFICITY: BW53,B49,BW78,B51									
10W223	BW52	526	7.9	15	0	73	438	87	0	82	100	0.38	76.9	5.27	0.66 BW52
10W223	BW53	511	7.3	6	1	67	437	86	14	91	86	0.24	29.6	4.44	0.64 BW53
10W223	B51	504	8.0	38	1	29	436	86	2	43	98	0.72	259.6	5.63	0.61 B51
10W223	BW77	465	7.4	7	2	22	434	68	22	75	78	0.42	80.3	3.66	0.50 BW77
10W223	BW4	456	7.2	19	211	3	223	63	91	13	9	0.16	11.9	1.13	0.10 BW4
10W225	TRAY:	4	POS: 34			LOCAL SPECIFICITY: B49,BW53,BW78									
10W225	BW52	524	8.0	15	0	86	423	80	0	85	100	0.35	64.7	6.01	0.77 BW52
10W225	BW53	509	8.0	7	0	79	423	76	0	91	100	0.26	34.9	5.10	0.75 BW53
10W225	BW63	502	7.0	8	1	71	422	74	11	89	89	0.27	37.0	5.11	0.72 BW63
10W225	B51	493	7.9	38	1	33	421	76	2	46	98	0.69	236.9	6.32	0.69 B51
10W225	BW77	454	8.0	8	1	25	420	51	11	75	89	0.45	90.8	4.29	0.60 BW77
10W225	B15.3	445	7.5	4	3	21	417	36	42	84	58	0.28	35.6	2.49	0.36 B15.3
10W225	B13	438	5.2	5	28	16	389	28	84	76	16	0.14	8.4	0.64	0.07 B13
10W225	BW6	405	5.7	14	335	2	54	37	95	12	5	0.01	0.0	1.03	0.17 BW6
10W226	TRAY:	6	POS: 56			LOCAL SPECIFICITY: B49,B5,TE78									
10W226	BW77	531	8.0	12	0	223	296	90	0	94	100	0.17	15.5	6.98	0.98 BW77
10W226	BW62	519	7.9	116	0	107	296	89	0	47	100	0.62	198.3	9.76	0.97 BW62
10W226	BW75	403	7.9	58	0	49	296	83	0	45	100	0.68	187.4	8.92	0.97 BW75
10W226	BW63	345	7.6	10	0	39	296	69	0	79	100	0.42	62.2	6.34	0.92 BW63
10W226	B15.3	335	7.3	6	1	33	295	66	14	84	86	0.34	38.1	4.57	0.71 B15.3
10W226	BW57	328	7.1	9	8	24	287	66	47	72	53	0.33	36.4	2.60	0.36 BW57
10W226	BW46	311	7.2	21	21	3	266	66	50	12	50	0.63	121.9	2.33	0.26 BW46
10W228	TRAY:	5	POS: 46			LOCAL SPECIFICITY: B35,BW62,BW70									
10W228	BW75	531	7.8	60	0	209	262	85	0	77	100	0.35	65.9	6.75	0.73 BW75
10W228	B35	471	7.8	43	0	166	262	84	0	79	100	0.35	59.3	6.42	0.73 B35
10W228	BW70	428	7.2	44	1	122	261	83	2	73	98	0.41	73.7	5.48	0.62 BW70
10W228	BW62	383	7.8	102	4	20	257	88	3	16	97	0.85	279.8	5.63	0.57 BW62
10W228	BW56	277	7.7	6	2	14	255	65	25	70	75	0.45	56.5	2.90	0.44 BW56
10W228	BW6	269	7.1	9	151	5	104	57	94	35	6	0.02	0.1	1.52	0.28 BW6
10W228	BW4	109	6.4	5	101	0	3	40	95	0	5	0.04	0.1	0.93	0.17 BW4

10W229	TRAY:	5	POS: 41			LOCAL	SPECIFICITY:	BW62,BW70,BW50,BW56							
10W229	BW75	534	7.9	60	0	211	263	81	0	77	100	0.35	65.6	7.96	0.87 BW75
10W229	BW62	474	7.8	114	1	97	262	78	0	45	100	0.62	183.4	7.90	0.79 BW62
10W229	BW77	359	7.6	11	1	86	261	62	8	88	92	0.27	26.3	5.02	0.71 BW77
10W229	BW56	347	6.6	7	2	79	259	60	22	91	78	0.20	13.9	3.86	0.58 BW56
10W229	B35	338	7.1	29	4	50	255	60	12	63	88	0.50	85.0	4.87	0.58 B35
10W229	BW70	305	7.0	41	5	9	250	60	10	18	90	0.83	209.1	5.07	0.57 BW70
10W229	B15.3	259	7.0	4	2	5	248	66	33	55	67	0.53	73.1	3.33	0.54 B15.3
10W229	BW6	253	6.8	5	149	0	99	60	96	0	4	0.11	3.3	1.42	0.14 BW6
10W230	TRAY:	5	POS: 42			LOCAL	SPECIFICITY:	B15,BW50,BW70							
10W230	BW76	527	7.9	18	0	262	247	83	0	93	100	0.18	16.4	5.46	0.73 BW76
10W230	BW75	509	7.8	54	2	208	245	82	3	79	97	0.32	50.9	5.43	0.61 BW75
10W230	BW70	453	7.8	45	2	163	243	80	4	78	96	0.34	52.4	5.18	0.59 BW70
10W230	B35	406	7.5	34	4	129	239	77	10	79	90	0.32	42.4	4.68	0.57 B35
10W230	BW62	368	7.7	99	6	30	233	76	5	23	95	0.78	226.4	5.07	0.52 BW62
10W230	B45	263	7.0	10	2	20	231	46	16	66	84	0.49	64.4	3.21	0.47 B45
10W230	BW41	251	6.0	4	3	16	228	40	42	80	58	0.31	23.7	2.09	0.34 BW41
10W230	BW6	244	6.7	15	125	1	103	43	89	6	11	0.19	9.3	1.26	0.24 BW6
10W231	TRAY:	5	POS: 45			LOCAL	SPECIFICITY:	B15,B17,B35,BW70							
10W231	BW75	533	8.0	60	0	268	205	87	0	81	100	0.28	42.3	7.81	0.91 BW75
10W231	BW76	473	8.0	14	0	254	205	85	0	94	100	0.15	11.0	6.05	0.90 BW76
10W231	BW77	459	8.0	13	0	241	205	84	0	94	100	0.15	10.8	5.96	0.90 BW77
10W231	B15.3	446	7.6	9	0	232	205	83	0	96	100	0.13	7.8	5.39	0.88 B15.3
10W231	BW62	437	8.0	110	1	122	204	84	0	52	100	0.54	126.5	7.80	0.83 BW62
10W231	B35	326	7.7	32	2	90	202	70	5	73	95	0.40	52.1	6.04	0.76 B35
10W231	BW63	292	8.0	7	2	83	200	65	22	92	78	0.18	9.6	4.33	0.71 BW63
10W231	B45	283	7.1	13	2	70	198	62	13	84	87	0.30	25.1	4.14	0.61 B45
10W231	BW57	268	7.7	13	3	57	195	61	18	81	82	0.32	26.8	3.89	0.56 BW57
10W231	BW70	252	7.0	31	11	26	184	56	26	45	74	0.55	75.5	3.28	0.40 BW70
10W231	BW58	210	7.1	15	17	11	167	53	53	42	47	0.44	41.4	1.64	0.21 BW58
10W231	BW60	178	6.3	6	21	5	146	36	77	45	23	0.28	14.1	0.71	0.09 BW60
10W233	TRAY:	5	POS: 43			LOCAL	SPECIFICITY:	BW62,B15.3,BW50,BW70							
10W233	BW75	531	8.0	60	0	209	262	92	0	77	100	0.35	65.9	6.97	0.76 BW75
10W233	BW70	471	7.6	46	1	163	261	90	2	77	98	0.36	60.5	5.67	0.64 BW70
10W233	B35	424	8.0	40	2	123	259	93	4	75	96	0.39	63.5	5.30	0.61 B35
10W233	BW62	382	7.9	103	4	20	255	91	3	16	97	0.86	279.4	5.76	0.59 BW62
10W233	BW56	275	8.0	7	1	13	254	70	12	65	88	0.53	78.6	3.72	0.37 BW56
10W233	BW6	267	6.5	12	147	1	107	53	92	7	8	0.15	6.1	2.36	0.22 BW6
10W234	TRAY:	5	POS: 44			LOCAL	SPECIFICITY:	B35,BW70,B15.3,BW62							
10W234	BW75	532	7.9	60	0	172	300	72	0	74	100	0.41	87.4	7.92	0.86 BW75
10W234	B35	472	7.8	40	3	132	297	65	6	76	94	0.37	65.4	5.73	0.65 B35
10W234	BW56	429	7.0	6	3	126	294	57	33	95	67	0.11	5.6	3.62	0.63 BW56
10W234	BW62	420	6.9	89	16	37	278	57	15	29	85	0.69	199.9	4.72	0.48 BW62
10W234	BW70	315	7.1	30	16	7	262	56	34	18	66	0.69	148.6	2.99	0.34 BW70
10W234	BW6	269	6.4	5	155	2	107	42	96	28	4	0.04	0.4	1.78	0.17 BW6

Table 5. *Continued*

10W235	TRAY:	6	POS: 20			LOCAL	SPECIFICITY:	B35,B15.3,BW53,B51							
10W235	BW53		531	8.0	7	0	212	312	78	0	96	100	0.14	10.1	6.25	1.00 BW53
10W235	B35		524	7.8	45	0	167	312	77	0	78	100	0.37	72.4	8.52	0.98 B35
10W235	BW75		479	7.8	55	1	112	311	73	1	67	99	0.48	112.1	7.89	0.88 BW75
10W235	BW77		423	8.0	10	1	102	310	65	9	91	91	0.24	24.1	5.69	0.83 BW77
10W235	B15.3		412	7.8	8	1	94	309	61	11	92	89	0.22	20.3	5.24	0.80 B15.3
10W235	B51		403	7.7	27	5	67	304	59	15	71	85	0.42	72.4	5.25	0.63 B51
10W235	BW52		371	7.1	7	6	60	298	47	46	89	54	0.18	11.7	2.80	0.[illegible] BW52
10W235	BW62		358	6.5	55	34	5	264	45	[illegible]	8	62	0.69	172.2	3.39	0.[illegible] BW62
10W235	BW70		269	7.2	5	40	0	224	80	[illegible]	0	12	0.31	25.4	0.77	0.[illegible] BW70
10W236	TRAY:	6	POS: 37			LOCAL	SPECIFICITY:	BW62,B15.3,SH.7							
10W236	BW75		530	7.9	[illegible]	0	150	321	92	0	71	100	0.44	102.0	9.17	0.98 BW75
10W236	BW62		471	7.9	[illegible]	0	35	321	90	0	23	100	0.83	325.6	9.79	0.97 BW62
10W236	BW77		356	7.8	[illegible]	0	24	321	77	0	68	100	0.54	104.1	6.71	0.95 BW77
10W236	BW76		345	7.2	[illegible]	0	11	321	70	0	45	100	0.72	180.7	6.56	0.90 BW76
10W236	B15.3		332	8.0	[illegible]	2	6	319	72	28	54	72	0.56	103.6	4.05	0.63 B15.3
10W236	BW6		325	6.8	5	221	1	98	50	97	16	3	0.04	0.5	0.61	0.06 BW6
10W237	TRAY:	6	POS: 41			LOCAL	SPECIFICITY:	B15							
10W237	BW77		523	8.0	12	0	179	332	83	0	93	100	0.20	21.3	6.18	0.85 BW77
10W237	BW75		511	7.8	60	0	119	332	82	0	66	100	0.50	126.1	8.00	0.85 BW75
10W237	B15.3		451	7.0	8	0	111	332	78	0	93	100	0.22	22.7	5.43	0.81 B15.3
10W237	BW62		443	7.5	99	7	12	325	79	6	10	94	0.88	346.6	6.52	0.65 BW62
10W237	B35		337	6.4	5	29	7	296	41	85	58	15	0.20	13.7	0.47	0.05 B35
10W238	TRAY:	6	POS: 50			LOCAL	SPECIFICITY:	B15							
10W238	BW76		530	8.0	18	0	195	317	93	0	91	100	0.23	27.7	7.75	0.99 BW76
10W238	BW77		512	8.0	12	0	183	317	92	0	93	100	0.20	20.0	7.18	0.99 BW77
10W238	BW75		500	7.9	55	1	128	316	92	1	69	99	0.45	103.2	9.00	0.97 BW75
10W238	BW62		444	8.0	108	5	20	311	91	4	15	96	0.86	329.1	8.08	0.80 BW62
10W238	BW63		331	7.1	9	1	11	310	55	10	55	90	0.62	128.0	5.32	0.76 BW63
10W238	B15.3		321	7.0	6	1	5	309	45	14	45	86	0.68	146.4	4.86	0.75 B15.3
10W238	BW57		314	6.0	4	13	1	296	20	76	20	24	0.42	55.2	1.23	0.16 BW57
10W239	TRAY:	6	POS: 36			LOCAL	SPECIFICITY:	B15							
10W239	BW62		529	6.3	80	35	26	388	36	30	24	70	0.65	225.0	3.42	0.33 BW62
10W239	BW75		414	6.0	21	37	5	351	30	63	19	37	0.50	102.6	1.18	0.13 BW75
10W240	TRAY:	6	POS: 40			LOCAL	SPECIFICITY:	B15							
10W240	BW75		531	7.9	60	0	139	332	93	0	69	100	0.46	112.9	9.27	0.98 BW75
10W240	BW62		471	7.9	115	0	24	332	92	0	17	100	0.88	363.4	10.02	0.98 BW62
10W240	B15.3		356	7.7	7	0	17	332	79	0	70	100	0.53	98.8	6.29	0.95 B15.3
10W240	BW77		349	8.0	10	1	7	331	76	9	41	91	0.72	181.4	5.89	0.82 BW77
10W240	BW63		338	7.2	5	5	2	326	42	50	28	50	0.59	116.7	3.22	0.45 BW63
10W241	TRAY:	6	POS: 46			LOCAL	SPECIFICITY:	B15							
10W241	BW77		531	8.0	12	0	191	328	83	0	94	100	0.19	19.8	7.19	0.99 BW77
10W241	BW75		519	7.8	60	0	131	328	82	0	68	100	0.47	116.5	9.07	0.96 BW75
10W241	BW62		459	7.6	112	2	19	326	79	1	14	99	0.89	361.3	9.65	0.95 BW62
10W241	B15.3		345	7.2	5	2	14	324	47	28	73	72	0.42	59.7	4.04	0.61 B15.3
10W241	BW63		338	7.4	7	3	7	321	42	30	50	70	0.58	112.6	3.97	0.56 BW63
10W241	BW57		328	5.6	5	12	2	309	14	70	28	30	0.44	63.9	1.15	0.16 BW57

10W242	TRAY:	6	POS: 47			LOCAL SPECIFICITY: B15									
10W242	BW75	531	8.0	60	0	180	291	93	0	75	100	0.39	82.0	7.84	0.84 BW75
10W242	BW62	471	7.8	115	0	65	291	91	0	36	100	0.72	246.0	8.34	0.83 BW62
10W242	BW76	356	8.0	13	0	52	291	92	0	80	100	0.41	60.4	6.00	0.83 BW76
10W242	BW77	343	8.0	11	0	41	291	90	0	78	100	0.43	63.6	5.80	0.82 BW77
10W242	BW63	332	7.4	10	0	31	291	87	0	75	100	0.47	73.2	5.54	0.80 BW63
10W242	B15.3	322	8.0	5	2	26	289	93	28	83	72	0.31	31.4	3.32	0.52 B15.3
10W242	BW46	315	7.9	22	19	4	270	92	46	15	54	0.64	128.3	2.17	0.25 BW46
10W243	TRAY:	6	POS: 58			LOCAL SPECIFICITY: B15									
10W243	BW62	530	8.0	117	0	100	313	95	0	46	100	0.64	216.6	9.98	0.99 BW62
10W243	BW77	413	8.0	11	0	89	313	[illegible]	0	89	100	0.29	35.4	6.90	0.98 BW77
10W243	BW75	402	7.9	57	0	32	313	[illegible]	0	35	100	0.76	233.6	9.04	0.98 BW75
10W243	BW63	345	7.8	9	1	23	312	[illegible]	10	71	90	0.48	79.7	5.46	0.79 BW63
10W243	B15.3	335	7.7	6	1	17	311	[illegible]	14	73	86	0.46	69.5	4.87	0.76 B15.3
10W243	BW57	328	7.7	6	11	11	300	[illegible]	64	64	36	0.32	33.1	1.83	0.24 BW57
10W243	BW46	311	7.2	10	32	1	268	63	76	9	24	0.43	58.5	0.98	0.11 BW46
10W245	TRAY:	6	POS: 48			LOCAL SPECIFICITY: BW62,BW63									
10W245	BW75	493	7.9	59	0	128	306	93	0	68	100	0.47	109.7	7.79	0.84 BW75
10W245	BW77	434	8.0	12	0	116	306	92	0	90	100	0.26	29.5	6.00	0.84 BW77
10W245	BW62	422	7.8	97	2	19	304	91	2	16	98	0.87	322.5	8.25	0.84 BW62
10W245	B15.3	323	7.7	6	1	13	303	89	14	68	86	0.50	82.4	4.27	0.66 B15.3
10W245	BW63	316	8.0	6	3	7	300	92	33	53	67	0.54	91.9	3.78	0.56 BW63
10W245	BW4	307	8.0	5	191	2	109	85	97	28	3	0.02	0.2	1.37	0.25 BW4
10W246	TRAY:	6	POS: 38			LOCAL SPECIFICITY: BW62									
10W246	BW62	531	7.9	117	0	87	327	91	0	42	100	0.67	240.5	10.02	0.98 BW62
10W246	BW75	414	7.9	58	0	29	327	83	0	33	100	0.78	253.5	9.12	0.97 BW75
10W246	BW76	356	5.8	13	0	16	327	62	0	55	100	0.65	152.1	6.61	0.89 BW76
10W246	BW77	343	7.8	10	1	6	326	81	9	37	91	0.74	190.1	5.65	0.78 BW77
10W246	B15.3	332	8.0	4	3	2	323	66	42	33	58	0.61	123.4	3.91	0.59 B15.3
10W247	TRAY:	6	POS: 49			LOCAL SPECIFICITY: BW62									
10W247	BW76	530	8.0	18	0	206	306	89	0	91	100	0.22	25.5	6.94	0.89 BW76
10W247	BW77	512	8.0	12	0	194	306	88	0	94	100	0.19	18.3	6.38	0.88 BW77
10W247	BW75	500	7.8	56	0	138	306	87	0	71	100	0.45	99.5	8.11	0.87 BW75
10W247	B15.3	444	7.5	8	1	130	305	86	11	94	89	0.18	14.3	5.67	0.83 B15.3
10W247	BW62	435	7.9	110	1	20	304	86	0	15	100	0.89	340.7	8.31	0.82 BW62
10W247	BW63	324	7.2	5	5	15	299	35	50	75	50	0.32	34.2	3.76	0.54 BW63
10W247	BW57	314	6.7	6	11	9	288	26	64	60	36	0.34	36.8	1.42	0.18 BW57
10W248	TRAY:	6	POS: 33			LOCAL SPECIFICITY: BW62									
10W248	BW76	523	7.7	18	0	156	349	70	0	89	100	0.27	37.4	7.21	0.91 BW76
10W248	BW62	505	7.8	108	6	48	343	68	5	30	95	0.75	281.1	7.72	0.75 BW62
10W248	BW75	391	5.5	42	12	6	331	22	22	12	78	0.80	249.6	4.16	0.45 BW75
10W248	BW6	337	5.2	5	225	1	106	0	97	16	3	0.04	0.6	1.08	0.10 BW6
10W249	TRAY:	6	POS: 39			LOCAL SPECIFICITY: BW62									
10W249	BW75	529	7.4	59	0	117	353	65	0	66	100	0.50	133.2	8.84	0.93 BW75
10W249	BW77	470	5.8	12	0	105	353	60	0	89	100	0.28	37.2	6.55	0.89 BW77
10W249	BW62	458	6.9	100	13	5	340	62	11	4	89	0.89	365.0	6.58	0.64 BW62
10W249	B15.3	345	6.5	4	3	1	337	40	42	20	58	0.67	155.2	3.21	0.48 B15.

Table 5. *Continued*

10W250	TRAY:	6	POS: 34			LOCAL SPECIFICITY: BW62									
10W250	BW76	531	7.8	18	0	88	425	60	0	83	100	0.38	74.7	7.85	0.97 BW76
10W250	BW62	513	6.8	88	28	0	397	54	24	0	76	0.84	363.5	4.76	0.46 BW62
10W252	TRAY:	6	POS: 35			LOCAL SPECIFICITY: BW62									
10W252	BW76	531	7.8	18	0	112	401	76	0	86	100	0.33	37.5	7.71	0.95 BW76
10W252	BW62	513	7.5	107	9	5	392	74	7	4	93	0.92	436.4	7.37	0.71 BW62
10W253	TRAY:	5	POS: 48			LOCAL SPECIFICITY: BW62									
10W253	BW76	534	7.2	18	0	159	357	63	0	89	100	0.27	27.6	5.65	0.72 BW76
10W253	BW62	516	7.1	100	16	59	341	63	13	37	87	0.65	215.4	4.63	0.45 BW62
10W253	BW77	400	7.8	9	3	50	338	59	25	84	75	0.30	35.7	3.18	0.43 BW77
10W253	BW75	388	7.0	35	19	15	319	54	35	30	65	0.62	150.7	2.33	0.25 BW75
10W254	TRAY:	6	POS: 57			LOCAL SPECIFICITY: B15,SH.7									
10W254	BW75	531	8.0	60	0	211	260	93	0	77	100	0.35	64.9	8.08	0.88 BW75
10W254	BW76	471	8.0	14	0	197	260	91	0	93	100	0.19	17.8	6.31	0.87 BW76
10W254	BW77	457	8.0	12	0	185	260	90	0	93	100	0.19	16.3	6.12	0.86 BW77
10W254	BW62	445	7.8	113	0	72	260	90	0	38	100	0.69	212.9	8.59	0.86 BW62
10W254	BW63	332	8.0	8	2	64	258	90	20	88	80	0.25	20.6	4.75	0.70 BW63
10W254	B15.3	322	7.7	6	1	58	257	89	14	90	86	0.25	19.5	4.29	0.67 B15.3
10W254	BW46	315	7.8	38	3	20	254	89	7	34	93	0.74	173.1	5.69	0.65 BW46
10W254	BW57	274	8.0	14	2	6	252	85	12	30	88	0.77	161.5	4.78	0.64 BW57
10W255	TRAY:	6	POS: 42			LOCAL SPECIFICITY: B15,SH.7									
10W255	BW75	529	7.9	60	0	79	390	77	0	56	100	0.60	189.9	8.82	0.95 BW75
10W255	BW77	469	7.6	11	1	68	389	65	8	86	92	0.32	49.2	5.52	0.77 BW77
10W255	B15.3	457	7.8	8	1	60	388	61	11	88	89	0.29	39.7	4.97	0.73 B15.3
10W255	B51	448	7.1	23	9	37	379	58	28	61	72	0.48	101.6	4.06	0.49 B51
10W255	BW62	416	6.8	24	78	13	301	51	76	35	24	0.29	35.7	0.70	0.07 BW62
10W255	B35	314	6.8	5	29	8	272	30	85	61	15	0.18	10.7	0.50	0.06 B35
10W256	TRAY:	6	POS: 60			LOCAL SPECIFICITY: BW76,B15									
10W256	BW76	529	8.0	18	0	11	500	72	0	37	100	0.78	321.3	7.06	0.85 BW76
10W257	TRAY:	4	POS: 50			LOCAL SPECIFICITY: BW46									
10W257	BW46	532	7.8	56	1	9	466	81	1	13	99	0.91	440.5	7.85	0.80 BW46
10W258	TRAY:	8	POS: 30			LOCAL SPECIFICITY: BW46									
10W258	BW46	518	7.9	56	1	8	453	84	1	12	99	0.92	436.3	7.89	0.81 BW46
10W259	TRAY:	7	POS: 40			LOCAL SPECIFICITY: BW46,CW1									
10W259	BW63	532	6.5	8	2	245	277	88	20	96	80	0.09	4.3	3.01	0.49 BW63
10W259	BW76	522	8.0	18	0	227	277	90	0	92	100	0.20	21.1	3.40	0.43 BW76
10W259	BW77	504	8.0	13	0	214	277	89	0	94	100	0.18	16.3	3.17	0.43 BW77
10W259	BW75	491	8.0	52	4	162	273	88	7	75	93	0.36	62.4	2.79	0.30 BW75
10W259	B15.3	435	8.0	7	2	155	271	85	22	95	78	0.12	6.5	1.66	0.24 B15.3
10W259	CW1	426	7.9	74	2	81	269	84	2	52	98	0.59	148.6	2.34	0.23 CW1
10W259	BW62	350	7.5	76	17	5	252	75	18	6	82	0.84	244.3	1.94	0.19 BW62

10W260	TRAY:	6	POS: 59			LOCAL SPECIFICITY: BW46,B15,B17,CW3.1									
10W260	BW62	530	8.0	116	0	177	237	97	0	60	100	0.48	120.1	6.28	0.63 BW62
10W260	BW75	414	8.0	58	0	119	237	96	0	67	100	0.47	90.3	5.59	0.61 BW75
10W260	BW76	356	8.0	13	0	106	237	94	0	89	100	0.27	26.9	4.15	0.58 BW76
10W260	B15.3	343	8.0	7	0	99	237	94	0	93	100	0.22	16.0	3.68	0.58 B15.3
10W260	BW77	336	8.0	11	0	88	237	93	0	88	100	0.28	27.2	4.01	0.58 BW77
10W260	BW63	325	8.0	10	0	78	237	93	0	88	100	0.29	27.8	3.93	0.58 BW63
10W260	BW57	315	7.9	16	1	62	236	92	5	79	95	0.38	46.4	3.54	0.47 BW57
10W260	BW46	298	7.9	36	4	26	232	91	10	41	90	0.67	134.3	3.32	0.38 BW46
10W260	CW3	258	7.7	23	37	3	195	84	61	11	39	0.52	68.9	0.95	0.09 CW3
10W261	TRAY:	5	POS: 47			LOCAL SPECIFICITY: B15,BW46,B13									
10W261	BW75	531	7.9	60	0	235	236	89	0	7[illegible]	100	0.32	54.1	6.94	0.76 BW75
10W261	BW46	471	7.9	52	0	183	236	88	0	7[illegible]	100	0.35	58.7	6.76	0.76 BW46
10W261	B13	419	7.9	29	0	154	236	85	0	8[illegible]	100	0.31	40.2	6.11	0.75 B13
10W261	BW77	390	8.0	12	0	142	236	83	0	92	100	0.22	19.0	5.15	0.74 BW77
10W261	BW76	378	7.8	12	0	130	236	82	0	91	100	0.23	20.6	5.01	0.72 BW76
10W261	BW63	366	7.5	8	1	122	235	81	11	93	89	0.18	11.5	4.72	0.71 BW63
10W261	B15.3	357	7.6	9	0	113	235	81	0	92	100	0.22	17.8	4.58	0.69 B15.3
10W261	BW62	348	7.7	95	3	18	232	81	3	15	97	0.86	258.6	5.75	0.59 BW62
10W261	BW57	250	6.0	5	11	13	221	38	68	72	32	0.24	14.8	0.85	0.11 BW57
10W261	CW3	234	6.8	10	42	3	179	38	80	23	20	0.32	23.8	0.82	0.07 CW3
10W262	TRAY:	6	POS: 54			LOCAL SPECIFICITY: BW62,BW57,BW63,BW46									
10W262	BW62	526	8.0	114	0	165	247	96	0	59	100	0.49	128.9	8.26	0.84 BW62
10W262	BW75	412	8.0	58	0	107	247	93	0	64	100	0.50	101.0	7.49	0.83 BW75
10W262	BW76	354	8.0	13	0	94	247	89	0	87	100	0.30	31.2	5.67	0.81 BW76
10W262	BW77	341	8.0	11	0	83	247	88	0	88	100	0.30	29.9	5.47	0.80 BW77
10W262	BW63	330	8.0	10	0	73	247	86	0	87	100	0.30	30.7	5.36	0.80 BW63
10W262	B15.3	320	8.0	7	0	66	247	84	0	90	100	0.28	24.2	4.96	0.80 B15.3
10W262	BW57	313	8.0	16	1	50	246	83	5	75	95	0.43	57.6	5.05	0.68 BW57
10W262	BW46	296	7.8	38	2	12	244	78	5	24	95	0.82	201.0	5.51	0.64 BW46
10W262	B35	256	5.6	5	26	7	218	41	83	58	17	0.20	10.3	0.49	0.06 B35
10W263	TRAY:	3	POS: 37			LOCAL SPECIFICITY: B8,BW46									
10W263	B8	53[illegible]	7.9	31	1	53	447	88	3	63	97	0.56	168.4	7.71	0.86 B8
10W263	BW46	50[illegible]	7.8	50	6	3	441	84	10	5	90	0.91	412.0	6.50	0.67 BW46
10W264	TRAY:	6	POS: 53			LOCAL SPECIFICITY: BW62,BW63,BW57,BW46									
10W264	BW62	5[illegible]	7.9	117	0	137	276	92	0	53	100	0.55	163.1	8.76	0.88 BW62
10W264	BW75	41[illegible]	8.0	58	0	79	276	89	0	57	100	0.57	135.9	7.99	0.87 BW75
10W264	BW76	355	8.0	13	0	66	276	82	0	83	100	0.36	47.1	6.22	0.86 BW76
10W264	B15.3	342	8.0	7	0	59	276	78	0	89	100	0.30	29.9	5.51	0.86 B15.3
10W264	BW77	335	8.0	11	0	48	276	76	0	81	100	0.40	53.2	6.02	0.86 BW77
10W264	BW63	324	8.0	10	0	38	276	70	0	79	100	0.43	59.3	5.90	0.86 BW63
10W264	BW57	314	7.5	16	1	22	275	63	5	57	95	0.60	113.7	5.37	0.71 BW57
10W264	BW46	297	6.8	16	23	6	252	50	58	27	42	0.50	74.0	1.54	0.18 BW46
10W266	TRAY:	6	POS: 43			LOCAL SPECIFICITY: B17,BW63									
10W266	BW58	531	8.0	52	0	54	425	91	0	50	100	0.66	231.1	7.28	0.77 BW58
10W266	BW57	479	7.9	24	0	30	425	85	0	55	100	0.64	198.9	6.39	0.76 BW57
10W266	BW77	455	7.0	8	4	22	421	76	33	73	67	0.40	72.2	4.54	0.61 BW77
10W266	BW63	443	8.0	6	2	16	419	77	25	72	75	0.44	84.7	3.35	0.48 BW63

Table 5. *Continued*

10W267	TRAY:	6	POS: 44		LOCAL SPECIFICITY: BW63,BW57,BW58										
10W267	BW57	530	7.8	26	0	51	453	71	0	66	100	0.55	160.9	7.40	0.85 BW57
10W267	BW77	504	6.7	9	3	42	450	62	25	82	75	0.34	56.9	5.85	0.77 BW77
10W267	BW58	492	7.1	35	15	7	435	61	30	16	70	0.74	269.3	3.55	0.37 BW58
10W323	TRAY:	4	POS: 57		LOCAL SPECIFICITY: BW50,BW41,BW62,BW70										
10W323	BW75	530	7.2	59	1	127	343	66	1	68	99	0.47	118.8	7.42	0.81 BW75
10W323	BW62	470	7.5	88	26	39	317	63	22	30	78	0.64	192.1	4.89	0.50 BW62
10W323	BW41	356	4.5	4	3	35	314	30	42	89	58	0.21	15.6	2.96	0.47 BW41
10W323	BW70	349	6.0	19	28	16	286	34	59	45	41	0.40	55.6	1.64	0.19 BW70
10W323	B35	302	6.7	9	25	7	261	43	73	43	27	0.34	34.2	0.85	0.10 B35
10W323	BW60	268	6.5	4	24	3	237	28	85	42	15	0.25	16.8	0.59	0.07 BW60
10W416	TRAY:	3	POS: 54		LOCAL SPECIFICITY: BW65										
10W416	BW77	532	6.7	11	2	47	472	50	15	81	85	0.37	74.5	2.94	0.38 BW77
10W416	CW8	519	7.1	31	25	16	447	46	44	34	56	0.56	163.4	2.71	0.27 CW8
10W418	TRAY:	4	POS: 48		LOCAL SPECIFICITY: B12										
10W418	B44	524	7.9	77	0	38	409	92	0	33	100	0.78	321.0	8.71	0.87 B44
10W418	BW76	447	8.0	16	0	22	409	84	0	57	100	0.63	178.6	6.87	0.86 BW76
10W418	B45	431	7.9	14	0	8	409	72	0	36	100	0.79	269.0	6.57	0.84 B45
10W422	TRAY:	8	POS: 42		LOCAL SPECIFICITY: B12,B44										
10W422	BW76	534	7.9	18	0	113	403	82	0	86	100	0.33	57.3	6.90	0.87 BW76
10W422	B45	516	7.8	16	0	97	403	80	0	85	100	0.34	58.9	6.60	0.85 B45
10W422	B44	500	7.9	76	1	21	402	79	1	21	99	0.86	366.1	7.97	0.81 B44
10W422	BW57	423	4.8	5	18	16	384	23	78	76	22	0.19	14.5	0.73	0.09 BW57
10W428	TRAY:	4	POS: 44		LOCAL SPECIFICITY: B45										
10W428	BW76	531	7.9	18	0	18	505	80	0	30	100	0.83	361.9	7.58	0.91 BW76
10W428	B45	513	7.2	5	11	3	494	50	68	37	32	0.43	94.8	2.06	0.25 B45
10W432	TRAY:	4	POS: 45		LOCAL SPECIFICITY: B45										
10W432	BW76	532	8.0	18	0	18	496	88	0	50	100	0.69	256.7	7.53	0.90 BW76
10W432	B45	514	7.7	14	2	4	494	77	12	22	88	0.82	344.8	5.42	0.66 B45
10W478	TRAY:	6	POS: 45		LOCAL SPECIFICITY: BW57,BW58,BW63,BW62										
10W478	BW58	530	7.9	52	0	60	418	83	0	53	100	0.64	215.2	8.98	0.97 BW58
10W478	BW57	478	7.9	24	0	36	418	71	0	60	100	0.61	176.0	7.84	0.95 BW57
10W478	BW77	454	6.7	12	0	24	418	55	0	66	100	0.56	143.1	6.34	0.87 BW77
10W478	BW63	442	8.0	7	1	17	417	50	12	70	88	0.49	106.9	5.08	0.75 BW63
10W478	BW6	434	6.1	17	365	0	52	29	95	0	5	0.07	2.4	0.91	0.09 BW6
10W493	TRAY:	3	POS: 14		LOCAL SPECIFICITY: BW4										
10W493	B27	526	7.5	26	2	266	232	84	7	91	93	0.18	16.7	2.74	0.33 B27
10W493	B38	498	7.8	24	2	242	230	85	7	90	93	0.18	16.7	2.02	0.25 B38
10W493	BW57	472	7.9	23	1	219	229	84	4	90	96	0.21	20.1	1.93	0.24 BW57
10W493	BW52	448	7.8	13	1	206	228	83	7	94	93	0.16	11.2	1.56	0.22 BW52
10W493	B44	434	7.8	67	4	139	224	83	5	67	95	0.42	74.9	1.96	0.21 B44
10W493	B51	363	7.5	31	3	108	221	78	8	77	92	0.35	44.4	1.56	0.19 B51
10W493	BW4	329	7.5	97	13	11	208	76	11	10	89	0.84	229.6	2.49	0.44 BW4

10W495	TRAY:	3	POS: 15			LOCAL	SPECIFICITY:	BW4								
10W495	B13	533	7.9	35	1	282	215	92	2	88	98	0.21	22.8	2.60	0.30	B13
10W495	B27	497	7.8	26	0	256	215	92	0	90	100	0.21	20.9	2.43	0.30	B27
10W495	BW57	471	8.0	26	0	230	215	91	0	89	100	0.22	23.1	2.40	0.30	BW57
10W495	BW52	445	7.9	14	0	216	215	90	0	93	100	0.17	13.5	2.06	0.28	BW52
10W495	B44	431	7.9	73	1	143	214	90	1	66	99	0.44	84.2	2.57	0.27	B44
10W495	B51	357	7.9	33	1	110	213	87	2	76	98	0.38	50.9	2.01	0.24	B51
10W495	BW4	323	7.9	95	5	15	208	84	5	13	95	0.86	239.5	4.19	0.75	BW4
10W496	TRAY:	3	POS: 17			LOCAL	SPECIFICITY:	BW4								
10W496	BW77	533	8.0	13	0	235	285	64	0	94	100	0.17	15.3	3.01	0.40	BW77
10W496	B27	520	7.2	26	1	209	284	62	3	88	97	0.24	30.0	3.14	0.37	B27
10W496	BW63	493	7.8	10	0	199	284	61	0	95	100	0.17	13.9	2.59	0.36	BW63
10W496	BW52	483	6.6	10	3	189	281	59	23	94	77	0.12	7.0	2.45	0.36	BW52
10W496	BW57	470	7.2	23	1	166	280	59	4	87	96	0.26	32.5	2.45	0.29	BW57
10W496	B51	446	7.4	29	4	137	276	58	12	82	88	0.30	39.1	2.31	0.27	B51
10W496	B38	413	7.6	20	3	117	273	55	13	85	87	0.28	31.8	2.10	0.25	B38
10W496	B13	390	7.2	23	9	94	264	51	28	80	72	0.27	29.1	1.89	0.22	B13
10W496	B37	358	6.0	7	2	87	262	46	22	92	78	0.19	12.7	1.29	0.18	B37
10W496	AW33	349	6.7	25	5	62	257	47	16	71	84	0.41	59.8	1.04	0.11	AW33
10W496	A32	319	6.9	7	4	55	253	45	36	88	64	0.21	14.2	0.66	0.09	A32
10W496	BW4	308	6.6	51	45	4	208	43	46	7	54	0.62	118.3	4.41	0.40	BW4
10W497	TRAY:	3	POS: 18			LOCAL	SPECIFICITY:	BW4								
10W497	B15.3	534	7.6	5	5	68	456	69	50	93	50	0.15	11.4	2.90	0.42	B15.3
10W497	BW63	524	8.0	5	5	63	451	69	50	92	50	0.15	12.4	2.53	0.36	BW63
10W497	B13	514	7.9	21	14	42	437	66	40	66	60	0.39	79.6	2.95	0.34	B13
10W497	B27	479	7.8	8	18	34	419	52	69	80	31	0.19	16.6	1.29	0.16	B27
10W497	BW61	453	5.7	7	18	27	401	44	72	79	28	0.19	16.0	1.06	0.13	BW61
10W497	B51	428	7.2	5	28	22	373	44	84	81	16	0.11	4.7	0.55	0.06	B51
10W497	B44	395	6.8	12	57	10	316	40	82	45	18	0.24	22.2	0.54	0.06	B44
10W498	TRAY:	3	POS: 16			LOCAL	SPECIFICITY:	BW4								
10W498	B51	532	7.9	40	0	272	220	94	0	87	100	0.24	30.5	2.61	0.29	B51
10W498	B13	492	8.0	35	0	237	220	94	0	87	100	0.25	30.5	2.54	0.29	B13
10W498	B27	457	8.0	23	0	214	220	93	0	90	100	0.22	22.5	2.27	0.28	B27
10W498	B44	434	7.9	73	2	141	218	92	2	65	98	0.44	83.7	2.25	0.23	B44
10W498	B38	359	7.9	21	1	12[illegible]	217	90	4	85	96	0.29	31.0	1.69	0.21	B38
10W498	BW4	337	7.9	108	9	12	208	90	7	10	93	0.86	251.3	3.92	0.70	BW4
10W499	TRAY:	3	POS: 19			LOCAL	SPECIFICITY:	BW6								
10W499	BW75	517	8.0	60	0	347	110	92	0	85	100	0.19	18.3	4.13	0.51	BW75
10W499	BW76	457	8.0	14	0	333	110	91	0	95	100	0.10	4.6	2.71	0.43	BW76
10W499	BW62	443	8.0	111	2	222	108	91	1	66	99	0.31	43.2	3.73	0.42	BW62
10W499	B39	330	7.6	16	0	206	108	87	0	92	100	0.16	8.2	2.59	0.40	B39
10W499	BW54	314	8.0	7	0	199	108	88	0	96	100	0.11	3.8	2.13	0.39	BW54
10W499	BW41	307	8.0	6	0	193	108	87	0	96	100	0.10	3.3	2.02	0.39	BW41
10W499	BW48	301	8.0	5	0	188	108	87	0	97	100	0.10	2.8	1.90	0.38	BW48
10W499	BW60	296	7.9	25	1	163	107	87	3	86	97	0.21	13.1	2.57	0.36	BW60
10W499	B8	270	7.9	20	1	143	106	85	4	87	96	0.21	11.6	2.34	0.34	B8
10W499	B35	249	7.9	29	2	114	104	84	6	79	94	0.28	18.9	2.30	0.31	B35
10W499	B7	218	7.8	35	3	79	101	82	7	69	93	0.37	29.2	2.15	0.28	B7
10W499	BW56	180	8.0	6	1	73	100	79	14	92	86	0.17	5.2	1.41	0.26	BW56
10W499	BW70	173	7.9	31	5	42	95	78	13	57	87	0.46	35.9	1.82	0.24	BW70
10W499	BW61	137	6.7	6	2	36	93	64	25	85	75	0.24	7.9	1.16	0.21	BW61
10W499	BW6	129	7.2	22	11	14	82	63	33	38	67	0.51	33.1	3.56	0.34	BW6

Table 5. *Continued*

10W500	TRAY:	3	POS: 20		LOCAL	SPECIFICITY:	BW6								
10W500	BW75	532	8.0	59	0	421	52	95	0	87	100	0.12	7.2	2.14	0.27 BW75
10W500	BW60	473	8.0	38	0	383	52	94	0	90	100	0.10	5.1	1.83	0.25 BW60
10W500	B35	435	8.0	42	0	341	52	94	0	89	100	0.12	6.3	1.80	0.24 B35
10W500	BW46	393	7.9	48	0	293	52	93	0	85	100	0.15	8.3	1.84	0.24 BW46
10W500	B18	345	8.0	23	0	270	52	93	0	92	100	0.11	4.4	1.49	0.22 B18
10W500	BW70	322	8.0	43	1	227	51	93	2	84	98	0.15	7.2	1.56	0.21 BW70
10W500	BW62	278	8.0	84	3	143	48	92	3	62	97	0.26	18.8	1.73	0.21 BW62
10W500	BW42	191	8.0	15	0	128	48	88	0	89	100	0.17	5.5	1.21	0.20 BW42
10W500	B8	176	7.9	15	0	113	48	86	0	88	100	0.19	6.1	1.14	0.18 B8
10W500	BW76	161	8.0	11	0	102	48	85	0	90	100	0.18	5.0	1.04	0.18 BW76
10W500	B39	150	7.7	14	0	88	48	84	0	86	100	0.22	7.3	1.08	0.18 B39
10W500	BW54	136	8.0	5	0	83	48	84	0	94	100	0.14	2.8	0.72	0.15 BW54
10W500	B7	131	8.0	28	2	55	46	83	6	66	94	0.34	15.1	1.03	0.15 B7
10W500	B45	101	8.0	6	1	49	45	74	14	89	86	0.17	3.0	0.42	0.08 B45
10W500	BW6	94	8.0	20	2	29	43	71	9	59	91	0.43	17.3	2.01	0.19 BW6
10W501	TRAY:	3	POS: 23		LOCAL	SPECIFICITY:	BW6								
10W501	B7	528	7.8	59	3	357	109	79	4	85	96	0.15	11.3	5.15	0.65 B7
10W501	BW76	466	8.0	18	0	339	109	77	0	94	100	0.11	5.7	4.06	0.63 BW76
10W501	B35	448	7.4	45	2	294	107	75	4	86	96	0.16	11.5	4.66	0.62 B35
10W501	BW60	401	7.3	46	0	248	107	75	0	84	100	0.22	18.9	4.70	0.62 BW60
10W501	B39	355	7.7	18	0	230	107	75	0	92	100	0.15	8.2	3.92	0.61 B39
10W501	BW54	337	8.0	10	0	220	107	75	0	95	100	0.12	4.8	3.40	0.60 BW54
10W501	BW41	327	8.0	7	0	213	107	74	0	96	100	0.10	3.5	3.04	0.59 BW41
10W501	BW75	320	6.7	35	1	178	106	73	2	83	98	0.23	17.1	3.70	0.51 BW75
10W501	B8	284	7.2	21	1	157	105	75	4	88	96	0.20	11.0	3.26	0.49 B8
10W501	BW46	262	7.3	33	5	124	100	76	13	78	87	0.23	13.4	3.18	0.44 BW46
10W501	BW42	224	8.0	16	2	108	98	76	11	87	89	0.20	8.9	2.79	0.44 BW42
10W501	BW61	206	7.6	14	2	94	96	73	12	87	88	0.20	8.6	2.47	0.40 BW61
10W501	BW62	190	7.3	52	10	42	86	71	16	44	84	0.48	43.6	2.76	0.35 BW62
10W501	BW70	128	7.2	22	8	20	78	69	26	47	74	0.48	29.2	2.08	0.31 BW70
10W501	B18	98	7.3	9	4	11	74	65	30	55	70	0.47	22.0	1.45	0.24 B18
10W501	BW6	85	7.4	7	4	4	70	54	36	36	64	0.58	28.8	2.54	0.24 BW6
10W502	TRAY:	8	POS: 52		LOCAL	SPECIFICITY:	BW6								
10W502	BW62	530	8.0	113	2	351	64	92	1	75	99	0.17	15.5	1.27	0.13 BW62
10W502	B18	415	8.0	28	0	323	64	90	0	92	100	0.11	5.5	0.93	0.12 B18
10W502	B35	387	7.9	37	0	286	64	90	0	88	100	0.14	8.1	0.97	0.12 B35
10W502	B8	350	8.0	25	0	261	64	89	0	91	100	0.13	6.0	0.88	0.12 B8
10W502	BW70	325	8.0	39	1	222	63	88	2	85	98	0.16	8.5	0.83	0.10 BW70
10W502	BW76	285	8.0	17	0	205	63	86	0	92	100	0.13	5.1	0.71	0.10 BW76
10W502	B39	268	7.8	18	0	187	63	85	0	91	100	0.15	5.9	0.69	0.10 B39
10W502	B45	250	8.0	11	0	176	63	85	0	94	100	0.12	3.9	0.56	0.09 B45
10W502	BW60	239	7.9	32	1	144	62	84	3	81	97	0.21	10.7	0.67	0.08 BW60
10W502	BW41	206	8.0	6	0	138	62	81	0	95	100	0.11	2.7	0.45	0.08 BW41
10W502	BW6	200	7.7	96	9	42	53	80	8	30	92	0.51	52.0	1.81	0.17 BW6

10W504	TRAY:	3	POS: 24			LOCAL SPECIFICITY: BW6										
10W504	BW75	530	7.8	60	0	348	122	84	0	85	100	0.20	20.2	3.35	0.39	BW75
10W504	B18	470	7.9	29	0	319	122	83	0	91	100	0.15	10.8	2.76	0.36	B18
10W504	BW62	441	7.8	105	3	214	119	82	2	67	98	0.32	44.3	3.30	0.35	BW62
10W504	B7	333	8.0	41	2	173	117	77	4	80	96	0.25	20.8	2.61	0.32	B7
10W504	BW60	290	7.5	26	1	147	116	72	3	84	97	0.24	16.6	2.46	0.32	BW60
10W504	BW76	263	7.8	13	0	134	116	70	0	91	100	0.20	10.8	2.06	0.31	BW76
10W504	B8	250	7.7	19	0	115	116	68	0	85	100	0.27	17.8	1.95	0.26	B8
10W504	BW64	231	8.0	4	0	111	116	65	0	96	100	0.13	4.1	1.30	0.25	BW64
10W504	BW67	227	7.0	4	0	107	116	63	0	96	100	0.14	4.3	1.25	0.24	BW67
10W504	BW56	223	6.9	7	1	100	115	63	12	93	88	0.15	5.2	1.04	0.17	BW56
10W504	BW41	215	7.6	5	1	95	114	63	16	95	84	0.13	3.4	0.83	0.15	BW41
10W504	BW42	209	7.7	14	4	81	110	62	22	85	78	0.20	8.3	0.95	0.13	BW42
10W504	BW54	191	7.5	4	1	77	109	56	20	95	80	0.12	3.0	0.73	0.13	BW54
10W504	BW61	185	6.7	12	3	65	106	55	20	84	80	0.23	10.0	0.74	0.11	BW61
10W504	B39	171	7.8	9	4	56	102	55	30	86	70	0.18	5.8	0.59	0.09	B39
10W504	BW6	158	6.7	45	26	11	76	50	36	19	64	0.53	44.0	2.80	0.27	BW6
10W507	TRAY:	3	POS: 21			LOCAL SPECIFICITY: BW6										
10W507	BW75	529	8.0	60	0	345	124	87	0	85	100	0.20	20.7	3.92	0.47	BW75
10W507	B35	469	7.9	43	0	302	124	85	0	87	100	0.19	17.0	3.57	0.45	B35
10W507	BW76	426	7.0	14	0	288	124	84	0	95	100	0.12	5.9	2.79	0.43	BW76
10W507	BW67	412	8.0	5	0	283	124	84	0	98	100	0.07	2.2	2.06	0.40	BW67
10W507	BW62	407	8.0	103	2	180	122	84	1	63	99	0.37	54.5	3.53	0.39	BW62
10W507	BW46	302	7.5	37	3	143	119	76	7	79	93	0.26	20.7	2.71	0.36	BW46
10W507	BW60	262	7.7	26	1	117	118	74	3	81	97	0.28	21.1	2.51	0.34	BW60
10W507	BW70	235	7.9	43	2	74	116	71	4	63	96	0.45	46.6	2.61	0.33	BW70
10W507	BW41	190	6.4	5	1	69	115	59	16	93	84	0.16	5.1	1.73	0.32	BW41
10W507	B8	184	6.9	11	5	58	110	59	31	84	69	0.20	7.3	1.11	0.17	B8
10W507	BW63	168	6.0	4	4	54	106	62	50	93	50	0.07	0.9	0.93	0.16	BW63
10W507	B39	160	6.5	8	5	46	101	64	38	85	62	0.17	4.9	0.97	0.15	B39
10W507	B7	147	6.5	17	15	29	86	69	46	63	54	0.25	9.1	0.62	0.08	B7
10W507	BW6	115	7.5	21	15	8	71	75	41	27	59	0.51	30.5	2.82	0.27	BW6
10W509	TRAY:	3	POS: 22			LOCAL SPECIFICITY: BW6,AN										
10W509	BW62	527	8.0	116	0	365	46	95	0	75	100	0.16	14.2	0.72	0.07	BW62
10W509	B7	411	8.0	47	0	318	46	94	0	87	100	0.13	6.7	0.53	0.06	B7
10W509	B35	364	8.0	38	0	280	46	93	0	88	100	0.13	6.1	0.48	0.06	B35
10W509	B37	326	7.6	10	1	270	45	92	9	96	91	0.03	0.2	0.35	0.05	B37
10W509	BW60	315	7.9	38	0	232	45	92	0	85	100	0.15	7.2	0.42	0.05	BW60
10W509	BW6	277	7.9	169	6	63	39	91	3	27	97	0.46	57.4	1.44	0.14	BW6
10W524	TRAY:	2	POS: 47			LOCAL SPECIFICITY: CW3										
10W524	CW3	533	8.0	219	1	25	288	98	0	10	100	0.90	436.3	6.46	0.59	CW3
10W524	BW46	313	8.0	19	1	6	287	92	5	24	95	0.84	220.1	2.18	0.22	BW46
10W525	TRAY:	2	POS: 48			LOCAL SPECIFICITY: CW3										
10W525	CW3	534	7.8	219	2	28	285	91	0	11	100	0.89	423.5	6.91	0.63	CW3
10W525	BW46	313	7.9	19	1	9	284	82	5	32	95	0.79	194.2	2.16	0.22	BW46
10W526	TRAY:	2	POS: 49			LOCAL SPECIFICITY: CW3										
10W526	CW3	530	7.6	211	6	58	255	78	2	21	98	0.77	317.6	5.20	0.49	CW3
10W526	BW46	313	7.6	15	5	43	250	55	25	74	75	0.38	45.1	0.99	0.10	BW46
10W526	CW1	293	7.2	5	21	38	229	44	80	88	20	0.04	0.5	0.72	0.08	CW1
10W526	BW77	267	6.0	4	7	34	222	42	63	89	37	0.13	4.6	0.47	0.06	BW77

Table 5. *Continued*

10W527	TRAY:	2	POS: 50			LOCAL SPECIFICITY: CW3									
10W527	CW3	533	7.7	218	3	64	248	84	1	22	99	0.77	316.9	5.37	0.49 CW3
10W527	BW46	312	7.6	18	2	46	246	65	10	71	90	0.45	63.3	2.61	0.28 BW46
10W527	BW77	292	7.6	10	1	36	245	58	9	78	91	0.41	48.6	1.78	0.25 BW77
10W527	BW62	281	5.7	6	16	30	229	52	72	83	28	0.13	4.5	1.66	0.17 BW62
10W527	BW75	259	7.5	16	11	14	218	56	40	46	60	0.51	66.9	1.18	0.13 BW75
10W528	TRAY:	2	POS: 51			LOCAL SPECIFICI		CW3							
10W528	CW3	532	7.9	212	8	33	2	90	3	13	97	0.85	382.2	4.98	0.46 CW3
10W528	BW46	312	7.0	10	10	23	2	51	50	69	50	0.34	35.1	0.52	0.05 BW46
10W544	TRAY:	7	POS: 49			LOCAL SPECIFICI		BW4,CW6,TEC10							
10W544	BW53	531	8.0	7	0	242	2	83	0	97	100	0.12	8.0	4.14	0.64 BW53
10W544	B15.3	524	7.5	8	2	234	2	82	20	96	80	0.09	4.7	4.23	0.62 B15.3
10W544	B51	514	7.9	36	1	198	2	82	2	84	98	0.29	43.1	5.19	0.60 B51
10W544	BW76	477	6.4	15	0	183	27	80	0	92	100	0.21	21.8	4.40	0.59 BW76
10W544	B44	462	7.9	72	3	111	27	83	4	60	96	0.51	119.0	5.20	0.55 B44
10W544	B45	387	7.8	13	1	98	27	74	7	88	93	0.27	29.2	3.84	0.52 B45
10W544	B35	373	7.9	35	6	63	269	72	14	64	86	0.47	83.0	3.53	0.40 B35
10W544	BW52	332	7.8	8	6	55	263	60	42	87	58	0.20	13.8	1.63	0.22 BW52
10W544	BW75	318	7.4	29	18	26	245	56	38	47	62	0.49	76.0	1.66	0.19 BW75
10W544	CW4	271	6.5	11	13	15	232	34	54	57	46	0.38	39.9	0.56	0.06 CW4
10W544	BW4	247	6.6	10	109	5	123	33	91	33	9	0.09	2.2	1.04	0.19 BW4
10W2064	TRAY:	15	POS: 26			LOCAL SPECIFICITY: A24,BW4									
10W2064	B44	404	8.0	56	0	232	116	96	0	80	100	0.25	26.2	2.18	0.24 B44
10W2064	B27	348	8.0	25	0	207	116	96	0	89	100	0.20	13.5	1.77	0.22 B27
10W2064	B38	323	8.0	23	0	184	116	96	0	88	100	0.21	13.9	1.73	0.22 B38
10W2064	BW58	300	7.9	16	0	168	116	95	0	91	100	0.19	10.7	1.60	0.22 BW58
10W2064	BW77	284	8.0	10	0	158	116	95	0	94	100	0.16	7.2	1.44	0.22 BW77
10W2064	BW52	274	8.0	13	0	145	116	95	0	91	100	0.19	10.0	1.51	0.22 BW52
10W2064	B51	261	8.0	26	1	119	115	95	3	82	97	0.28	20.2	1.36	0.17 B51
10W2064	A23	234	8.0	5	0	114	115	94	0	95	100	0.15	4.9	1.12	0.17 A23
10W2064	B13	229	7.9	21	1	93	114	93	4	81	96	0.30	20.3	1.26	0.16 B13
10W2064	A24	207	7.9	65	6	28	108	93	8	30	92	0.68	94.9	0.99	0.11 A24
10W2064	BW4	136	7.8	23	1	5	107	92	4	17	96	0.86	100.9	4.01	0.75 BW4
10W2065	TRAY:	15	POS: 28			LOCAL SPECIFICITY: A24,BW4									
10W2065	B38	403	7.9	26	0	256	121	96	0	90	100	0.17	11.9	2.08	0.26 B38
10W2065	BW53	377	8.0	6	0	250	121	96	0	97	100	0.09	2.9	1.54	0.26 BW53
10W2065	BW58	371	8.0	16	0	234	121	96	0	93	100	0.15	8.1	1.85	0.26 BW58
10W2065	BW52	355	8.0	14	0	220	121	96	0	94	100	0.15	7.5	1.79	0.25 BW52
10W2065	BW77	341	8.0	13	0	207	121	96	0	94	100	0.15	7.4	1.76	0.25 BW77
10W2065	B51	328	8.0	29	1	178	120	96	3	85	97	0.22	16.0	1.70	0.21 B51
10W2065	B13	298	8.0	26	1	152	119	95	3	85	97	0.24	16.5	1.63	0.21 B13
10W2065	B44	271	8.0	44	2	108	117	94	4	71	96	0.36	35.2	1.65	0.19 B44
10W2065	B27	225	8.0	16	1	92	116	93	5	85	95	0.26	15.7	1.36	0.19 B27
10W2065	BW57	208	8.0	13	1	79	115	92	7	85	93	0.26	14.4	1.26	0.18 BW57
10W2065	A24	194	7.9	63	8	16	107	91	11	20	89	0.74	106.9	0.89	0.10 A24
10W2065	A23	123	8.0	2	0	14	107	81	0	87	100	0.33	13.6	0.51	0.08 A23
10W2065	BW4	121	7.6	9	0	5	107	78	0	35	100	0.78	74.3	3.87	0.72 BW4

10W2097	TRAY:	16	POS: 11		LOCAL SPECIFICITY: B44,B45,B51,BW52											
10W2097	BW76	395	8.0	18	0	64	313	71	0	78	100	0.43	72.0	4.98	0.65	BW76
10W2097	B45	377	6.0	4	2	60	311	64	33	93	67	0.17	10.7	2.37	0.38	B45
10W2097	BW77	371	8.0	6	7	54	304	66	53	90	47	0.16	8.9	1.25	0.17	BW77
10W2097	B44	358	6.6	22	24	32	280	62	52	59	48	0.35	44.2	1.17	0.13	B44
10W2097	BW52	312	7.0	4	10	28	270	65	71	87	29	0.13	5.3	0.78	0.11	BW52
10W2097	B51	298	7.3	8	21	20	249	67	72	71	28	0.20	12.5	0.48	0.06	B51
10W2101	TRAY:	16	POS: 21		LOCAL SPECIFICITY: BW4											
10W2101	BW77	396	7.0	12	1	64	319	68	7	84	93	0.34	46.3	4.62	0.64	BW77
10W2101	BW63	383	7.3	8	1	56	318	68	11	87	89	0.30	34.5	4.22	0.63	BW63
10W2101	B15.3	374	7.5	4	4	52	314	67	50	92	50	0.15	7.9	3.31	0.50	B15.3
10W2101	BW57	366	7.4	7	8	45	306	67	53	86	47	0.19	13.5	2.12	0.29	BW57
10W2101	B13	351	7.7	15	13	30	293	64	46	66	54	0.36	45.2	2.34	0.28	B13
10W2101	B37	323	7.6	5	7	25	286	50	58	83	42	0.22	15.5	1.73	0.24	B37
10W2101	B27	311	6.3	8	14	17	272	44	63	68	37	0.29	25.7	1.06	0.14	B27
10W2101	B51	289	6.8	8	19	9	253	47	70	52	30	0.32	30.3	0.93	0.11	B51
10W2101	BW4	262	5.2	5	89	4	164	33	94	44	6	0.08	1.6	0.96	0.09	BW4
10W2102	TRAY:	16	POS: 12		LOCAL SPECIFICITY: BW4											
10W2102	B44	395	8.0	51	0	178	166	96	0	77	100	0.33	42.5	5.47	0.69	B44
10W2102	B27	344	8.0	25	0	153	166	95	0	85	100	0.27	25.1	4.70	0.67	B27
10W2102	BW58	319	8.0	16	0	137	166	94	0	89	100	0.24	18.3	4.23	0.66	BW58
10W2102	BW57	303	8.0	15	0	122	166	94	0	89	100	0.25	19.1	4.16	0.66	BW57
10W2102	BW52	288	8.0	13	0	109	166	93	0	89	100	0.25	18.5	4.01	0.66	BW52
10W2102	BW77	275	8.0	10	0	99	166	92	0	90	100	0.24	15.8	3.75	0.65	BW77
10W2102	B37	265	8.0	10	0	89	166	91	0	89	100	0.26	17.4	3.75	0.65	B37
10W2102	BW63	255	8.0	5	0	84	166	91	0	94	100	0.19	9.5	3.17	0.64	BW63
10W2102	BW53	250	8.0	5	0	79	166	90	0	94	100	0.20	10.1	3.17	0.64	BW53
10W2102	B51	245	8.0	24	2	55	164	89	7	69	93	0.44	48.0	3.93	0.56	B51
10W2102	B38	219	7.9	14	1	41	163	85	6	74	94	0.43	39.8	2.83	0.44	B38
10W2102	B13	204	7.9	18	3	23	160	82	14	56	86	0.55	62.8	2.77	0.41	B13
10W2102	BW4	183	8.0	10	6	13	154	73	37	56	63	0.47	39.8	4.93	0.48	BW4
10W2103	TRAY:	16	POS: 22		LOCAL SPECIFICITY: BW6											
10W2103	B7	395	7.7	31	3	237	124	81	8	88	92	0.15	9.3	1.34	0.17	B7
10W2103	B8	361	8.0	18	0	219	124	80	0	92	100	0.17	9.9	1.15	0.16	B8
10W2103	BW76	343	7.8	17	1	202	123	78	5	92	95	0.15	7.7	0.72	0.10	BW76
10W2103	B35	325	7.5	34	5	168	118	77	12	83	88	0.19	11.8	0.74	0.09	B35
10W2103	BW54	286	8.0	9	2	159	116	77	18	94	82	0.09	2.5	0.49	0.07	BW54
10W2103	CW8	275	7.5	44	7	115	109	76	13	72	87	0.27	20.8	0.63	0.07	CW8
10W2103	AW34	224	6.7	9	2	106	107	73	18	92	82	0.14	4.3	0.49	0.07	AW34
10W2103	AW33	213	7.0	21	4	85	103	75	16	80	84	0.25	13.3	0.53	0.06	AW33
10W2103	CW1	188	7.6	34	15	51	88	76	30	60	70	0.29	15.6	0.48	0.05	CW1
10W2105	TRAY:	16	POS: 24		LOCAL SPECIFICITY: BW6											
10W2105	BW70	393	6.4	20	3	219	151	71	13	91	87	0.13	7.0	2.00	0.30	BW70
10W2105	BW76	370	7.8	18	0	201	151	75	0	91	100	0.19	13.0	1.83	0.28	BW76
10W2105	BW77	352	7.8	12	1	189	150	73	7	94	93	0.14	6.8	1.42	0.23	BW77
10W2105	B38	339	6.6	13	7	176	143	72	35	93	65	0.05	0.7	0.78	0.13	B38
10W2105	B8	319	6.2	9	5	167	138	73	35	94	65	0.04	0.5	0.67	0.12	B8
10W2105	CW8	305	7.7	38	7	129	131	76	15	77	85	0.25	18.8	1.05	0.12	CW8
10W2105	BW61	260	7.5	8	3	121	128	72	27	93	73	0.10	2.5	0.52	0.08	BW61
10W2105	BW46	249	7.6	36	10	85	118	71	21	70	79	0.28	19.9	0.59	0.07	BW46
10W2105	B37	203	7.7	6	2	79	116	68	25	92	75	0.14	3.8	0.44	0.07	B37
10W2105	A31	195	7.6	10	1	69	115	67	9	87	91	0.25	12.3	0.46	0.06	A31

Table 5. *Continued*

10W2106	TRAY:	16	POS:	14		LOCAL	SPECIFICITY:	BW6							
10W2106	B18	396	7.9	26	0	280	90	94	0	91	100	0.14	8.2	3.28	0.46 B18
10W2106	BW76	370	8.0	18	0	262	90	94	0	93	100	0.13	6.1	2.98	0.45 BW76
10W2106	BW70	352	8.0	21	1	241	89	94	4	91	96	0.12	5.4	3.10	0.45 BW70
10W2106	BW67	330	8.0	5	0	236	89	93	0	97	100	0.08	1.9	2.23	0.45 BW67
10W2106	B8	325	8.0	14	0	222	89	93	0	94	100	0.13	5.5	2.80	0.45 B8
10W2106	BW56	311	8.0	9	0	213	89	93	0	95	100	0.11	3.7	2.51	0.44 BW56
10W2106	B35	302	7.7	35	1	178	88	92	2	83	98	0.22	14.0	3.02	0.40 B35
10W2106	BW60	266	8.0	39	1	139	87	94	2	78	98	0.27	19.9	3.02	0.40 BW60
10W2106	B7	226	7.8	30	1	109	86	92	3	78	97	0.29	18.9	2.79	0.38 B7
10W2106	BW61	195	7.9	14	1	95	85	92	6	87	94	0.22	9.2	2.20	0.35 BW61
10W2106	BW62	180	7.6	51	6	44	79	92	10	46	90	0.50	45.1	2.11	0.26 BW62
10W2106	BW75	123	7.9	25	4	19	75	97	13	43	87	0.58	42.0	1.77	0.25 BW75
10W2106	BW6	94	8.0	17	11	2	64	100	39	10	61	0.66	40.6	3.81	0.39 BW6
10W2107	TRAY:	16	POS:	15		LOCAL	SPECIFICITY:	BW6							
10W2107	BW56	395	7.8	9	0	325	61	95	0	97	100	0.07	1.7	1.03	0.18 BW56
10W2107	B7	386	8.0	34	0	291	61	96	0	89	100	0.13	7.0	1.28	0.17 B7
10W2107	BW70	352	8.0	24	0	267	61	95	0	91	100	0.12	5.4	1.14	0.16 BW70
10W2107	B18	328	8.0	22	0	245	61	95	0	91	100	0.13	5.4	1.11	0.16 B18
10W2107	BW76	306	8.0	18	0	227	61	94	0	92	100	0.12	4.8	1.04	0.15 BW76
10W2107	BW54	288	8.0	11	0	216	61	94	0	95	100	0.10	3.1	0.93	0.15 BW54
10W2107	B39	277	8.0	14	0	202	61	93	0	93	100	0.12	4.2	0.98	0.15 B39
10W2107	B8	263	8.0	14	0	188	61	93	0	93	100	0.13	4.5	0.98	0.15 B8
10W2107	BW60	249	7.9	36	1	152	60	93	2	80	98	0.21	11.2	0.98	0.13 BW60
10W2107	BW75	212	7.9	32	1	120	59	92	3	78	97	0.24	12.3	0.94	0.12 BW75
10W2107	B35	179	7.9	29	2	91	57	92	6	75	94	0.26	11.9	0.71	0.09 B35
10W2107	CW2	148	7.7	7	0	84	57	92	0	92	100	0.18	4.6	0.53	0.07 CW2
10W2107	BW6	141	7.8	70	6	14	51	92	7	16	93	0.72	72.4	2.86	0.30 BW6

Table 6. Seratyping of Antigen Society Sera Defining B15 and Its Subgroups

SERUM	ANTIGEN	NO. REAC	AVE	++	MISS +-	EXTR -+	--	PERCENTAGE STR	MISS	EXTR	INCL	R	CHI	QSCORE	QNORM	ANTIGEN
	TRAY: 280	POS: 3			LOCAL SPECIFICITY: B17,BW63											
10W265	BW57	680	7.5	22	8	64	586	67	26	74	74	0.39	104.6	3.16	0.35	BW57
10W265	BW58	650	7.4	36	18	28	568	60	33	43	67	0.57	214.2	2.76	0.28	BW58
10W265	BW63	596	5.7	6	22	22	546	39	78	78	22	0.18	18.4	0.50	0.06	BW63
	TRAY: 280	POS: 4			LOCAL SPECIFICITY: B15											
10W4486	BW77	684	8.0	13	0	319	352	73	0	96	100	0.14	14.1	5.69	0.78	BW77
10W4486	BW75	671	7.8	61	1	258	351	72	1	80	99	0.32	70.8	6.72	0.72	BW75
10W4486	BW76	609	5.7	14	0	244	351	67	0	94	100	0.18	19.5	5.20	0.70	BW76
10W4486	BW62	595	7.6	157	8	87	343	70	4	35	96	0.68	276.7	6.23	0.59	BW62
10W4486	B15.3	430	6.6	10	1	77	342	48	9	88	91	0.29	34.9	3.99	0.56	B15.3
10W4486	BW63	419	7.4	22	6	55	336	48	21	71	79	0.42	72.5	3.77	0.47	BW63
10W4486	BW57	391	6.7	14	6	41	330	38	30	74	70	0.37	54.6	2.64	0.33	BW57
10W4486	BW46	371	5.7	29	50	12	280	31	63	29	37	0.43	67.2	1.14	0.12	BW46
	TRAY: 280	POS: 5			LOCAL SPECIFICITY: B15											
10W4487	BW76	682	8.0	18	0	15	649	75	0	45	100	0.73	363.6	6.65	0.77	BW76
	TRAY: 280	POS: 6			LOCAL SPECIFICITY: BW62,BW63											
10W4488	BW75	681	7.8	62	0	218	401	81	0	77	100	0.38	97.7	8.07	0.83	BW75
10W4488	BW77	619	8.0	13	0	205	401	78	0	94	100	0.20	24.4	6.32	0.83	BW77
10W4488	B15.3	606	7.4	13	0	192	401	77	0	93	100	0.21	26.0	5.92	0.77	B15.3
10W4488	BW62	593	7.7	151	11	41	390	78	6	21	94	0.80	376.7	6.11	0.56	BW62
10W4488	BW63	431	7.3	24	4	17	386	43	14	41	86	0.68	202.0	4.50	0.52	BW63
10W4488	A31	399	4.9	7	25	9	358	18	78	56	22	0.27	28.8	0.59	0.06	A31
	TRAY: 280	POS: 7			LOCAL SPECIFICITY: BW62											
10W4489	BW76	684	8.0	18	0	263	403	83	0	93	100	0.20	26.5	6.51	0.84	BW76
10W4489	BW77	666	8.0	13	0	250	403	82	0	95	100	0.17	20.3	6.08	0.83	BW77
10W4489	B15.3	653	7.3	9	3	241	400	82	25	96	75	0.10	7.0	5.06	0.78	B15.3
10W4489	BW75	641	7.8	52	6	189	394	82	10	78	90	0.34	73.7	5.79	0.63	BW75
10W4489	BW62	583	7.7	146	17	43	377	78	10	22	90	0.76	337.3	5.79	0.55	BW62
10W4489	BW63	420	6.7	9	19	34	358	41	67	79	33	0.19	15.7	1.01	0.12	BW63
10W4489	BW57	392	6.3	6	15	28	343	41	71	82	29	0.17	11.1	0.72	0.09	BW57
10W4489	BW46	371	5.9	22	57	6	286	39	72	21	28	0.40	59.3	0.70	0.07	BW46
	TRAY: 280	POS: 8			LOCAL SPECIFICITY: BW62											
10W4490	BW77	683	8.0	13	0	226	444	84	0	94	100	0.19	24.6	6.15	0.83	BW77
10W4490	BW76	670	7.8	18	0	208	444	83	0	92	100	0.23	36.3	6.46	0.82	BW76
10W4490	BW75	652	7.8	50	8	158	436	83	13	75	87	0.36	86.4	5.19	0.56	BW75
10W4490	BW62	594	7.6	138	27	20	409	79	16	12	84	0.80	380.7	4.89	0.46	BW62
10W4490	B15.3	429	6.7	6	5	14	404	45	45	70	55	0.38	63.2	2.60	0.41	B15.3
10W4490	BW63	418	6.4	5	23	9	381	42	82	64	18	0.22	19.5	0.67	0.08	BW63
10W4491	TRAY: 280	POS: 9			LOCAL SPECIFICITY: BW62											
10W4491	BW77	653	8.0	13	0	221	419	75	0	94	100	0.19	23.8	6.56	0.89	BW77
10W4491	BW76	640	6.6	18	0	203	419	73	0	91	100	0.23	35.1	6.15	0.79	BW76
10W4491	BW75	622	7.9	50	6	153	413	75	10	75	90	0.38	89.8	6.10	0.66	BW75
10W4491	BW62	566	7.4	129	29	24	384	69	18	15	82	0.77	331.4	4.61	0.44	BW62
10W4491	B15.3	408	6.7	6	5	18	379	33	45	75	55	0.34	48.4	2.57	0.36	B15.3
10W4491	BW63	397	6.0	4	17	14	362	22	80	77	20	0.16	10.8	0.71	0.09	BW63
10W4491	CW6	303	6.0	4	73	6	220	20	94	60	6	0.06	1.2	0.56	0.06	CW6
10W4491	BW6	299	5.8	8	215	2	74	20	96	20	4	0.02	0.2	0.76	0.10	BW6

Table 6. *Continued*

	TRAY: 280	POS: 10			LOCAL SPECIFICITY: BW62										
10W4492	BW77	684	8.0	13	0	240	431	80	0	94	100	0.18	22.6	6.84	0.90 BW77
10W4492	BW75	671	7.8	60	1	180	430	79	1	75	99	0.41	114.4	8.63	0.89 BW75
10W4492	BW76	610	4.9	11	3	169	427	74	21	93	79	0.16	16.6	6.13	0.79 BW76
10W4492	BW62	596	7.5	152	13	17	414	78	7	10	93	0.88	459.7	6.69	0.62 BW62
10W4492	B15.3	431	7.0	6	5	11	409	47	45	64	55	0.42	70.3	2.81	0.43 B15.3
10W4492	BW63	420	6.5	8	20	3	389	36	71	27	29	0.43	79.2	1.45	0.17 BW63
	TRAY: 280	POS: 11			LOCAL SPECIFICITY: BW62										
10W4493	BW76	658	7.7	18	0	161	479	82	0	89	100	0.27	49.5	7.68	0.96 BW76
10W4493	BW62	640	7.6	133	34	28	445	81	20	17	80	0.75	356.3	5.41	0.50 BW62
10W4493	BW75	473	7.5	19	36	9	409	60	65	32	35	0.44	91.6	1.56	0.17 BW75
10W4493	BW60	414	5.6	5	32	4	373	22	86	44	14	0.24	24.6	0.60	0.07 BW60
10W4493	BW6	381	6.0	4	239	0	138	25	98	0	2	0.08	2.3	0.88	0.08 BW6
	TRAY: 280	POS: 12			LOCAL SPECIFICITY: BW62,B15.3										
10W4494	BW76	683	8.0	18	0	271	394	85	0	93	100	0.19	25.2	5.81	0.75 BW76
10W4494	BW75	665	8.0	55	3	216	391	84	5	79	95	0.34	77.0	5.61	0.60 BW75
10W4494	BW77	607	7.7	12	1	204	390	80	7	94	93	0.18	18.6	4.29	0.59 BW77
10W4494	BW62	594	7.8	155	10	49	380	79	6	24	94	0.78	359.9	5.53	0.53 BW62
10W4494	B15.3	429	7.1	7	4	42	376	38	36	85	64	0.27	30.4	2.58	0.41 B15.3
10W4494	B27	414	6.4	5	16	36	357	31	76	87	24	0.11	4.8	0.43	0.06 B27
10W4494	B13	393	5.6	5	29	31	328	30	85	86	15	0.06	1.4	0.47	0.05 B13
10W4494	BW6	363	5.9	26	231	6	100	31	89	18	11	0.07	1.9	1.45	0.20 BW6
	TRAY: 280	POS: 13			LOCAL SPECIFICITY: BW62,B15.3										
10W4495	BW77	686	7.0	12	1	170	503	67	7	93	93	0.21	29.4	5.76	0.74 BW77
10W4495	BW75	673	7.4	49	13	121	490	67	20	71	80	0.39	104.6	5.24	0.54 BW75
10W4495	BW62	611	7.0	113	54	8	436	61	32	6	68	0.74	331.5	4.02	0.37 BW62
	TRAY: 280	POS: 14			LOCAL SPECIFICITY: B51										
10W4496	B51	682	7.6	38	13	8	623	76	25	17	75	0.77	402.4	4.66	0.47 B51
10W4496	BW6	631	5.8	8	503	0	120	37	98	0	2	0.05	1.9	0.64	0.09 BW6
	TRAY: 280	POS: 15			LOCAL SPECIFICITY: BW62,BW63,8W66										
10W4497	BW77	685	8.0	12	1	139	533	70	7	92	93	0.24	38.1	6.42	0.84 BW77
10W4497	BW76	672	7.2	18	0	121	533	67	0	87	100	0.32	70.9	6.58	0.81 BW76
10W4497	BW75	654	7.3	46	12	75	521	66	20	61	80	0.49	156.1	4.44	0.47 BW75
10W4497	BW62	596	6.8	62	104	13	417	57	62	17	38	0.46	128.3	1.39	0.13 BW62
10W4497	BW63	430	5.5	4	23	9	394	30	85	69	15	0.18	13.7	0.56	0.06 BW63
	TRAY: 280	POS: 16			LOCAL SPECIFICITY: BW62,B13										
10W4498	BW76	685	8.0	18	0	194	473	68	0	91	100	0.25	41.2	6.95	0.90 BW76
10W4498	BW77	667	7.8	13	0	181	473	65	0	93	100	0.22	32.3	6.43	0.88 BW77
10W4498	BW75	654	7.7	46	12	135	461	63	20	74	80	0.36	84.8	4.66	0.51 BW75
10W4498	B13	586	7.0	23	19	109	435	56	45	82	55	0.21	26.9	2.36	0.27 B13
10W4498	BW62	554	6.6	90	69	22	373	52	43	19	57	0.57	183.1	2.49	0.24 BW62
10W4498	BW57	395	6.7	9	14	13	359	45	60	59	40	0.36	52.3	1.62	0.20 BW57
10W4498	BW63	372	6.0	5	19	8	340	30	79	61	21	0.25	22.9	0.76	0.10 BW63

	TRAY: 280	POS: 17			LOCAL SPECIFICITY: BW62,B35,B50,BW77										
10W4499	BW75	666	7.9	61	0	287	318	75	0	82	100	0.30	61.4	7.14	0.75 BW75
10W4499	BW77	605	7.7	13	0	274	318	71	0	95	100	0.16	14.7	5.03	0.68 BW77
10W4499	BW70	592	7.1	48	2	226	316	70	4	82	96	0.30	54.3	6.07	0.67 BW70
10W4499	B15.3	542	6.7	11	1	215	315	73	8	95	92	0.15	12.6	4.34	0.59 B15.3
10W4499	BW62	530	7.6	148	11	67	304	73	6	31	94	0.70	259.8	5.74	0.54 BW62
10W4499	BW56	367	7.3	8	1	57	301	46	11	87	89	0.30	32.1	3.40	0.49 BW56
10W4499	B35	362	7.1	33	7	26	296	42	17	44	83	0.63	144.5	3.56	0.41 B35
	TRAY: 280	POS: 18			LOCAL SPECIFICITY: BW62,CW4,CW7										
10W4500	BW76	679	8.0	18	0	348	313	84	0	95	100	0.15	15.8	3.89	0.51 BW76
10W4500	B15.3	661	7.1	14	0	334	313	83	0	95	100	0.14	12.9	3.47	0.48 B15.3
10W4500	BW75	647	7.8	56	1	278	312	84	1	93	99	0.29	54.4	4.23	0.47 BW75
10W4500	BW62	590	7.9	159	5	119	307	82	3	92	97	0.62	226.4	4.68	0.45 BW62
10W4500	BW77	426	7.6	10	1	109	306	67	9	91	91	0.23	22.2	2.55	0.37 BW77
10W4500	B35	415	7.6	36	6	73	300	65	14	86	86	0.45	85.3	2.39	0.27 B35
10W4500	CW4	371	7.7	26	3	47	295	54	10	64	90	0.51	97.5	2.26	0.22 CW4
10W4500	BW70	344	7.4	14	31	33	266	38	68	70	32	0.20	13.4	0.53	0.06 BW70
10W4500	BW46	299	4.6	16	61	17	205	21	79	51	21	0.18	10.0	0.53	0.06 BW46
10W4500	BW6	222	5.8	13	114	4	91	41	89	23	11	0.11	2.8	0.85	0.08 BW6
	TRAY: 280	POS: 19			LOCAL SPECIFICITY: BW4,BW62										
10W4501	BW62	680	7.9	166	3	380	131	85	1	69	99	0.26	45.7	3.23	0.33 BW62
10W4501	B38	511	7.9	26	0	354	131	81	0	93	100	0.14	9.4	2.31	0.31 B38
10W4501	BW76	485	8.0	15	0	339	131	80	0	95	100	0.11	5.7	1.97	0.29 BW76
10W4501	BW58	470	8.0	52	1	287	130	79	1	84	99	0.21	20.1	2.44	0.29 BW58
10W4501	BW77	417	8.0	12	0	275	130	75	0	95	100	0.12	5.6	1.80	0.28 BW77
10W4501	BW52	405	7.6	18	0	257	130	74	0	93	100	0.15	8.9	1.88	0.27 BW52
10W4501	BW57	387	8.0	21	1	236	129	74	4	91	96	0.15	8.8	1.75	0.24 BW57
10W4501	BW75	365	7.9	40	2	196	127	72	4	83	96	0.23	19.4	1.86	0.23 BW75
10W4501	BW63	323	7.8	18	1	178	126	67	5	90	95	0.17	9.8	1.56	0.22 BW63
10W4501	B51	304	7.6	24	1	154	125	64	4	86	96	0.23	15.7	1.59	0.21 B51
10W4501	B15.3	279	7.7	7	1	147	124	61	12	95	88	0.11	3.5	0.90	0.15 B15.3
10W4501	B27	270	6.9	13	2	133	122	60	13	91	87	0.16	6.8	0.91	0.13 B27
10W4501	B44	256	7.2	38	13	96	109	60	25	71	75	0.22	12.5	1.04	0.13 B44
10W4501	AW34	145	8.0	7	0	58	80	56	0	89	100	0.25	9.1	0.63	0.09 AW34
10W4501	A10	138	8.0	9	1	49	79	51	10	84	90	0.27	10.2	0.60	0.07 A10
10W4501	AW33	174	6.5	8	3	64	99	45	27	88	73	0.17	4.8	0.44	0.05 AW33
10W4501	BW4	177	6.8	28	17	44	88	47	37	61	63	0.26	11.6	2.78	0.40 BW4
	TRAY: 280	POS: 20			LOCAL SPECIFICITY: B49,BW51,BW52,BW63,BW66										
10W4502	BW52	678	7.4	23	2	81	572	75	8	77	92	0.42	117.5	6.30	0.73 BW52
10W4502	B51	653	7.9	47	4	34	568	75	7	41	93	0.70	323.8	5.72	0.58 B51
10W4502	BW77	602	7.1	7	2	27	566	50	22	79	78	0.38	89.2	3.59	0.48 BW77
10W4502	BW63	593	7.1	9	12	18	554	48	57	66	43	0.35	73.5	2.03	0.23 BW63
10W4502	BW4	572	6.4	14	249	4	305	44	94	22	6	0.12	7.6	0.97	0.08 BW4
	TRAY: 280	POS: 21			LOCAL SPECIFICITY: B17,BW63										
10W4504	BW57	686	7.9	29	2	80	575	84	6	73	94	0.46	146.5	6.16	0.68 BW57
10W4504	BW58	655	8.0	52	2	28	573	80	3	35	97	0.77	388.1	6.32	0.63 BW58
10W4504	BW63	601	6.8	10	18	18	555	42	64	64	36	0.33	63.8	1.06	0.12 BW63
10W4504	AW33	551	5.3	6	42	12	491	33	87	66	13	0.16	14.2	0.50	0.05 AW33
10W4504	BW4	525	5.8	8	223	4	290	41	96	33	4	0.07	2.6	0.83	0.07 BW4

Table 6. *Continued*

10W4505	TRAY: 280	POS: 22			LOCAL SPECIFICITY: B17,BW63,B49,BN										
10W4505	BW77	685	7.8	9	4	87	585	64	30	90	70	0.22	33.5	3.88	0.49 BW77
10W4505	BW57	672	7.0	25	6	62	579	62	19	71	81	0.44	132.2	4.28	0.47 BW57
10W4505	BW58	641	7.2	38	15	24	564	59	28	38	72	0.63	254.4	3.61	0.37 BW58
10W4505	BW63	588	6.8	8	20	16	544	37	71	66	29	0.28	45.0	1.20	0.13 BW63
10W4505	B51	560	4.8	8	33	8	511	31	80	50	20	0.28	44.2	0.57	0.06 B51
10W4505	BW4	519	7.0	6	204	2	307	50	97	25	3	0.09	4.0	0.87	0.08 BW4
	TRAY: 280	POS: 23			LOCAL SPECIFICITY: B5,B49,BW63										
10W4506	BW52	681	7.6	25	0	126	530	80	0	83	100	0.37	91.1	6.14	0.70 BW52
10W4506	BW77	656	8.0	13	0	113	530	80	0	89	100	0.29	55.8	5.50	0.70 BW77
10W4506	BW53	643	7.7	13	1	100	529	78	7	88	93	0.30	56.0	5.48	0.69 BW53
10W4506	B51	629	8.0	45	1	55	528	78	2	55	98	0.63	249.1	6.14	0.64 B51
10W4506	BW63	583	7.8	17	5	38	523	61	22	69	78	0.46	123.1	3.63	0.42 BW63
10W4506	BW57	561	6.0	7	20	31	503	50	74	81	26	0.17	16.5	0.76	0.09 BW57
10W4506	BW4	534	6.8	25	201	6	302	54	88	19	12	0.19	19.8	0.62	0.09 BW4
	TRAY: 280	POS: 24			LOCAL SPECIFICITY: B15,BW46,CW3,B17										
10W4507	BW77	680	8.0	13	0	402	265	91	0	96	100	0.11	8.5	4.55	0.65 BW77
10W4507	B15.3	667	7.8	13	0	389	265	90	0	96	100	0.11	8.7	4.52	0.64 B15.3
10W4507	BW76	654	7.4	18	0	371	265	90	0	95	100	0.14	12.6	4.78	0.64 BW76
10W4507	BW46	636	7.9	89	3	282	262	91	3	76	97	0.32	65.3	5.89	0.62 BW46
10W4507	BW62	544	8.0	155	2	127	260	90	1	45	99	0.60	194.3	6.13	0.60 BW62
10W4507	BW75	387	8.0	50	1	77	259	80	1	60	99	0.54	113.3	5.29	0.60 BW75
10W4507	BW63	336	7.3	25	2	52	257	68	7	67	93	0.49	80.7	3.79	0.47 BW63
10W4507	B38	309	6.5	8	8	44	249	65	50	84	50	0.21	13.3	1.15	0.16 B38
10W4507	BW61	293	7.4	7	9	37	240	68	56	84	44	0.19	10.9	1.08	0.16 BW61
10W4507	A31	273	7.5	4	5	32	232	63	55	88	45	0.17	7.9	0.91	0.10 A31
10W4507	B51	268	7.3	8	12	25	223	63	60	75	40	0.24	15.3	0.63	0.09 B51
10W4507	B35	248	6.8	8	21	17	202	60	72	68	28	0.21	11.1	0.58	0.07 B35
	TRAY: 280	POS: 25			LOCAL SPECIFICITY: B15,BW57,BW46										
10W4508	BW76	677	8.0	18	0	365	294	92	0	95	100	0.14	14.2	6.25	0.82 BW76
10W4508	BW77	659	8.0	13	0	352	294	91	0	96	100	0.13	10.7	5.86	0.82 BW77
10W4508	B15.3	646	7.7	13	0	339	294	91	0	96	100	0.13	11.1	5.77	0.81 B15.3
10W4508	BW62	633	8.0	164	1	175	293	91	0	51	100	0.55	188.5	8.06	0.78 BW62
10W4508	BW75	468	7.9	55	1	120	292	84	1	68	99	0.46	100.5	6.76	0.75 BW75
10W4508	BW63	412	7.7	27	1	93	291	78	3	77	97	0.40	65.9	5.75	0.70 BW63
10W4508	BW57	384	8.0	19	2	74	289	75	9	79	91	0.37	53.1	4.88	0.63 BW57
10W4508	BW46	363	7.4	62	16	12	273	68	20	16	80	0.77	213.8	3.96	0.42 BW46
	TRAY: 280	POS: 26			LOCAL SPECIFICITY: BW46,CW1,CW3										
10W4509	CW1	670	7.8	95	20	184	371	74	17	65	83	0.38	95.9	4.10	0.40 CW1
10W4509	CW3	555	7.3	159	52	25	319	65	24	13	76	0.70	273.6	3.34	0.30 CW3
10W4509	BW46	354	7.6	5	2	27	320	37	28	84	72	0.31	33.8	2.96	0.29 BW46
10W4509	BW62	347	5.8	16	33	11	287	29	67	40	33	0.38	49.2	0.98	0.09 BW62
10W4509	BW4	298	5.5	8	182	3	105	18	95	27	5	0.04	0.4	1.07	0.16 BW4
	TRAY: 280	POS: 27			LOCAL SPECIFICITY: B15,BW22,CW3										
10W4510	CW3	675	7.7	225	37	24	389	85	14	9	86	0.81	441.4	4.24	0.37 CW3
10W4510	BW62	423	7.3	12	49	20	342	62	80	62	20	0.19	14.9	0.73	0.07 BW62
10W4510	AW33	344	6.3	7	21	13	303	55	75	65	25	0.24	20.5	0.52	0.05 AW33

	TRAY: 280	POS: 28			LOCAL SPECIFICITY: B5,B35,BW53,B18,B15.3,BW62,BW70											
10W4511	BW52	664	8.0	21	0	398	245	89	0	94	100	0.14	12.7	5.54	0.75	BW52
10W4511	B15.3	643	8.0	13	0	385	245	89	0	96	100	0.11	8.2	4.96	0.74	B15.3
10W4511	BW53	630	8.0	13	0	372	245	89	0	96	100	0.12	8.4	4.96	0.74	BW53
10W4511	BW75	617	8.0	59	1	313	244	88	1	84	99	0.26	40.2	6.05	0.69	BW75
10W4511	B35	557	8.0	50	1	263	243	86	1	84	99	0.27	39.9	5.85	0.69	B35
10W4511	B51	506	7.9	45	1	218	242	84	2	82	98	0.29	42.6	5.65	0.67	B51
10W4511	B18	460	7.5	32	1	186	241	81	3	85	97	0.28	35.0	4.93	0.62	B18
10W4511	BW62	427	7.8	119	6	67	235	81	4	36	96	0.67	191.7	5.26	0.55	BW62
10W4511	BW77	302	8.0	4	1	63	234	59	20	94	80	0.18	9.8	2.82	0.51	BW77
10W4511	BW70	297	7.2	33	14	30	220	57	29	47	71	0.52	80.2	2.31	0.28	BW70
10W4511	B38	250	7.3	8	9	22	211	50	52	73	48	0.29	21.2	1.78	0.27	B38
	TRAY: 280	POS: 29			LOCAL SPECIFICITY: B15,B35,B18,B5,4C											
10W4512	B51	683	8.0	51	0	338	294	78	0	86	100	0.25	41.7	5.05	0.57	B51
10W4512	B35	632	7.9	60	0	278	294	74	0	82	100	0.30	57.7	5.02	0.55	B35
10W4512	BW52	572	8.0	24	0	254	294	70	0	91	100	0.22	26.5	4.22	0.54	BW52
10W4512	BW53	548	8.0	10	0	244	294	68	0	96	100	0.15	11.8	3.28	0.49	BW53
10W4512	BW76	538	7.1	15	0	229	294	66	0	93	100	0.19	18.6	3.46	0.48	BW76
10W4512	B37	516	7.2	12	0	213	291	66	0	94	100	0.18	15.9	3.26	0.47	B37
10W4512	B18	511	7.9	36	1	181	293	65	2	83	98	0.31	49.1	3.93	0.47	B18
10W4512	B15.3	474	6.9	9	2	172	291	59	18	95	82	0.14	9.1	2.75	0.46	B15.3
10W4512	BW75	463	7.8	40	7	132	284	59	14	76	86	0.33	51.5	3.13	0.36	BW75
10W4512	B21	410	6.4	5	1	124	280	50	16	96	84	0.14	7.6	1.89	0.31	B21
10W4512	B38	410	7.1	13	2	114	281	50	13	89	87	0.23	22.6	2.17	0.30	B38
10W4512	BW41	389	6.8	5	2	106	276	49	28	95	72	0.13	6.4	1.42	0.23	BW41
10W4512	B13	382	7.1	23	12	83	264	49	34	78	66	0.27	27.7	1.35	0.17	B13
10W4512	CW8	347	7.6	10	3	73	261	42	23	87	77	0.25	20.9	1.58	0.17	CW8
10W4512	B39	334	5.8	8	9	65	252	35	52	89	48	0.14	6.7	0.83	0.12	B39
10W4512	CW4	317	6.8	17	18	48	234	36	51	73	49	0.24	19.0	0.55	0.06	CW4
10W4512	BW6	288	5.8	46	185	5	52	29	80	9	20	0.12	3.9	0.59	0.09	BW6
	TRAY: 280	POS: 30			LOCAL SPECIFICITY: B15,B35,4C											
10W4513	BW76	680	8.0	18	0	400	262	75	0	95	100	0.13	11.6	5.57	0.79	BW76
10W4513	B21	653	7.7	6	0	387	260	74	0	98	100	0.08	4.0	4.33	0.77	B21
10W4513	BW62	656	7.7	163	4	231	258	74	2	58	98	0.45	131.6	6.66	0.68	BW62
10W4513	BW75	489	7.9	53	3	178	255	65	5	77	95	0.34	57.0	5.19	0.61	BW75
10W4513	BW77	433	7.5	11	1	167	254	56	8	93	92	0.17	13.0	3.91	0.60	BW77
10W4513	BW41	417	7.0	6	1	159	251	53	14	96	86	0.12	6.3	3.24	0.55	BW41
10W4513	B15.3	414	8.0	8	3	153	250	54	27	95	73	0.11	5.4	3.29	0.51	B15.3
10W4513	BW70	403	6.6	43	8	110	242	51	15	71	85	0.36	53.3	3.64	0.44	BW70
10W4513	B35	352	7.0	30	11	80	231	51	26	72	74	0.33	38.0	2.76	0.34	B35
10W4513	BW53	311	7.0	4	5	76	226	47	55	95	45	0.07	1.7	1.84	0.30	BW53
10W4513	B45	296	6.3	6	6	70	214	46	50	92	50	0.11	3.9	1.70	0.26	B45
10W4513	BW60	288	6.3	16	16	54	202	47	50	77	50	0.21	12.9	1.52	0.20	BW60
10W4513	B44	258	7.0	26	19	28	185	50	42	51	58	0.42	44.7	1.69	0.20	B44
10W4513	BW61	213	6.5	4	7	24	178	35	63	85	37	0.16	5.5	1.13	0.18	BW61
10W4513	B51	202	5.5	8	16	16	162	33	66	66	34	0.24	12.0	0.97	0.14	B51
10W4513	BW57	178	6.5	4	14	12	148	37	77	75	23	0.16	4.3	0.57	0.08	BW57
10W4513	BW6	160	5.4	10	84	2	64	33	89	16	11	0.14	3.2	1.35	0.19	BW6

Table 6. *Continued*

	TRAY: 280	POS: 31		LOCAL SPECIFICITY: B15,B17										
10W4514	BW76	680	8.0	18	0	448	214	91	0	96	100	0.11	8.5	5.91	0.84	BW76
10W4514	B15.3	662	8.0	14	0	434	214	91	0	96	100	0.10	6.8	5.60	0.84	B15.3
10W4514	BW77	648	8.0	12	0	422	214	91	0	97	100	0.10	6.0	5.41	0.83	BW77
10W4514	BW62	636	8.0	163	1	259	213	90	0	61	100	0.41	108.0	7.78	0.80	BW62
10W4514	BW75	472	8.0	55	1	204	212	85	1	78	99	0.32	48.2	6.50	0.76	BW75
10W4514	BW57	416	8.0	22	1	182	211	81	4	89	96	0.23	21.2	5.32	0.72	BW57
10W4514	BW63	393	7.9	24	2	158	209	79	7	86	93	0.25	23.7	4.82	0.64	BW63
10W4514	BW46	367	7.5	68	8	90	2[illegible]	76	10	56	90	0.48	84.2	4.99	0.57	BW46
10W4514	BW70	291	7.1	41	8	49	1[illegible]	72	16	54	84	0.51	76.7	4.38	0.53	BW70
10W4514	B35	242	7.5	30	8	19	1[illegible]	75	21	38	79	0.63	96.2	3.85	0.49	B35
10W4514	B51	204	7.3	8	14	11	1[illegible]	68	63	57	37	0.32	21.4	1.07	0.15	B51
10W4514	BW4	182	6.8	10	109	1	8[illegible]	63	91	9	9	0.14	3.4	1.32	0.20	BW4
	TRAY: 280	POS: 32		LOCAL SPECIFICITY: B17,B15										
10W4515	BW76	682	8.0	18	0	249	415	85	0	93	100	0.21	28.7	6.95	0.90	BW76
10W4515	BW77	664	8.0	13	0	236	413	84	0	94	100	0.18	22.1	6.50	0.89	BW77
10W4515	B15.3	651	7.3	11	2	225	413	83	15	95	85	0.14	13.4	5.04	0.69	B15.3
10W4515	BW62	638	7.8	141	24	84	389	83	14	37	86	0.62	245.6	5.46	0.52	BW62
10W4515	BW75	473	7.6	39	16	45	373	67	29	53	71	0.50	120.4	3.76	0.41	BW75
10W4515	BW63	418	6.5	12	16	33	357	51	57	73	43	0.28	32.2	2.00	0.25	BW63
10W4515	BW57	390	6.4	5	15	28	342	51	75	84	25	0.14	7.4	0.97	0.12	BW57
10W4515	BW46	370	6.6	21	57	7	285	53	73	25	27	0.38	52.9	0.81	0.08	BW46
10W4515	BW6	292	6.0	6	209	1	76	28	97	14	3	0.04	0.5	0.83	0.11	BW6
	TRAY: 280	POS: 33		LOCAL SPECIFICITY: BW62										
10W9228	BW77	527	8.0	13	0	246	268	76	0	94	100	0.16	13.8	5.56	0.84	BW77
10W9228	BW76	514	6.7	18	0	228	268	75	0	92	100	0.20	20.3	5.53	0.79	BW76
10W9228	B15.3	496	6.4	11	0	217	268	76	0	95	100	0.16	13.2	4.80	0.75	B15.3
10W9228	BW62	485	7.6	133	17	84	251	77	11	38	89	0.59	169.5	5.01	0.52	BW62
10W9228	BW75	335	7.8	36	12	48	239	64	25	57	75	0.47	74.3	3.37	0.41	BW75
10W9228	BW63	287	7.1	13	14	35	225	43	51	72	49	0.27	21.1	1.45	0.20	BW63
10W9228	BW57	260	6.8	5	9	30	216	37	64	85	36	0.16	6.3	1.11	0.17	BW57
10W9228	BW46	246	6.2	18	51	12	165	33	73	40	27	0.27	17.3	0.46	0.05	BW46
	TRAY: 280	POS: 34		LOCAL SPECIFICITY: BW62,BW62S,BW76,B45CR										
10W9229	BW76	521	8.0	18	0	180	323	80	0	90	100	0.24	30.4	6.37	0.82	BW76
10W9229	BW62	503	7.7	133	18	47	305	78	11	26	89	0.71	256.8	5.23	0.50	BW62
10W9229	BW75	352	6.7	34	14	13	291	48	29	27	71	0.67	158.7	2.91	0.32	BW75
10W9229	BW6	304	5.3	11	192	2	99	23	94	15	6	0.08	1.9	0.65	0.11	BW6
	TRAY: 281	POS: 3		LOCAL SPECIFICITY: B5										
10W9230	BW52	562	7.7	23	3	61	475	82	11	72	89	0.45	115.9	6.60	0.79	BW52
10W9230	B51	536	7.6	47	2	14	473	78	4	22	96	0.84	382.2	6.97	0.74	B51
10W9230	BW61	487	7.0	4	30	10	443	50	88	71	12	0.15	10.3	0.57	0.06	BW61
10W9230	BW6	453	6.7	9	370	1	73	50	97	10	3	0.03	0.3	0.99	0.16	BW6
	TRAY: 281	POS: 4		LOCAL SPECIFICITY: B5,B35										
10W9231	B51	562	7.8	45	4	45	468	63	8	50	92	0.64	229.5	7.09	0.76	B51
10W9231	BW52	513	6.6	19	7	26	461	33	26	57	74	0.53	141.5	4.12	0.50	BW52
10W9231	BW53	487	6.3	6	7	20	454	23	53	76	47	0.30	44.0	3.26	0.43	BW53
10W9231	B35	474	5.4	13	38	7	416	25	74	35	26	0.37	64.0	0.92	0.10	B35
10W9231	BW61	423	6.4	5	26	2	390	57	83	28	17	0.32	43.1	0.77	0.09	BW61

	TRAY: 281	POS: 5			LOCAL SPECIFICITY: B5											
10W9232	B51	561	7.6	41	7	8	505	77	14	16	86	0.83	387.2	5.22	0.54	B51
	TRAY: 281	POS: 6			LOCAL SPECIFICITY: B5											
10W9234	BW53	561	7.4	10	3	126	422	70	23	92	77	0.19	20.1	2.61	0.35	BW53
10W9234	B51	548	7.6	37	10	89	412	70	21	70	79	0.41	90.2	2.37	0.26	B51
10W9234	BW52	501	6.8	18	9	71	403	64	33	79	67	0.31	46.7	1.73	0.21	BW52
10W9234	BW54	474	6.4	5	8	66	395	63	61	92	39	0.11	5.8	0.77	0.10	BW54
10W9234	B38	461	7.3	6	17	60	378	63	73	90	27	0.08	2.7	0.51	0.06	B38
10W9234	A30	287	7.6	5	6	45	231	66	54	90	46	0.15	6.2	0.40	0.06	A30
	TRAY: 281	POS: 7			LOCAL SPECIFICITY: B15,B35											
10W9235	BW75	563	8.0	47	0	184	332	69	0	79	100	0.36	73.7	5.01	0.57	BW75
10W9235	BW76	516	6.0	14	0	170	332	61	0	92	100	0.22	26.0	3.47	0.49	BW76
10W9235	B35	502	7.8	50	5	120	327	64	9	70	91	0.42	89.7	3.52	0.39	B35
10W9235	BW54	447	6.9	7	6	113	321	52	46	94	54	0.11	5.0	0.89	0.14	BW54
10W9235	B39	434	7.0	8	9	105	312	52	52	92	48	0.10	4.1	0.91	0.12	B39
10W9235	B15.3	417	5.6	5	6	100	306	52	54	95	46	0.08	2.5	0.70	0.10	B15.3
10W9235	A28	406	6.6	20	12	80	294	53	37	80	63	0.26	26.8	0.80	0.09	A28
10W9235	BW60	374	5.5	16	22	64	272	51	57	80	43	0.17	10.8	0.61	0.07	BW60
10W9235	BW6	336	6.8	59	175	5	97	57	74	7	26	0.24	19.0	1.20	0.11	BW6
	TRAY: 281	POS: 8			LOCAL SPECIFICITY: B5,B35											
10W9236	B51	563	7.9	29	20	67	447	47	40	69	60	0.35	67.4	2.23	0.24	B51
10W9236	BW53	514	6.0	6	7	61	440	28	53	91	47	0.16	12.9	1.39	0.20	BW53
10W9236	A30	330	5.0	4	7	37	282	39	63	90	37	0.13	6.0	0.75	0.10	A30
10W9236	BW52	490	6.4	9	17	48	416	29	65	84	35	0.17	14.1	0.61	0.07	BW52
10W9236	A23	464	8.0	4	16	44	400	29	80	91	20	0.07	2.1	0.54	0.07	A23
10W9236	B35	444	5.9	18	34	26	366	22	65	59	35	0.30	40.3	0.54	0.06	B35
10W9236	BW4	392	5.7	12	154	14	212	15	92	53	8	0.02	0.2	0.82	0.15	BW4
	TRAY: 281	POS: 9			LOCAL SPECIFICITY: B5,B35											
10W9237	B51	564	7.7	45	4	31	484	69	8	40	92	0.71	282.6	6.90	0.73	B51
10W9237	BW52	515	6.7	12	15	19	469	38	55	61	45	0.38	74.4	2.27	0.27	BW52
10W9237	B35	488	5.6	15	40	4	429	26	72	21	28	0.43	90.5	1.02	0.11	B35
	TRAY: 281	POS: 10			LOCAL SPECIFICITY: B5,B35											
10W9238	B51	564	7.0	36	11	23	492	59	26	38	74	0.64	227.4	3.55	0.37	B51
10W9238	BW52	515	7.0	10	3	13	475	43	62	56	38	0.37	70.8	1.43	0.16	BW52
	TRAY: 281	POS: 11			LOCAL SPECIFICITY: B5,B35											
10W9239	BW53	557	7.3	12	2	60	483	75	14	83	86	0.35	67.6	4.55	0.58	BW53
10W9239	B35	543	7.7	32	20	28	463	76	38	46	62	0.52	149.1	3.19	0.34	B35
10W9239	B51	491	7.3	21	27	7	436	64	56	25	44	0.54	143.2	2.09	0.22	B51
	TRAY: 281	POS: 12			LOCAL SPECIFICITY: B15											
10W9240	BW76	562	8.0	18	0	274	270	80	0	93	100	0.17	17.2	4.29	0.60	BW76
10W9240	BW77	544	8.0	13	0	261	270	78	0	95	100	0.16	13.1	3.93	0.58	BW77
10W9240	BW75	531	7.9	41	2	220	268	77	4	84	96	0.27	40.0	4.39	0.53	BW75
10W9240	B15.3	488	7.6	10	1	210	267	74	9	95	91	0.14	9.5	2.77	0.43	B15.3
10W9240	BW62	477	7.6	138	21	72	246	73	13	34	87	0.61	177.0	3.15	0.32	BW62
10W9240	BW63	318	6.5	16	11	56	235	54	40	77	60	0.27	22.6	1.40	0.19	BW63
10W9240	B39	291	6.3	6	7	50	228	58	53	89	47	0.15	6.3	0.76	0.11	B39

Table 6. *Continued*

	TRAY: 281	POS: 13			LOCAL SPECIFICITY: B15										
10W9241	BW75	560	7.3	45	2	138	375	54	4	75	96	0.41	9[illegible]	6.13	0.71 BW75
10W9241	BW76	513	6.1	14	0	124	375	47	0	89	100	0.28	3[illegible]	4.73	0.66 BW76
10W9241	B21	486	7.0	4	1	119	362	46	20	96	80	0.13	[illegible]	2.75	0.47 B21
10W9241	B35	494	6.9	33	22	87	352	45	40	72	60	0.29	4[illegible]	1.80	0.20 B35
10W9241	B18	439	5.7	13	16	74	336	41	55	85	45	0.17	1[illegible]	1.07	0.14 B18
10W9241	CW2	252	5.7	6	10	52	184	48	62	89	38	0.09	[illegible]	0.86	0.13 CW2
10W9241	B39	394	6.4	5	11	63	315	50	68	92	32	0.08	2[illegible]	0.78	0.11 B39
10W9241	BW62	378	6.6	45	86	18	229	49	65	28	35	0.35	45[illegible]	0.78	0.08 BW62
10W9241	BW6	247	6.3	16	128	2	101	44	88	11	12	0.17	7[illegible]	1.08	0.10 BW6
	TRAY: 281	POS: 14			LOCAL SPECIFICITY: B15										
10W9242	BW76	563	5.5	13	5	34	511	27	27	72	73	0.42	99.2	3.05	0.38 BW76
10W9242	BW6	545	5.6	30	399	4	112	26	93	11	7	0.06	2.3	0.72	0.12 BW6
	TRAY: 281	POS: 15			LOCAL SPECIFICITY: B15										
10W9243	BW76	553	8.0	18	0	123	412	59	0	87	100	0.31	54.4	6.34	0.86 BW76
10W9243	B45	496	8.0	8	0	110	378	53	0	93	100	0.23	26.0	5.20	0.82 B45
10W9243	BW55	527	7.0	4	5	111	407	50	55	96	45	0.07	2.7	2.01	0.31 BW55
10W9243	BW75	518	7.6	23	19	88	388	50	45	79	55	0.24	30.2	2.11	0.25 BW75
10W9243	B39	476	8.0	5	12	83	376	42	70	94	30	0.05	1.4	1.38	0.19 B39
10W9243	BW54	459	6.0	5	8	78	368	38	61	93	39	0.09	3.8	1.14	0.16 BW54
10W9243	BW62	446	6.2	53	98	25	270	38	64	32	36	0.33	49.1	0.84	0.08 BW62
10W9243	BW6	295	5.6	21	170	4	100	28	89	16	11	0.12	4.4	0.53	0.05 BW6
	TRAY: 281	POS: 16			LOCAL SPECIFICITY: B15										
10W9244	BW76	562	7.7	18	0	360	184	76	0	95	100	0.13	9.1	2.28	0.32 BW76
10W9244	B35	544	7.8	53	4	307	180	76	7	85	93	0.19	20.4	1.90	0.22 B35
10W9244	BW55	487	6.2	9	1	298	179	73	10	97	90	0.08	3.2	1.34	0.21 BW55
10W9244	B39	477	7.0	14	4	284	175	73	22	95	78	0.06	1.9	1.38	0.20 B39
10W9244	BW70	459	7.3	27	5	257	170	75	15	90	85	0.13	7.4	1.33	0.17 BW70
10W9244	BW62	427	7.5	121	21	136	149	75	14	52	86	0.36	55.6	1.54	0.16 BW62
10W9244	BW56	285	7.0	8	1	128	148	70	11	94	89	0.15	6.3	0.97	0.15 BW56
10W9244	B8	276	8.0	13	5	115	143	71	27	89	73	0.14	5.2	0.68	0.10 B8
10W9244	BW60	258	7.6	23	10	92	133	67	30	80	70	0.19	9.7	0.73	0.09 BW60
10W9244	CW8	225	7.8	34	3	58	130	65	8	63	92	0.46	47.7	0.90	0.09 CW8
10W9244	B7	188	5.7	13	10	45	120	50	43	77	57	0.21	8.1	0.61	0.08 B7
10W9244	BW61	165	7.4	13	7	32	113	57	35	71	65	0.31	16.3	0.55	0.08 BW61
10W9244	AW34	80	6.7	3	1	17	59	55	25	85	75	0.26	5.6	0.30	0.06 AW34
	TRAY: 281	POS: 17			LOCAL SPECIFICITY: BW76										
10W9245	BW76	564	7.2	18	0	24	522	45	0	57	100	0.64	231.1	6.99	0.88 BW76

	TRAY: 281	POS: 18			LOCAL SPECIFICITY: BW46,B17											
10W9246	BW76	563	7.7	18	0	339	206	62	0	94	100	0.14	10.7	1.91	0.27	BW76
10W9246	BW77	545	7.2	13	0	326	206	61	0	96	100	0.12	8.1	1.61	0.25	BW77
10W9246	BW46	532	7.7	88	11	238	195	60	11	73	89	0.27	39.1	1.92	0.21	BW46
10W9246	BW75	433	7.4	31	8	207	187	50	20	86	80	0.16	10.4	1.01	0.13	BW75
10W9246	BW56	394	6.4	5	2	202	185	46	28	97	72	0.05	1.0	0.70	0.12	BW56
10W9246	B13	387	6.8	19	8	183	177	46	29	90	71	0.10	3.8	0.87	0.12	B13
10W9246	B18	360	5.6	17	9	166	168	44	34	90	66	0.08	2.4	0.53	0.07	B18
10W9246	AW34	219	6.0	3	1	106	109	44	25	97	75	0.07	1.0	0.43	0.07	AW34
10W9246	BW60	330	7.1	19	9	144	158	46	32	88	68	0.11	4.2	0.50	0.07	BW60
10W9246	BW58	302	6.9	7	5	137	153	44	41	95	59	0.04	0.6	0.44	0.07	BW58
10W9246	BW54	290	7.7	6	3	131	150	43	33	95	67	0.07	1.4	0.40	0.07	BW54
10W9246	CW8	281	6.4	9	7	122	143	41	43	93	57	0.05	0.6	0.58	0.06	CW8
10W9246	CW1	265	7.3	8	9	114	134	41	52	93	48	0.01	0.0	0.56	0.06	CW1
10W9246	A29	165	5.4	7	2	73	83	40	22	91	78	0.14	3.3	0.34	0.06	A29
	TRAY: 281	POS: 19			LOCAL SPECIFICITY: CW3,CW1.3											
10W9247	BW77	557	8.0	13	0	222	322	73	0	94	100	0.18	18.2	3.88	0.54	BW77
10W9247	B51	544	7.9	41	3	181	319	71	6	81	94	0.32	54.4	3.60	0.41	B51
10W9247	BW63	500	7.9	22	4	159	315	66	15	87	85	0.24	27.8	2.87	0.35	BW63
10W9247	BW52	474	7.8	19	4	140	311	62	17	88	83	0.23	26.1	2.47	0.31	BW52
10W9247	BW53	451	7.0	6	4	134	307	58	40	95	60	0.09	4.0	1.33	0.22	BW53
10W9247	BW57	441	6.2	12	6	122	301	58	33	91	67	0.16	11.7	1.33	0.17	BW57
10W9247	BW76	423	7.6	9	6	113	295	59	40	92	60	0.13	7.4	1.16	0.16	BW76
10W9247	BW46	408	7.0	60	30	53	265	58	33	46	67	0.46	87.6	1.42	0.15	BW46
10W9247	BW58	318	7.0	6	7	47	258	49	53	88	47	0.16	8.5	0.91	0.13	BW58
10W9247	A32	220	6.4	5	4	33	178	52	44	86	56	0.21	9.6	0.48	0.06	A32
	TRAY: 281	POS: 20			LOCAL SPECIFICITY: CW3,CW1.3											
10W9248	CW3	564	7.9	228	7	78	251	84	2	25	98	0.73	296.9	4.75	0.44	CW3
10W9248	BW57	329	6.2	9	6	69	245	57	40	88	60	0.19	11.4	1.44	0.19	BW57
10W9248	BW46	314	7.0	33	18	36	227	62	35	52	65	0.45	64.8	1.24	0.13	BW46
10W9248	BW62	263	7.1	11	38	25	189	61	77	69	23	0.12	3.9	0.82	0.08	BW62
10W9248	BW54	214	8.0	4	3	21	186	60	42	84	58	0.26	14.5	0.53	0.08	BW54
10W9248	B8	207	5.2	5	14	16	172	52	73	76	27	0.17	6.0	0.55	0.07	B8
	TRAY: 281	POS: 21			LOCAL SPECIFICITY: CW3,CW1.3											
10W9249	CW1	562	7.9	92	19	185	236	80	17	66	83	0.33	62.5	3.22	0.33	CW1
10W9249	CW3	451	7.4	157	30	28	236	72	16	15	84	0.73	243.4	3.41	0.33	CW3
10W9249	BW46	264	7.1	9	3	19	233	42	25	67	75	0.46	55.0	2.27	0.23	BW46
10W9249	BW62	252	5.1	7	37	12	196	26	84	63	16	0.15	5.4	0.66	0.06	BW62
	TRAY: 281	POS: 22			LOCAL SPECIFICITY: CW1,CW3,CW3.1											
10W9250	CW3	561	7.9	230	3	128	200	92	1	35	99	0.61	210.2	4.06	0.39	CW3
10W9250	BW46	328	8.0	51	0	77	200	82	0	60	100	0.54	94.4	3.35	0.35	BW46
10W9250	CW1	277	7.7	20	5	57	195	72	20	74	80	0.37	37.3	2.79	0.31	CW1
10W9250	BW56	252	4.0	1	0	56	195	66	0	98	100	0.12	3.4	1.87	0.31	BW56
10W9250	B51	251	7.3	18	10	38	185	67	35	67	65	0.36	32.0	0.86	0.10	B51
10W9250	BW62	223	6.8	10	28	28	157	65	73	73	27	0.11	2.8	0.83	0.08	BW62
10W9250	BW75	185	6.9	9	14	19	143	75	60	67	40	0.25	11.8	0.43	0.05	BW75

Table 6. *Continued*

	TRAY: 281	POS: 23			LOCAL SPECIFICITY: B15,BW46											
10W9251	BW76	563	6.9	18	0	291	254	79	0	94	100	0.16	15.3	5.25	0.72	BW76
10W9251	BW77	545	8.0	13	0	278	254	81	0	95	100	0.15	11.6	4.92	0.72	BW77
10W9251	BW46	532	8.0	97	2	181	252	80	2	65	98	0.44	101.9	5.94	0.63	BW46
10W9251	BW75	433	7.8	36	3	145	249	69	7	80	93	0.32	44.9	4.32	0.52	BW75
10W9251	BW63	394	7.4	19	9	126	240	63	32	86	68	0.18	12.5	2.97	0.39	BW63
10W9251	BW54	366	6.9	7	4	119	236	61	36	94	64	0.11	4.3	2.53	0.38	BW54
10W9251	B15.3	355	8.0	6	5	113	231	63	45	94	55	0.08	2.3	2.21	0.33	B15.3
10W9251	BW62	344	6.9	96	48	17	183	51	33	15	67	0.61	128.4	2.58	0.26	BW62
10W9251	BW57	200	7.3	6	8	11	175	10	57	64	43	0.34	22.8	1.40	0.20	BW57
10W9251	CW1	186	7.0	4	11	7	164	3	73	63	27	0.26	12.6	0.83	0.08	CW1
	TRAY: 281	POS: 24			LOCAL SPECIFICITY: BW75											
10W9252	BW75	561	6.6	37	10	107	407	[illegible]	21	74	79	0.37	75.7	3.13	0.37	BW75
10W9252	BW55	514	5.0	4	5	103	402	[illegible]	55	96	45	0.08	3.1	1.89	0.30	BW55
10W9252	BW54	505	6.3	7	6	96	396	[illegible]	46	93	54	0.13	9.2	1.67	0.24	BW54
10W9252	B35	492	6.7	29	26	67	370	[illegible]	47	69	53	0.30	43.5	1.84	0.21	B35
10W9252	B51	437	6.5	13	35	54	335	[illegible]	72	80	28	0.11	5.7	0.64	0.08	B51
	TRAY: 281	POS: 25			LOCAL SPECIFICITY: CW1,CW1.3											
10W9253	BW41	560	6.5	4	2	192	362	55	33	97	67	0.07	2.7	1.91	0.43	BW41
10W9253	BW54	554	7.0	14	2	178	360	59	12	92	88	0.19	20.3	2.31	0.32	BW54
10W9253	BW77	538	5.8	11	2	167	358	60	15	93	85	0.17	16.0	2.06	0.30	BW77
10W9253	CW1	525	7.6	83	17	84	341	62	17	50	83	0.53	149.2	2.24	0.22	CW1
10W9253	BW46	425	6.2	9	14	75	327	39	60	89	40	0.12	5.8	1.72	0.18	BW46
10W9253	AW34	280	7.0	6	5	48	221	38	45	88	55	0.18	9.1	0.55	0.08	AW34
10W9253	B13	391	6.3	8	14	61	308	37	63	88	37	0.12	5.6	0.57	0.07	B13
10W9253	BW6	369	6.1	51	257	10	51	37	83	16	17	0.00	0.0	2.16	0.35	BW6
	TRAY: 281	POS: 26			LOCAL SPECIFICITY: TS1,B35,BW62											
10W9254	B35	566	7.9	54	3	266	243	81	5	83	95	0.26	37.6	4.52	0.53	B35
10W9254	BW75	509	8.0	47	3	219	240	78	6	82	94	0.28	38.7	4.31	0.51	BW75
10W9254	BW62	459	7.7	138	10	81	230	73	6	36	94	0.63	181.5	4.52	0.47	BW62
10W9254	BW70	311	6.8	26	5	55	225	51	16	67	84	0.44	59.8	3.50	0.46	BW70
10W9254	BW76	280	6.8	10	3	45	222	49	23	81	77	0.32	28.3	2.32	0.35	BW76
10W9254	B15.3	267	6.4	5	4	40	218	46	44	88	56	0.19	10.0	1.63	0.27	B15.3
10W9254	BW60	258	5.7	13	17	27	201	45	56	67	44	0.28	20.1	0.95	0.12	BW60
10W9254	B39	228	6.5	4	9	23	192	59	69	85	31	0.14	4.7	0.70	0.11	B39
10W9254	BW6	215	7.3	16	94	7	98	60	85	30	15	0.13	3.5	1.69	0.16	BW6
	TRAY: 281	POS: 27			LOCAL SPECIFICITY: TS1,B35,BW46,B51,BW52,B15											
10W9255	B35	511	7.9	51	2	286	172	86	3	84	97	0.22	24.1	5.63	0.72	B35
10W9255	BW52	458	8.0	24	0	262	172	84	0	91	100	0.18	15.2	5.09	0.71	BW52
10W9255	BW77	434	8.0	12	0	250	172	83	0	95	100	0.14	8.1	4.34	0.70	BW77
10W9255	BW53	422	7.6	10	0	240	172	82	0	96	100	0.13	7.0	4.13	0.67	BW53
10W9255	B18	412	8.0	26	2	214	170	82	7	89	93	0.19	14.8	4.14	0.60	B18
10W9255	B51	384	7.8	40	2	174	168	80	4	81	96	0.28	29.8	4.11	0.54	B51
10W9255	B15.3	342	8.0	7	0	167	168	77	0	95	100	0.14	6.9	3.15	0.54	B15.3
10W9255	BW75	335	7.9	41	2	126	166	76	4	75	96	0.35	40.8	4.12	0.52	BW75
10W9255	BW76	292	6.9	7	3	119	163	70	30	94	70	0.10	3.0	2.11	0.36	BW76
10W9255	BW63	282	6.7	9	4	110	159	70	30	92	70	0.12	4.1	1.90	0.31	BW63
10W9255	BW62	269	7.2	66	33	44	126	71	33	40	67	0.40	43.1	1.72	0.20	BW62
10W9255	BW46	170	7.5	36	35	8	91	75	49	18	51	0.48	39.2	0.96	0.12	BW46

	TRAY: 281	POS: 28		LOCAL SPECIFICITY: TS1,B35,B51,BW52,B15											
10W9256	B35	571	8.0	55	3	243	270	82	5	81	95	0.29	47.0	5.53	0.63 B35
10W9256	BW52	513	7.8	25	0	218	270	78	0	89	100	0.24	29.2	4.71	0.59 BW52
10W9256	BW53	488	7.6	11	0	207	270	77	0	94	100	0.17	13.9	4.04	0.57 BW53
10W9256	B15.3	477	7.6	9	1	198	269	76	10	95	90	0.14	9.0	3.69	0.55 B15.3
10W9256	BW75	467	8.0	47	2	151	267	75	4	76	96	0.37	64.2	4.10	0.46 BW75
10W9256	BW77	418	8.0	10	1	141	266	68	9	93	91	0.19	14.7	3.02	0.44 BW77
10W9256	B51	407	7.9	38	4	103	262	65	9	73	91	0.40	64.5	3.73	0.43 B51
10W9256	B18	365	7.3	19	7	84	255	54	26	81	74	0.28	27.8	3.03	0.39 B18
10W9256	BW62	339	6.7	54	65	30	190	50	54	35	46	0.35	41.7	0.74	0.08 BW62
	TRAY: 281	POS: 29		LOCAL SPECIFICITY: TS1,B35,B51,BW52,B15											
10W9257	BW76	551	8.0	18	0	324	209	82	0	94	100	0.14	11.4	4.49	0.70 BW76
10W9257	BW77	533	8.0	13	0	311	209	81	0	95	100	0.13	8.6	4.15	0.69 BW77
10W9257	B15.3	520	7.6	9	0	302	209	80	0	97	100	0.11	6.2	3.79	0.68 B15.3
10W9257	BW53	511	7.5	11	2	291	207	80	15	96	85	0.08	3.6	4.03	0.67 BW53
10W9257	BW75	498	7.9	45	4	246	203	80	8	84	92	0.22	25.0	4.30	0.56 BW75
10W9257	B35	449	8.0	43	5	203	198	77	10	82	90	0.24	26.3	3.70	0.48 B35
10W9257	BW62	401	7.6	124	14	79	184	72	10	38	90	0.57	129.6	3.61	0.41 BW62
10W9257	BW70	263	6.5	19	11	60	173	53	36	75	64	0.26	17.9	2.41	0.35 BW70
10W9257	BW52	233	6.7	12	6	48	167	58	33	80	67	0.27	17.1	1.69	0.26 BW52
10W9257	B51	215	6.8	17	9	31	158	58	34	64	66	0.38	31.6	1.69	0.25 B51
10W9257	BW57	189	6.0	5	8	26	150	54	61	83	39	0.16	5.0	0.88	0.15 BW57
10W9257	BW63	176	6.0	4	9	22	141	57	69	84	31	0.13	2.9	0.68	0.11 BW63
	TRAY: 281	POS: 30		LOCAL SPECIFICITY: TS1,BW46											
10W9258	BW76	568	6.7	18	0	196	354	63	0	91	100	0.23	30.8	4.15	0.58 BW76
10W9258	BW77	550	7.3	12	1	184	353	64	7	93	93	0.18	18.6	3.79	0.56 BW77
10W9258	BW46	537	7.6	86	17	98	336	63	16	53	84	0.51	137.1	3.11	0.33 BW46
10W9258	BW55	434	6.0	4	3	94	333	44	42	95	58	0.11	4.9	1.41	0.24 BW55
10W9258	BW58	427	6.0	8	6	86	327	44	42	91	58	0.16	10.4	1.40	0.21 BW58
10W9258	BW54	413	7.0	8	4	78	323	45	33	90	67	0.20	15.8	1.38	0.20 BW54
10W9258	BW75	401	7.1	20	18	58	305	43	47	74	53	0.27	29.5	1.04	0.13 BW75
10W9258	CW1	363	6.6	10	13	48	292	36	56	82	44	0.20	13.8	1.30	0.12 CW1
	TRAY: 281	POS: 31		LOCAL SPECIFICITY: TS1,BW62											
10W9259	B7	558	6.3	28	19	45	466	38	40	61	60	0.42	97.6	2.44	0.27 B7
10W9259	BW60	511	4.9	19	28	26	438	37	59	57	41	0.36	64.4	1.77	0.20 BW60
10W9259	BW75	464	7.5	8	35	18	403	50	81	69	19	0.18	15.1	0.51	0.06 BW75
	TRAY: 281	POS: 32		LOCAL SPECIFICITY: BW62,BW63											
10W9260	BW76	569	7.8	18	0	190	361	70	0	91	100	0.24	32.3	6.58	0.90 BW76
10W9260	BW77	551	7.2	12	1	178	360	68	7	93	93	0.19	19.7	5.05	0.73 BW77
10W9260	BW75	538	7.0	38	11	140	349	67	22	78	78	0.30	48.1	4.06	0.48 BW75
10W9260	BW62	489	7.2	119	40	21	309	67	25	15	75	0.71	246.3	3.84	0.38 BW62
10W9260	BW63	330	5.3	6	19	15	290	23	76	71	24	0.21	14.1	0.76	0.10 BW63
10W9260	BW60	305	5.3	6	26	9	264	26	81	60	19	0.22	14.6	0.70	0.09 BW60
	TRAY: 281	POS: 33		LOCAL SPECIFICITY: BW62											
10W9261	BW76	567	8.0	18	0	183	366	68	0	91	100	0.24	33.9	6.11	0.78 BW76
10W9261	BW77	549	6.8	10	3	173	363	65	23	94	77	0.14	11.4	4.74	0.70 BW77
10W9261	BW75	536	7.0	39	10	134	353	65	20	77	80	0.32	55.2	3.91	0.43 BW75
10W9261	BW62	487	7.2	115	42	19	311	65	26	14	74	0.71	243.0	3.38	0.32 BW62

Table 6. *Continued*

	TRAY: 281	POS: 34			LOCAL SPECIFICITY: TS1,B35,B51,BW52										
10W9262	BW77	568	8.0	12	1	179	376	81	7	93	93	0.19	20.5	5.35	0.75 BW77
10W9262	B51	555	8.0	41	4	138	372	79	8	77	92	0.37	77.6	5.78	0.66 B51
10W9262	B35	510	7.8	47	8	91	364	74	14	65	86	0.46	106.5	5.40	0.61 B35
10W9262	BW52	455	7.6	21	4	70	360	64	16	76	84	0.39	67.7	4.84	0.61 BW52
10W9262	BW53	430	7.1	7	2	63	358	60	22	90	78	0.24	25.5	3.75	0.57 BW53
10W9262	BW75	421	7.2	39	9	24	349	60	18	38	82	0.67	187.1	4.47	0.51 BW75
10W9262	B15.3	373	8.0	4	5	20	344	45	55	83	45	0.24	22.1	3.14	0.47 B15.3
10W9262	CW4	339	7.7	6	25	13	295	36	80	68	20	0.19	12.2	0.56	0.06 CW4
	TRAY: 281	POS: 35			LOCAL SPECIFICITY: B15										
10W9263	BW76	566	8.0	18	0	181	367	77	0	90	100	0.25	34.3	6.10	0.81 BW76
10W9263	BW77	548	7.1	13	0	168	367	75	0	92	100	0.22	27.0	5.58	0.78 BW77
10W9263	BW62	535	7.5	118	40	50	327	76	25	29	75	0.60	195.0	3.58	0.35 BW62
10W9263	BW75	377	7.2	33	16	17	311	60	32	34	68	0.62	143.2	2.75	0.31 BW75
	TRAY: 281	POS: 36			LOCAL SPECIFICITY: CW1,CX46										
10W9264	CW1	529	7.2	78	26	24	401	66	25	23	75	0.70	258.2	2.88	0.28 CW1
10W9264	BW46	425	6.3	6	13	18	388	45	68	75	32	0.24	25.1	2.34	0.23 BW46
	TRAY: 282	POS: 3			LOCAL SPECIFICITY: B51,BW52,BW4										
10W9265	B44	559	7.6	72	5	37	445	72	6	33	94	0.75	311.6	5.47	0.54 B44
10W9265	BW77	482	6.0	7	4	30	441	48	36	81	64	0.32	49.7	2.25	0.30 BW77
10W9265	BW58	471	7.0	8	9	22	432	50	52	73	48	0.32	49.0	1.59	0.20 BW58
10W9265	AW33	419	6.2	9	21	10	379	47	70	52	30	0.34	48.4	1.19	0.12 AW33
10W9265	BW4	424	6.4	11	169	2	242	53	93	15	7	0.15	9.8	1.11	0.10 BW4
	TRAY: 282	POS: 4			LOCAL SPECIFICITY: B51,BW52										
10W9266	BW52	574	6.0	4	25	25	520	44	86	86	14	0.09	4.9	0.40	0.05 BW52
10W9266	BW4	545	6.2	17	255	8	265	44	93	32	7	0.08	3.4	0.40	0.07 BW4
	TRAY: 282	POS: 5			LOCAL SPECIFICITY: TS1										
10W9267	BW46	547	5.3	6	96	6	439	16	94	50	6	0.12	8.0	0.14	0.01 BW46
	TRAY: 282	POS: 6			LOCAL SPECIFICITY: BW46										
10W9268	BW46	568	5.8	11	90	10	457	38	89	47	11	0.18	17.9	0.18	0.02 BW46
	TRAY: 282	POS: 7			LOCAL SPECIFICITY: BW55,B35										
10W9269	BW52	576	7.7	24	5	112	435	68	17	82	83	0.32	59.2	3.71	0.43 BW52
10W9269	B44	547	7.2	55	21	57	414	64	27	50	73	0.52	146.0	3.17	0.33 B44
10W9269	B51	471	7.2	26	23	31	391	56	46	54	54	0.43	86.3	1.77	0.19 B51
10W9269	A23	420	6.0	4	9	27	380	38	69	87	31	0.16	10.7	1.02	0.12 A23
10W9269	B38	409	6.3	7	13	20	369	40	65	74	35	0.26	27.5	0.92	0.11 B38
10W9269	BW4	389	6.5	13	110	7	259	45	89	35	11	0.17	10.9	1.49	0.13 BW4
	TRAY: 282	POS: 8			LOCAL SPECIFICITY: BW46										
10W9270	CW4	531	7.0	4	82	14	431	44	95	77	5	0.03	0.5	0.07	0.01 CW4

	TRAY: 282	POS: 9			LOCAL SPECIFICITY: B5,B35,BW60,BW62										
10W9271	B18	582	7.8	35	0	404	143	91	0	92	100	0.14	12.1	4.33	0.58 B18
10W9271	BW76	547	7.7	18	0	386	143	90	0	95	100	0.11	6.6	3.80	0.58 BW76
10W9271	B15.3	529	8.0	11	0	375	143	91	0	97	100	0.09	4.2	3.27	0.55 B15.3
10W9271	BW53	518	7.6	14	0	361	143	90	0	96	100	0.10	5.5	3.44	0.55 BW53
10W9271	B35	504	8.0	53	2	308	141	91	3	85	97	0.19	18.6	4.09	0.52 B35
10W9271	BW75	449	8.0	41	1	267	140	89	2	86	98	0.20	18.1	3.95	0.51 BW75
10W9271	BW70	407	7.4	27	1	240	139	88	3	89	97	0.18	12.7	3.51	0.49 BW70
10W9271	BW52	379	7.9	25	0	215	139	90	0	89	100	0.20	15.5	3.50	0.48 BW52
10W9271	B51	354	7.8	39	4	176	135	89	9	81	91	0.23	18.4	3.38	0.45 B51
10W9271	BW62	311	7.9	116	9	60	126	88	7	34	93	0.60	111.5	3.62	0.41 BW62
10W9271	BW77	186	8.0	4	1	56	125	71	20	93	80	0.17	5.1	1.92	0.37 BW77
10W9271	BW58	181	8.0	7	2	49	123	69	22	87	78	0.23	9.1	1.73	0.30 BW58
10W9271	BW46	172	6.8	34	38	15	85	65	52	30	48	0.35	21.3	0.57	0.07 BW46
	TRAY: 282	POS: 10			LOCAL SPECIFICITY: B5,B35										
10W9272	AW33	517	5.7	7	57	12	441	52	89	63	11	0.15	10.9	0.38	0.04 AW33
	TRAY: 282	POS: 11			LOCAL SPECIFICITY: B51,BW52,B35,TS1,BW59,B37										
10W9273	A2	530	8.0	278	6	90	156	95	2	24	98	0.66	233.4	3.03	0.28 A2
10W9273	BW53	255	7.6	5	0	91	159	84	0	94	100	0.18	8.4	1.30	0.18 BW53
10W9273	BW63	250	7.9	15	0	76	159	84	0	83	100	0.33	27.9	1.27	0.16 BW63
10W9273	BW77	235	8.0	2	0	74	159	82	0	97	100	0.13	4.2	1.11	0.15 BW77
10W9273	B51	233	8.0	20	1	54	158	82	4	72	96	0.43	42.9	1.04	0.12 B51
10W9273	BW58	212	8.0	6	1	48	157	75	14	88	86	0.26	13.8	0.77	0.10 BW58
10W9273	BW57	205	7.3	9	2	39	155	72	18	81	82	0.33	22.1	0.80	0.10 BW57
10W9273	BW52	194	8.0	12	2	27	153	71	14	69	86	0.46	40.4	0.63	0.08 BW52
10W9273	BW70	180	6.0	5	11	22	142	59	68	81	32	0.14	3.6	0.45	0.05 BW70
	TRAY: 282	POS: 12			LOCAL SPECIFICITY: B44,B45,B49,B50,BW63										
10W9274	BW76	512	7.4	18	0	99	395	79	0	84	100	0.35	63.0	4.79	0.60 BW76
10W9274	B44	494	8.0	59	6	40	389	79	9	40	91	0.69	233.7	4.30	0.44 B44
10W9274	B21	429	8.0	4	1	36	388	50	20	90	80	0.26	29.9	2.69	0.42 B21
	TRAY: 282	POS: 13			LOCAL SPECIFICITY: B5,B35,BW53,BW63										
10W9275	BW77	553	7.2	13	0	89	451	86	0	87	100	0.33	58.9	6.27	0.83 BW77
10W9275	BW53	540	7.6	10	4	79	447	88	28	88	72	0.24	31.5	3.62	0.47 BW53
10W9275	B35	526	7.9	31	19	48	428	89	38	60	62	0.43	95.5	3.14	0.34 B35
10W9275	B51	476	7.9	24	17	24	411	85	41	50	59	0.49	116.2	2.81	0.31 B51
10W9275	BW52	435	7.6	9	14	15	397	75	60	62	40	0.35	52.6	1.74	0.21 BW52
10W9275	BW63	412	7.7	6	12	9	385	66	66	60	34	0.34	47.3	1.62	0.20 BW63
10W9275	B18	394	7.2	5	25	4	360	55	83	44	17	0.28	30.1	0.68	0.08 B18
	TRAY: 282	POS: 14			LOCAL SPECIFICITY: BW62										
10W9276	BW77	574	8.0	13	0	251	310	69	0	95	100	0.16	15.6	5.58	0.82 BW77
10W9276	BW76	561	6.8	18	0	233	310	67	0	92	100	0.20	23.0	5.63	0.78 BW76
10W9276	BW75	543	7.8	47	2	186	308	69	4	79	96	0.34	61.8	5.75	0.67 BW75
10W9276	BW62	494	7.4	136	25	50	283	63	15	26	85	0.67	223.0	4.25	0.43 BW62
10W9276	BW46	333	5.8	33	57	17	226	32	63	34	37	0.37	45.3	1.00	0.11 BW46
10W9276	BW52	243	6.5	4	13	13	213	29	76	76	24	0.18	7.7	0.77	0.11 BW52
10W9276	B51	226	5.6	5	21	8	192	23	80	61	20	0.21	9.8	0.56	0.07 B51
10W9276	B35	200	5.2	5	27	3	165	25	84	37	16	0.26	13.4	0.54	0.07 B35

Table 6. *Continued*

	TRAY: 282	POS: 15			LOCAL SPECIFICITY: BW62										
10W9277	BW46	498	5.7	6	90	4	398	40	93	40	7	0.15	10.9	0.11	0.01 BW46
10W9277	BW6	402	6.5	4	324	0	74	50	98	0	2	0.05	0.9	1.29	0.20 BW6
	TRAY: 282	POS: 16			LOCAL SPECIFICITY: B35										
10W9278	CW4	533	7.7	85	1	30	417	75	1	26	99	0.82	361.8	6.34	0.62 CW4
10W9278	B35	483	7.5	4	14	36	429	55	77	90	23	0.10	4.8	2.02	0.23 B35
10W9278	BW6	465	6.3	31	327	5	102	52	91	13	9	0.06	1.8	0.36	0.05 BW6
	TRAY: 282	POS: 17			LOCAL SPECIFICITY: CW3,B35,A2										
10W9279	A2	378	7.9	127	52	26	173	93	29	16	71	0.59	131.1	1.38	0.19 A2
10W9279	A28	199	6.5	11	19	15	154	84	63	57	37	0.30	17.3	0.84	0.11 A28
10W9279	B35	169	8.0	5	7	10	147	100	58	66	42	0.32	17.2	0.[illegible]	0.06 B35
	TRAY: 282	POS: 18			LOCAL SPECIFICITY: CW3,A2										
10W9280	A2	573	8.0	295	3	178	37	89	1	37	99	0.45	116.6	[illegible]	0.27 A2
10W9280	A28	275	8.0	36	2	142	95	74	5	79	95	0.25	17.4	[illegible]	0.22 A28
10W9280	AW34	169	8.0	11	0	109	49	71	0	90	100	0.17	4.8	[illegible]	0.19 AW34
10W9280	A10	158	8.0	21	1	88	48	68	4	80	96	0.23	8.4	[illegible]	0.18 A10
10W9280	BW60	204	6.7	11	0	99	94	59	0	90	100	0.22	9.9	0.[illegible]	0.06 BW60
10W9280	BW55	193	8.0	4	3	95	91	59	42	95	58	0.02	0.1	0.37	0.05 BW55
	TRAY: 282	POS: 19			LOCAL SPECIFICITY: A2,BW62										
10W9281	A2	570	7.9	279	17	68	206	84	5	19	95	0.71	288.1	3.78	0.35 A2
10W9281	BW41	274	6.0	5	1	63	205	41	16	92	84	0.20	11.3	1.46	0.23 BW41
10W9281	BW60	268	6.8	10	3	53	202	41	23	84	77	0.28	21.7	1.39	0.16 BW60
10W9281	B18	255	5.4	7	12	46	190	37	63	86	37	0.11	3.2	0.51	0.06 B18
10W9281	B7	236	6.8	18	9	28	181	41	33	60	67	0.43	43.2	0.52	0.06 B7
	TRAY: 282	POS: 20			LOCAL SPECIFICITY: B5										
10W9282	BW77	569	7.7	7	6	69	487	51	46	90	54	0.18	18.8	1.90	0.27 BW77
10W9282	B37	556	6.0	4	6	.65	481	47	60	94	40	0.11	7.1	1.44	0.21 B37
10W9282	B51	546	7.0	22	23	43	458	47	51	66	49	0.34	64.0	1.41	0.16 B51
10W9282	BW52	501	6.3	6	20	37	438	41	76	86	24	0.12	7.3	0.40	0.05 BW52
	TRAY: 282	POS: 21			LOCAL SPECIFICITY: BW6										
10W9283	BW76	572	7.6	18	0	301	253	66	0	94	100	0.16	14.7	3.99	0.58 BW76
10W9283	B7	554	8.0	46	2	255	251	65	4	84	96	0.26	36.5	4.20	0.51 B7
10W9283	BW55	506	6.4	9	1	246	250	59	10	96	90	0.11	6.4	2.48	0.40 BW55
10W9283	BW75	496	7.3	43	4	203	246	59	8	82	92	0.27	36.4	3.11	0.38 BW75
10W9283	BW60	449	7.7	37	5	166	241	56	11	81	89	0.28	34.4	2.93	0.36 BW60
10W9283	B8	407	6.3	21	8	145	233	50	27	87	73	0.18	12.9	2.11	0.29 B8
10W9283	BW61	378	7.2	25	7	120	226	51	21	82	79	0.25	23.4	1.98	0.26 BW61
10W9283	B39	346	7.0	10	5	110	221	46	33	91	67	0.14	7.1	1.50	0.22 B39
10W9283	B18	331	6.4	15	10	95	211	46	40	86	60	0.16	8.7	1.21	0.17 B18
10W9283	BW54	306	7.0	8	6	87	205	45	42	91	58	0.12	4.7	1.03	0.16 BW54
10W9283	BW62	292	6.3	49	52	38	153	43	51	43	49	0.30	25.9	0.66	0.07 BW62
10W9283	B35	191	6.0	13	17	25	136	44	56	65	44	0.25	12.3	0.50	0.07 B35
10W9283	BW6	161	6.5	20	41	5	95	48	67	20	33	0.37	22.3	1.02	0.15 BW6
	TRAY: 282	POS: 22			LOCAL SPECIFICITY: CW3										
10W9284	CW4	533	5.0	4	82	7	440	18	95	63	5	0.08	3.4	0.17	0.02 CW4

	TRAY: 282	POS: 23			LOCAL SPECIFICITY: B5,B21											
10W9285	BW77	549	8.0	5	8	32	504	54	61	86	39	0.20	21.3	1.79	0.25	BW77
10W9285	BW52	536	6.4	5	18	27	486	46	78	84	22	0.14	10.6	0.82	0.10	BW52
10W9285	B51	513	6.8	5	38	22	448	48	88	81	12	0.09	3.8	0.52	0.06	B51
10W9285	BW6	470	6.3	19	377	3	71	50	95	13	5	0.01	0.1	0.57	0.08	BW6
	TRAY: 282	POS: 24			LOCAL SPECIFICITY: BW4											
10W9286	BW57	370	7.6	9	6	64	291	61	40	87	60	0.21	16.0	1.78	0.24	BW57
10W9286	BW53	355	7.2	5	5	59	286	59	50	92	50	0.14	7.1	1.46	0.21	BW53
10W9286	BW52	345	7.8	9	8	50	278	59	47	84	53	0.22	16.2	1.50	0.20	BW52
10W9286	B44	328	7.4	19	16	31	262	54	45	62	55	0.38	46.2	1.68	0.20	B44
10W9286	B51	293	6.4	9	14	22	248	45	60	79	40	0.27	21.5	0.69	0.09	B51
10W9286	BW4	270	6.5	15	51	7	197	40	77	31	23	0.30	24.8	1.54	0.15	BW4
	TRAY: 282	POS: 25			LOCAL SPECIFICITY: B5,B18,B35,BW62											
10W9287	B35	571	8.0	58	0	390	123	91	0	87	100	0.18	17.7	5.35	0.68	B35
10W9287	BW75	513	8.0	50	0	340	123	90	0	87	100	0.18	17.5	5.19	0.67	BW75
10W9287	BW52	463	8.0	24	0	316	123	88	0	92	100	0.14	9.2	4.35	0.64	BW52
10W9287	B18	439	7.9	29	0	287	123	87	0	90	100	0.17	12.1	4.34	0.62	B18
10W9287	BW53	410	8.0	11	0	276	123	87	0	96	100	0.11	4.8	3.47	0.61	BW53
10W9287	B15.3	399	8.0	11	0	265	123	86	0	96	100	0.11	5.0	3.47	0.61	B15.3
10W9287	BW77	388	8.0	10	0	255	123	86	0	96	100	0.11	4.8	3.37	0.60	BW77
10W9287	BW76	378	7.1	14	0	241	123	85	0	94	100	0.14	7.0	3.62	0.60	BW76
10W9287	B38	364	7.7	15	0	226	123	85	0	93	100	0.15	8.0	3.54	0.58	B38
10W9287	BW62	349	7.9	122	4	104	119	85	3	46	97	0.50	88.9	4.31	0.50	BW62
10W9287	B51	223	7.9	23	2	81	117	75	8	77	92	0.32	23.3	3.57	0.49	B51
10W9287	BW70	198	7.6	28	1	53	116	69	3	65	97	0.47	43.5	3.43	0.49	BW70
10W9287	B37	169	8.0	4	1	49	115	62	20	92	80	0.18	5.7	1.79	0.37	B37
10W9287	B39	164	7.3	9	4	40	111	59	30	81	70	0.25	10.4	1.36	0.23	B39
	TRAY: 282	POS: 26			LOCAL SPECIFICITY: B35											
10W9288	BW77	570	6.8	12	1	105	452	54	7	89	93	0.27	42.0	5.60	0.75	BW77
10W9288	BW53	557	6.8	8	6	97	446	54	42	92	58	0.16	13.8	2.41	0.34	BW53
10W9288	B15.3	543	6.8	5	6	92	440	54	54	94	46	0.10	5.8	1.80	0.28	B15.3
10W9288	B35	532	6.7	32	22	60	418	54	40	65	60	0.37	74.0	2.37	0.26	B35
10W9288	BW75	478	6.9	28	22	32	396	51	44	53	56	0.45	96.0	2.10	0.23	BW75
10W9288	BW54	428	5.7	6	8	26	388	46	57	81	43	0.25	26.2	1.57	0.22	BW54
10W9288	BW52	414	6.0	5	18	21	370	50	78	80	22	0.15	9.9	0.74	0.09	BW52
10W9288	B51	391	7.1	11	29	10	341	52	88	47	28	0.33	42.9	0.71	0.08	B51
	TRAY: 282	POS: 27			LOCAL SPECIFICITY: B5,BW63											
10W9289	BW77	573	8.0	13	0	103	457	66	0	88	100	0.30	52.4	5.34	0.69	BW77
10W9289	B51	560	7.4	43	3	60	454	62	6	58	94	0.58	188.2	4.89	0.52	B51
10W9289	BW53	514	7.3	8	5	52	449	50	38	86	62	0.25	32.2	2.68	0.35	BW53
10W9289	BW52	501	6.8	19	8	33	441	48	29	63	71	0.47	110.4	2.93	0.35	BW52
10W9289	BW63	474	6.8	5	16	28	425	45	76	84	24	0.14	9.6	1.17	0.15	BW63
10W9289	BW57	453	6.0	6	13	22	412	42	68	78	32	0.22	22.1	0.73	0.09	BW57
	TRAY: 282	POS: 28			LOCAL SPECIFICITY: B5,B35											
10W9290	B51	572	7.5	42	8	35	487	68	16	45	84	0.64	234.0	4.93	0.52	B51
10W9290	BW52	522	7.2	21	6	14	481	54	22	40	78	0.66	229.9	3.78	0.43	BW52
10W9290	BW6	495	5.7	12	406	2	75	28	97	14	3	0.01	0.0	0.51	0.07	BW6
	TRAY: 282	POS: 29			LOCAL SPECIFICITY: B35											
10W9291	B51	562	6.3	6	44	22	490	28	88	78	12	0.10	5.7	0.33	0.04	B51

Table 6. *Continued*

	TRAY: 282	POS: 30			LOCAL SPECIFICITY: B5										
10W9292	BW77	572	7.1	13	0	106	453	47	0	89	100	0.30	50.6	4.82	0.65 BW77
10W9292	BW76	559	6.6	13	5	93	448	44	27	87	73	0.25	34.3	2.41	0.31 BW76
10W9292	BW58	541	7.3	8	9	85	439	43	52	91	48	0.14	11.0	1.71	0.22 BW58
10W9292	BW57	524	7.3	11	10	74	429	41	47	87	53	0.20	21.0	1.60	0.20 BW57
10W9292	B27	503	6.2	9	12	65	417	36	57	87	43	0.17	13.8	1.12	0.14 B27
10W9292	B44	488	6.5	26	40	39	377	33	60	60	40	0.30	44.3	1.07	0.11 B44
10W9292	B38	416	7.0	4	12	35	365	25	75	89	25	0.11	4.3	0.73	0.10 B38
10W9292	BW4	400	5.5	23	115	12	250	22	83	34	17	0.20	16.3	0.85	0.08 BW4
	TRAY: 282	POS: 31			LOCAL SPECIFICITY: B5										
10W9293	BW77	515	7.7	12	0	65	439	79	0	84	100	0.37	69.9	5.49	0.76 BW77
10W9293	BW52	503	7.9	16	7	49	419	78	30	75	70	0.37	68.7	2.94	0.36 BW52
10W9293	B51	480	7.8	26	10	23	403	73	27	46	73	0.58	163.3	3.12	0.36 B51
10W9293	BW53	444	7.5	4	7	19	374	52	63	82	37	0.22	22.3	1.51	0.21 BW53
10W9293	BW4	433	6.8	13	156	6	258	47	92	31	8	0.13	7.2	0.91	0.08 BW4
	TRAY: 282	POS: 32			LOCAL SPECIFICITY: BW4										
10W9294	B44	572	8.0	73	0	246	253	84	0	77	100	0.34	66.4	3.62	0.39 B44
10W9294	BW76	499	7.8	16	0	230	253	79	0	93	100	0.18	17.0	2.29	0.31 BW76
10W9294	BW77	483	8.0	11	0	219	253	78	0	95	100	0.16	12.4	2.10	0.31 BW77
10W9294	BW53	472	7.7	14	0	205	253	77	0	93	100	0.19	16.7	2.15	0.30 BW53
10W9294	B27	458	7.8	18	2	187	251	76	10	91	90	0.19	17.3	1.95	0.26 B27
10W9294	BW57	438	7.8	18	1	169	250	74	5	90	95	0.22	22.0	1.79	0.24 BW57
10W9294	BW52	419	8.0	23	2	146	248	73	8	86	92	0.27	29.5	1.80	0.23 BW52
10W9294	BW55	394	5.6	5	4	141	244	69	44	96	56	0.06	1.4	1.13	0.17 BW55
10W9294	B51	385	7.3	32	6	109	238	70	15	77	85	0.33	41.1	1.11	0.13 B51
10W9294	A23	347	8.0	6	0	103	238	67	0	94	100	0.20	13.3	0.77	0.10 A23
10W9294	BW4	341	7.3	72	15	31	223	66	17	30	83	0.67	153.0	3.04	0.47 BW4
	TRAY: 282	POS: 33			LOCAL SPECIFICITY: B5,B49										
10W9295	BW77	461	8.0	4	4	39	414	67	50	90	50	0.19	15.9	2.25	0.33 BW77
10W9295	BW52	453	7.4	13	11	26	403	64	45	66	55	0.38	66.8	2.11	0.25 BW52
10W9295	BW57	429	7.0	4	13	22	390	57	76	84	24	0.15	9.5	1.15	0.15 BW57
10W9295	B51	412	7.4	7	24	15	366	59	77	68	23	0.22	19.7	0.80	0.09 B51
	TRAY: 282	POS: 34			LOCAL SPECIFICITY: BW6										
10W9296	BW76	529	7.4	18	0	237	274	59	0	92	100	0.19	20.0	3.98	0.64 BW76
10W9296	BW48	316	7.0	4	0	169	143	61	0	97	100	0.10	3.3	2.65	0.60 BW48
10W9296	BW75	507	7.2	41	7	192	267	57	14	82	86	0.26	33.2	2.58	0.34 BW75
10W9296	B8	459	7.2	24	8	168	259	54	25	87	75	0.18	15.6	2.10	0.31 B8
10W9296	B7	427	6.9	34	9	134	250	52	20	79	80	0.27	31.6	2.08	0.28 B7
10W9296	B18	384	6.8	17	9	117	241	50	34	87	66	0.17	11.4	1.52	0.24 B18
10W9296	BW54	358	6.7	9	5	108	236	47	35	92	65	0.14	6.6	1.32	0.22 BW54
10W9296	BW60	344	7.1	22	12	86	224	47	35	79	65	0.24	19.4	1.51	0.21 BW60
10W9296	BW56	310	6.7	6	5	80	219	45	45	93	55	0.11	4.1	1.05	0.19 BW56
10W9296	B39	299	5.8	8	8	72	211	43	50	90	50	0.12	4.7	0.92	0.15 B39
10W9296	B35	283	6.2	23	21	49	190	44	47	68	53	0.26	19.8	0.87	0.12 B35
10W9296	A29	131	6.0	4	1	32	94	50	20	88	80	0.23	7.2	0.47	0.07 A29
10W9296	BW61	234	7.0	10	17	35	172	46	62	77	38	0.16	6.2	0.40	0.06 BW61
10W9296	BW6	207	6.5	34	86	1	86	42	71	2	29	0.36	26.5	1.02	0.16 BW6

	TRAY: 282	POS: 35			LOCAL SPECIFICITY: BW4										
10W9297	B44	573	8.0	72	1	398	102	86	1	84	99	0.17	15.6	1.00	0.11 B44
10W9297	B51	500	7.9	48	0	350	102	83	0	87	100	0.16	13.6	0.92	0.11 B51
10W9297	BW52	452	7.8	27	0	323	102	82	0	92	100	0.14	8.4	0.65	0.08 BW52
10W9297	BW4	425	7.8	147	4	176	98	81	2	54	98	0.37	58.5	3.78	0.58 BW4
	TRAY: 282	POS: 36			LOCAL SPECIFICITY: B5,B18,B35,BW53										
10W9298	B18	570	7.9	34	1	282	253	81	2	89	98	0.21	26.3	5.35	0.71 B18
10W9298	B15.3	535	8.0	10	1	272	252	79	9	96	91	0.11	6.6	4.16	0.69 B15.3
10W9298	BW77	524	7.6	11	0	261	252	79	0	95	100	0.14	10.4	4.12	0.48 BW77
10W9298	BW65	333	7.0	4	0	175	154	83	[illegible]	97	100	0.10	3.5	3.29	[illegible] BW65
10W9298	B35	509	7.9	54	1	203	251	78	[illegible]	78	99	0.33	56.1	5.20	[illegible] B35
10W9298	B51	454	7.9	40	2	163	249	74	[illegible]	80	96	0.32	47.8	4.86	[illegible] B51
10W9298	BW52	412	7.9	24	1	139	248	69	4	85	96	0.29	35.5	4.28	0.60 BW52
10W9298	BW75	387	7.7	42	2	97	246	64	4	69	96	0.44	76.5	4.30	0.55 BW75
10W9298	B37	343	8.0	7	0	90	246	53	0	92	100	0.23	18.1	3.08	0.53 B37
10W9298	BW53	336	8.0	8	1	82	245	50	11	91	89	0.23	18.2	3.08	0.53 BW53
10W9298	BW62	327	6.5	62	52	20	193	45	45	24	55	0.49	80.0	1.24	0.14 BW62
10W9298	BW70	213	5.5	4	25	16	168	35	86	80	14	0.06	0.8	0.48	0.07 BW70
10W9298	BW4	184	5.8	9	92	7	76	37	91	43	9	0.01	0.0	1.05	0.16 BW4
	TRAY: 283	POS: 3			LOCAL SPECIFICITY: A2,BW62										
10W9299	A2	567	7.2	191	104	3	269	74	35	1	65	0.67	254.7	3.60	0.33 A2
	TRAY: 283	POS: 4			LOCAL SPECIFICITY: CW3										
10W9300	CW3	565	7.5	212	27	24	302	79	11	10	89	0.81	375.1	5.08	0.47 CW3
10W9300	CW1	326	6.3	19	44	5	258	54	69	20	31	0.43	59.5	0.74	0.08 CW1
	TRAY: 283	POS: 5			LOCAL SPECIFICITY: CW3										
10W9301	CW3	568	7.6	232	8	25	303	82	3	9	97	0.88	443.6	5.84	0.53 CW3
	TRAY: 283	POS: 6			LOCAL SPECIFICITY: BW4										
10W9302	A29	376	7.3	14	1	303	58	90	6	95	94	0.05	1.0	0.68	0.11 A29
10W9302	BW57	551	8.0	19	0	406	126	88	0	95	100	0.10	5.8	0.59	0.07 BW57
10W9302	B27	532	7.8	26	0	380	126	87	0	93	100	0.13	8.5	0.61	0.07 B27
10W9302	A24	324	7.7	103	9	163	49	89	8	61	92	0.19	11.3	0.59	0.07 A24
10W9302	B44	394	8.0	51	2	226	115	86	3	81	97	0.22	19.7	0.63	0.07 B44
10W9302	CW2	170	7.2	5	2	116	47	88	28	95	72	0.00	0.0	0.44	0.06 CW2
10W9302	BW4	334	7.9	111	17	110	96	84	13	49	87	0.34	39.2	1.09	0.10 BW4
	TRAY: 283	POS: 7			LOCAL SPECIFICITY: B5										
10W9303	BW77	567	7.5	13	0	95	459	57	0	87	100	0.32	56.5	4.95	0.66 BW77
10W9303	BW63	554	6.6	19	10	76	449	53	34	80	66	0.30	50.4	2.63	0.31 BW63
10W9303	B15.3	525	7.3	6	5	70	444	53	45	92	55	0.17	14.6	1.85	0.25 B15.3
10W9303	B37	513	6.0	5	5	64	439	52	50	92	50	0.15	11.7	1.71	0.24 B37
10W9303	BW52	504	6.5	12	11	53	428	53	47	81	53	0.26	33.1	1.76	0.22 BW52
10W9303	BW58	481	7.0	6	8	47	420	56	57	88	43	0.18	14.9	1.39	0.18 BW58
10W9303	B51	467	6.6	20	18	27	402	55	47	57	53	0.42	82.8	1.59	0.18 B51
10W9303	BW57	429	6.8	5	12	22	390	51	70	81	30	0.19	16.0	1.04	0.13 BW57
	TRAY: 283	POS: 8			LOCAL SPECIFICITY: B35										
10W9304	B35	567	7.1	46	12	33	476	59	20	41	80	0.64	230.3	4.56	0.48 B35
10W9304	BW53	509	7.3	6	5	27	471	48	45	81	55	0.29	42.8	3.50	0.45 BW53
10W9304	B15.3	498	7.2	5	7	22	464	44	58	81	42	0.25	31.5	2.33	0.31 B15.3
10W9304	B51	486	6.0	10	36	12	428	40	78	54	22	0.27	34.8	0.70	0.08 B51

Table 6. *Continued*

	TRAY: 283	POS: 9			LOCAL SPECIFICITY: BW6										
10W9305	BW60	567	7.7	51	1	381	134	83	1	88	99	0.16	15.1	3.78	0.45 BW60
10W9305	BW76	515	7.9	18	0	363	134	82	0	95	100	0.11	6.6	3.01	0.43 BW76
10W9305	BW56	497	7.5	11	0	352	134	81	0	96	100	0.09	4.2	2.63	0.41 BW56
10W9305	BW75	486	7.8	40	1	312	133	82	2	88	98	0.17	14.2	3.04	0.38 BW75
10W9305	B7	445	7.6	42	3	270	130	80	6	86	94	0.17	12.9	2.99	0.37 B7
10W9305	B8	400	7.9	27	1	243	129	80	3	90	97	0.17	11.5	2.76	0.36 B8
10W9305	BW70	372	7.3	23	1	220	128	79	4	90	96	0.17	10.5	2.50	0.34 BW70
10W9305	B18	348	7.3	21	1	199	127	80	4	90	96	0.17	10.5	2.45	0.34 B18
10W9305	BW55	326	7.0	8	1	191	126	79	11	85	89	0.10	3.0	1.78	0.29 BW55
10W9305	BW61	317	7.9	26	4	165	122	80	13	86	87	0.17	9.7	2.13	0.28 BW61
10W9305	BW62	287	7.5	95	8	70	114	78	7	92	93	0.53	79.3	2.34	0.25 BW62
10W9305	B39	184	7.6	11	2	59	112	72	15	84	85	0.26	12.[illegible]	1.58	0.24 B39
10W9305	B35	171	7.4	25	4	34	108	71	13	87	87	0.49	41.[illegible]	1.70	0.22 B35
10W9305	CW2	82	8.0	1	0	15	66	93	0	93	100	0.23	4.[illegible]	0.45	0.06 CW2
10W9305	BW6	141	7.4	26	14	7	94	69	35	21	65	0.62	53.[illegible]	3.12	0.46 BW6
	TRAY: 283	POS: 10			LOCAL SPECIFICITY: B15										
10W9306	BW75	558	8.0	47	0	272	239	86	0	85	100	0.26	38.5	5.70	0.66 BW75
10W9306	BW76	511	8.0	14	0	258	239	84	0	94	100	0.16	12.6	4.36	0.62 BW76
10W9306	BW62	497	7.9	153	6	105	233	83	3	40	97	0.61	183.9	5.41	0.54 BW62
10W9306	BW77	338	7.1	11	1	94	232	66	8	89	92	0.25	21.3	3.24	0.47 BW77
10W9306	B15.3	326	7.7	6	3	88	229	65	33	93	67	0.14	6.5	2.23	0.34 B15.3
10W9306	BW63	317	6.6	18	8	70	221	64	30	79	70	0.28	24.3	2.51	0.33 BW63
10W9306	BW46	291	7.3	51	35	19	186	71	40	27	60	0.53	83.0	1.39	0.15 BW46
10W9306	CW3	205	7.3	12	36	7	150	63	75	36	25	0.30	18.4	0.78	0.07 CW3
10W9306	BW6	157	6.8	5	106	2	44	57	95	28	5	0.00	0.0	0.65	0.06 BW6
	TRAY: 283	POS: 11			LOCAL SPECIFICITY: CW1,CX46										
10W9307	CW1	534	7.7	100	10	15	409	76	9	13	91	0.86	394.6	6.49	0.63 CW1
10W9307	BW46	424	5.0	4	17	11	392	13	80	73	20	0.19	15.6	2.22	0.21 BW46
	TRAY: 283	POS: 12			LOCAL SPECIFICITY: CW1,CX46										
10W9308	CW1	516	7.7	89	16	40	371	76	15	31	85	0.70	251.1	3.38	0.33 CW1
10W9308	BW77	411	7.6	5	7	35	364	52	58	87	42	0.19	14.3	0.42	0.06 BW77
	TRAY: 283	POS: 13			LOCAL SPECIFICITY: BW62										
10W9309	BW76	559	6.0	18	0	152	389	54	0	89	100	0.28	42.6	6.32	0.81 BW76
10W9309	BW62	541	7.1	113	46	39	343	56	28	25	72	0.62	205.8	3.89	0.37 BW62
10W9309	BW75	382	5.6	27	16	12	327	25	37	30	63	0.62	146.1	2.66	0.30 BW75
10W9309	BW60	339	5.2	5	27	7	300	16	84	58	16	0.21	15.1	0.50	0.06 BW60
	TRAY: 283	POS: 14			LOCAL SPECIFICITY: B15,BW57,BW46										
10W9310	BW62	558	8.0	160	0	181	217	91	0	53	100	0.51	142.8	8.69	0.87 BW62
10W9310	BW76	398	8.0	17	0	164	217	84	0	90	100	0.23	21.3	6.09	0.84 BW76
10W9310	BW77	381	8.0	12	0	152	217	82	0	92	100	0.21	16.4	5.66	0.84 BW77
10W9310	BW57	369	7.9	19	0	133	217	81	0	87	100	0.28	28.6	6.16	0.84 BW57
10W9310	BW63	350	7.5	26	0	107	217	79	0	80	100	0.36	45.8	6.46	0.83 BW63
10W9310	B15.3	324	7.8	9	0	98	217	80	0	91	100	0.24	18.8	5.26	0.83 B15.3
10W9310	BW75	315	7.9	39	1	59	216	79	2	60	98	0.55	94.2	6.28	0.75 BW75
10W9310	BW46	275	7.0	54	31	5	185	67	36	8	64	0.69	129.2	2.57	0.28 BW46

	TRAY: 283	POS: 15			LOCAL SPECIFICITY: BW62,BW63,8W66											
10W9311	BW77	559	8.0	13	0	262	284	81	0	95	100	0.16	13.7	6.77	0.98	BW77
10W9311	BW75	546	7.8	47	0	215	284	80	0	82	100	0.32	55.7	8.21	0.95	BW75
10W9311	B15.3	499	7.1	11	0	204	284	78	0	94	100	0.17	14.9	6.06	0.91	B15.3
10W9311	BW76	488	5.9	14	0	190	284	78	0	93	100	0.20	20.1	5.95	0.85	BW76
10W9311	BW62	474	7.8	151	5	39	279	81	3	20	97	0.81	311.4	8.39	0.84	BW62
10W9311	BW63	318	6.0	15	12	24	267	41	44	61	56	0.40	51.4	2.47	0.33	BW63
10W9311	BW57	291	7.5	4	11	20	256	41	73	83	27	0.16	7.1	1.50	0.21	BW57
10W9311	BW46	276	5.9	18	67	2	189	35	78	10	22	0.36	35.5	0.55	0.06	BW46
	TRAY: 283	POS: 16			LOCAL SPECIFICITY: BW62,8W66,BW74											
10W9312	BW76	557	7.9	18	0	187	352	81	0	91	100	0.24	31.9	6.30	0.94	BW76
10W9312	BW62	539	7.7	140	18	47	334	80	11	25	89	0.73	286.8	5.45	0.84	BW62
10W9312	BW75	381	6.5	33	10	14	324	51	23	29	77	0.70	185.9	3.30	0.53	BW75
10W9312	BW63	338	5.5	4	23	10	301	35	85	71	15	0.16	8.4	0.49	0.06	BW63
	TRAY: 283	POS: 17			LOCAL SPECIFICITY: BW62,BW76											
10W9313	BW76	554	8.0	18	0	143	393	81	0	88	100	0.29	45.4	6.90	0.87	BW76
10W9313	BW62	536	7.7	128	28	15	365	79	17	10	83	0.80	344.9	4.94	0.47	BW62
10W9313	BW6	380	5.3	14	254	1	111	13	94	6	6	0.10	3.9	0.86	0.08	BW6
	TRAY: 283	POS: 18			LOCAL SPECIFICITY: B15											
10W9314	BW62	558	8.0	159	0	213	186	94	0	57	100	0.45	111.2	7.20	0.72	BW62
10W9314	BW75	399	8.0	47	0	166	186	90	0	77	100	0.34	46.5	5.90	0.69	BW75
10W9314	BW63	352	7.9	27	0	139	186	87	0	83	100	0.31	32.8	5.19	0.66	BW63
10W9314	BW76	325	8.0	13	0	126	186	85	0	90	100	0.24	18.1	4.40	0.64	BW76
10W9314	BW77	312	8.0	12	0	114	186	84	0	90	100	0.24	18.4	4.31	0.64	BW77
10W9314	BW57	300	7.7	15	0	99	186	82	0	86	100	0.29	25.8	4.48	0.63	BW57
10W9314	B15.3	285	7.8	9	0	90	186	80	0	90	100	0.25	17.5	3.93	0.62	B15.3
10W9314	BW46	276	7.6	74	10	16	176	80	11	17	89	0.78	169.2	3.78	0.41	BW46
10W9314	A31	192	7.5	4	7	12	169	56	63	75	37	0.25	12.0	0.57	0.06	A31
10W9314	BW4	181	6.4	10	100	2	69	50	90	16	10	0.12	2.7	1.19	0.19	BW4
	TRAY: 283	POS: 19			LOCAL SPECIFICITY: B15,A32,BW76											
10W9315	BW76	557	8.0	18	0	196	343	71	0	91	100	0.23	29.8	5.18	0.71	BW76
10W9315	BW75	539	7.1	36	7	160	336	68	16	81	84	0.29	45.3	3.94	0.47	BW75
10W9315	BW62	496	7.3	125	33	35	303	68	29	21	80	0.69	232.9	3.27	0.33	BW62
10W9315	B39	338	6.6	7	8	28	295	45	55	80	47	0.26	22.3	1.25	0.18	B39
10W9315	BW4	323	6.5	19	180	9	115	42	90	32	10	0.04	0.5	0.48	0.08	BW4
	TRAY: 283	POS: 20			LOCAL SPECIFICITY: BW62,B15											
10W9316	BW77	556	7.7	13	0	232	311	69	0	94	100	0.17	16.9	5.78	0.83	BW77
10W9316	BW76	543	6.2	18	0	214	311	68	0	92	100	0.21	25.0	5.93	0.80	BW76
10W9316	BW75	525	7.5	41	2	173	309	69	4	80	96	0.33	57.8	5.72	0.67	BW75
10W9316	BW62	482	7.4	135	21	38	288	65	13	21	87	0.73	257.1	4.96	0.49	BW62
10W9316	BW63	326	5.9	18	9	20	279	31	33	52	67	0.52	86.5	3.68	0.48	BW63
10W9316	B15.3	299	6.8	5	4	15	275	45	44	75	56	0 34	35.5	2.48	0.45	B15.3
	TRAY: 283	POS: 21			LOCAL SPECIFICITY: BW62											
10W9317	BW76	551	7.7	18	0	182	351	68	0	91	100	0.24	32.7	5.39	0.72	BW76
10W9317	BW75	533	7.2	35	8	147	343	67	18	80	82	0.30	46.4	4.28	0.50	BW75
10W9317	BW77	490	7.4	10	2	137	341	65	16	93	84	0.18	16.7	3.29	0.47	BW77
10W9317	BW62	478	7.1	107	49	30	292	64	31	21	69	0.61	180.6	2.70	0.27	BW62
10W9317	B39	322	6.5	4	11	26	281	46	73	86	27	0.13	5.6	1.15	0.16	B39

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Table 6. *Continued*

	TRAY: 283	POS: 22			LOCAL SPECIFICITY: B15,8W66,TS1,BW57,BW46									
10W9318	BW75	558	7.9	47	0	352	159	90	0	88	100	0.19	20.5	5.61	0.69	BW75
10W9318	BW76	511	8.0	14	0	338	159	90	0	96	100	0.11	6	4.29	0.66	BW76
10W9318	BW77	497	8.0	13	0	325	159	89	0	96	100	0.11	6	4.21	0.66	BW77
10W9318	BW63	484	7.8	29	0	296	159	89	0	91	100	0.18	15	4.88	0.65	BW63
10W9318	B15.3	455	7.8	11	0	285	159	89	0	96	100	0.12	6	3.90	0.63	B15.3
10W9318	BW62	444	8.0	152	2	133	157	89	1	46	99	0.52	122.1	5.79	0.61	BW62
10W9318	BW57	290	8.0	14	1	119	156	78	6	89	94	0.22	14.4	3.52	0.53	BW57
10W9318	BW46	275	7.8	79	5	40	151	76	5	33	95	0.68	127.0	4.15	0.47	BW46
10W9318	BW53	191	6.3	6	3	34	148	45	33	85	67	0.25	11.9	2.07	0.41	BW53
10W9318	BW70	182	6.7	9	18	25	130	44	66	73	34	0.16	-1.5	1.40	0.21	BW70
	TRAY: 283	POS: 23			LOCAL SPECIFICITY: B15									
10W9319	BW77	513	8.0	12	0	256	245	88	0	95	100	0.15	11.2	5.51	0.82	BW77
10W9319	BW76	501	7.8	18	0	238	245	87	0	92	100	0.19	17.9	5.92	0.82	BW76
10W9319	BW75	483	8.0	36	1	202	244	87	2	84	98	0.28	37.0	6.11	0.74	BW75
10W9319	BW62	446	7.9	144	5	58	239	85	3	28	97	0.73	238.1	6.39	0.65	BW62
10W9319	B15.3	297	7.4	7	1	51	238	58	12	87	88	0.29	24.2	3.59	0.58	B15.3
10W9319	BW57	289	7.6	9	4	42	234	56	30	82	70	0.29	24.9	2.80	0.41	BW57
10W9319	BW63	276	6.8	16	9	26	225	50	36	61	64	0.43	50.7	2.14	0.28	BW63
	TRAY: 283	POS: 24			LOCAL SPECIFICITY: B15,BW57									
10W9320	BW75	556	8.0	47	0	271	238	85	0	85	100	0.26	38.4	7.35	0.85	BW75
10W9320	B15.3	509	8.0	11	1	260	237	83	8	95	92	0.12	7.3	5.57	0.82	B15.3
10W9320	BW76	497	7.8	13	0	247	237	82	0	95	100	0.16	12.2	5.56	0.81	BW76
10W9320	BW77	484	7.8	12	0	235	237	81	0	95	100	0.16	11.8	5.46	0.80	BW77
10W9320	BW62	472	7.8	144	11	91	226	81	7	38	93	0.60	171.6	5.78	0.58	BW62
10W9320	BW63	317	7.0	24	3	67	223	63	11	73	89	0.41	52.2	4.10	0.52	BW63
10W9320	BW57	290	6.6	10	5	57	218	64	33	85	67	0.24	16.9	2.77	0.39	BW57
10W9320	BW46	275	7.4	50	34	7	184	70	40	12	60	0.63	110.8	2.12	0.23	BW46
	TRAY: 283	POS: 25			LOCAL SPECIFICITY: B15,8W66,TS1,BW57									
10W9321	BW75	557	8.0	47	0	371	139	90	0	88	100	0.18	17.1	5.83	0.76	BW75
10W9321	BW63	510	7.8	28	0	343	139	89	0	92	100	0.15	11.1	5.12	0.73	BW63
10W9321	BW57	482	8.0	16	0	327	139	89	0	95	100	0.12	6.7	4.56	0.73	BW57
10W9321	BW76	466	8.0	14	0	313	139	88	0	95	100	0.11	6.1	4.41	0.73	BW76
10W9321	BW77	452	8.0	13	0	300	139	88	0	95	100	0.11	5.9	4.32	0.72	BW77
10W9321	B15.3	439	8.0	11	0	289	139	87	0	96	100	0.11	5.2	4.13	0.72	B15.3
10W9321	BW62	428	7.9	152	1	137	138	87	0	47	100	0.51	110.0	6.40	0.71	BW62
10W9321	BW46	275	7.8	83	2	54	136	75	2	39	98	0.64	112.6	5.23	0.63	BW46
10W9321	BW53	190	6.0	6	3	48	133	50	33	88	67	0.19	6.8	2.20	0.48	BW53
10W9321	BW52	181	6.9	7	5	41	128	52	41	85	59	0.19	6.7	1.69	0.29	BW52
10W9321	BW70	169	5.8	13	14	28	114	53	51	68	49	0.24	10.0	1.86	0.28	BW70
10W9321	B13	142	6.5	8	10	20	104	67	55	71	45	0.24	8.0	1.11	0.17	B13
10W9321	B35	124	7.2	10	14	10	90	70	58	50	42	0.34	14.3	0.83	0.12	B35
10W9321	B51	100	6.7	6	13	4	77	60	68	40	32	0.35	12.1	0.61	0.09	B51

	TRAY: 283	POS: 26			LOCAL SPECIFICITY: B15,BW70,B35										
10W9322	BW77	540	8.0	13	0	320	207	85	0	96	100	0.12	8.3	4.81	0.77 BW77
10W9322	BW76	527	7.9	18	0	302	207	85	0	94	100	0.15	12.1	5.08	0.76 BW76
10W9322	B15.3	509	7.8	10	1	292	206	84	9	96	91	0.10	4.6	3.55	0.59 B15.3
10W9322	BW75	498	8.0	39	4	253	202	84	9	86	91	0.20	19.9	4.51	0.58 BW75
10W9322	BW62	455	7.8	142	11	111	191	81	7	43	93	0.53	129.3	4.69	0.50 BW62
10W9322	BW57	302	7.5	11	5	100	186	65	31	90	69	0.16	7.4	2.38	0.36 BW57
10W9322	B35	286	7.2	26	11	74	175	65	29	74	71	0.29	23.3	2.59	0.35 B35
10W9322	BW63	249	8.0	12	11	62	164	63	47	83	53	0.16	6.1	1.51	0.21 BW63
10W9322	BW46	226	7.0	42	32	20	132	56	43	32	57	0.46	47.5	1.49	0.18 BW46
10W9322	BW70	152	5.3	9	17	11	115	35	65	55	35	0.29	12.6	0.87	0.13 BW70
10W9322	B51	126	6.3	6	14	5	101	54	70	45	30	0.33	13.5	0.52	0.08 B51
	TRAY: 283	POS: 27			LOCAL SPECIFICITY: B15										
10W9323	BW76	541	5.8	16	2	213	310	69	11	93	89	0.17	16.5	5.74	0.78 BW76
10W9323	BW77	523	8.0	13	0	200	310	71	0	93	100	0.19	19.4	5.07	0.73 BW77
10W9323	B15.3	510	7.3	11	0	189	310	70	0	94	100	0.18	17.4	4.77	0.71 B15.3
10W9323	BW75	499	7.7	42	1	147	309	69	2	77	98	0.38	71.5	5.48	0.64 BW75
10W9323	BW63	456	6.8	16	11	131	298	63	40	89	60	0.15	9.6	2.08	0.27 BW63
10W9323	BW62	429	7.1	99	47	32	251	64	32	24	68	0.58	144.9	2.31	0.23 BW62
10W9323	BW46	283	6.3	24	59	8	192	56	71	25	29	0.36	36.3	0.66	0.07 BW46
	TRAY: 283	POS: 28			LOCAL SPECIFICITY: B15										
10W9324	BW77	559	8.0	13	0	292	254	83	0	95	100	0.14	11.1	5.00	0.76 BW77
10W9324	BW76	546	6.7	18	0	274	254	82	0	93	100	0.17	16.2	5.02	0.72 BW76
10W9324	B13	528	8.0	35	1	239	253	83	2	87	98	0.25	31.8	5.46	0.69 B13
10W9324	BW75	492	7.8	39	2	200	251	81	4	83	96	0.28	38.8	5.35	0.66 BW75
10W9324	B15.3	451	7.6	9	2	191	249	79	18	95	82	0.12	6.4	3.51	0.55 B15.3
10W9324	BW62	440	7.8	141	10	50	239	79	6	26	94	0.73	233.7	5.29	0.55 BW62
10W9324	BW57	289	7.3	8	8	42	231	40	50	84	50	0.21	12.7	1.79	0.26 BW57
10W9324	BW63	273	7.4	10	14	32	217	35	58	76	42	0.23	14.0	1.44	0.20 BW63
10W9324	B38	249	5.5	4	8	28	209	21	66	87	34	0.14	4.7	0.73	0.13 B38
10W9324	BW60	237	5.1	9	19	19	190	21	67	67	33	0.23	12.6	0.66	0.09 BW60
	TRAY: 283	POS: 29			LOCAL SPECIFICITY: B35,B51,BW53										
10W9325	BW53	563	7.0	10	3	75	475	56	23	88	77	0.27	39.7	4.85	0.70 BW53
10W9325	B35	550	6.7	31	22	44	453	56	41	58	59	0.43	100.2	2.95	0.32 B35
10W9325	B51	497	7.0	25	24	19	429	54	48	43	52	0.49	119.8	2.61	0.29 B51
10W9325	B13	448	8.0	4	29	15	400	31	87	78	13	0.11	5.4	0.58	0.07 B13
10W9325	BW60	415	5.0	8	41	7	359	15	83	46	17	0.25	25.8	0.48	0.05 BW60
	TRAY: 283	POS: 30			LOCAL SPECIFICITY: BW62S										
10W9326	BW46	511	5.5	12	90	8	401	20	88	40	12	0.20	20.9	0.19	0.02 BW46
	TRAY: 283	POS: 31			LOCAL SPECIFICITY: CW3										
10W9327	CW3	566	7.5	222	17	40	287	74	7	15	93	0.80	361.3	3.97	0.36 CW3
10W9327	BW77	327	7.7	6	6	34	281	57	50	85	50	0.22	16.6	0.39	0.05 BW77
10W9327	BW6	315	6.9	26	214	8	67	52	89	23	11	0.00	0.0	0.99	0.15 BW6

Table 6. *Continued*

	TRAY: 283	POS: 32			LOCAL SPECIFICITY: CW3										
10W9328	CW3	567	7.8	232	7	77	251	82	2	24	98	0.73	302.0	4.89	0.45 CW3
10W9328	BW77	328	7.5	11	1	66	250	49	8	85	92	0.31	32.2	1.67	0.24 BW77
10W9328	BW46	316	7.0	30	19	36	231	43	38	54	62	0.43	57.1	1.08	0.11 BW46
10W9328	BW62	267	6.0	8	40	28	191	36	83	77	17	0.04	0.5	0.81	0.08 BW62
10W9328	BW75	219	7.1	9	18	19	173	35	66	67	34	0.23	11.7	0.54	0.06 BW75
10W9328	CW1	192	5.5	4	13	15	160	26	76	78	24	0.14	3.9	0.57	0.06 CW1
10W9328	BW63	175	6.0	8	18	7	142	26	69	46	31	0.33	19.2	0.41	0.05 BW63
	TRAY: 283	POS: 33			LOCAL SPECIFICITY: CW3,BW46										
10W9329	CW3	557	7.9	229	4	62	262	87	1	21	99	0.78	340.3	6.25	0.58 CW3
10W9329	CW1	324	7.0	41	21	21	241	59	3	33	67	0.58	109.4	1.99	0.22 CW1
10W9329	BW46	262	6.0	4	8	17	233	42	26	80	34	0.20	10.9	1.36	0.14 BW46
10W9329	BW62	250	5.6	5	37	12	196	47	33	70	12	0.09	2.1	0.64	0.06 BW62
	TRAY: 283	POS: 34			LOCAL SPECIFICITY: B5,BW49,BW63										
10W9330	BW77	564	8.0	13	0	145	406	72	0	91	100	0.25	34.2	5.28	0.70 BW77
10W9330	BW53	551	7.4	13	1	132	405	69	7	91	93	0.24	32.8	5.23	0.69 BW53
10W9330	B51	537	7.6	43	2	89	403	68	4	67	96	0.50	133.5	5.15	0.56 B51
10W9330	BW52	492	7.6	23	4	66	399	60	14	74	86	0.42	86.8	3.70	0.43 BW52
10W9330	BW58	465	7.5	11	4	55	395	53	26	83	74	0.31	44.5	2.87	0.37 BW58
10W9330	BW63	450	6.4	15	6	40	389	49	28	72	72	0.40	72.0	2.76	0.34 BW63
10W9330	BW57	429	7.1	9	8	31	381	52	47	77	53	0.30	39.8	1.78	0.24 BW57
10W9330	B35	412	6.0	13	36	18	345	48	73	58	27	0.26	28.9	0.63	0.07 B35
10W9330	BW4	363	7.2	15	122	3	223	61	89	16	11	0.21	16.8	1.69	0.15 BW4
	TRAY: 283	POS: 35			LOCAL SPECIFICITY: B5,BW49,BW58,BW63										
10W9331	BW52	564	8.0	27	0	147	390	82	0	84	100	0.34	63.6	5.35	0.62 BW52
10W9331	BW53	537	8.0	14	0	133	390	78	0	90	100	0.27	38.1	4.57	0.59 BW53
10W9331	BW77	523	8.0	13	0	120	390	76	0	90	100	0.27	39.1	4.49	0.59 BW77
10W9331	B51	510	7.9	43	2	77	388	74	4	64	96	0.53	142.3	4.62	0.49 B51
10W9331	BW57	465	7.8	16	3	61	385	62	15	79	85	0.38	65.6	2.96	0.36 BW57
10W9331	BW58	446	7.3	11	3	50	382	55	21	81	79	0.34	51.6	2.42	0.31 BW58
10W9331	BW63	432	6.6	14	5	36	377	52	26	72	74	0.42	74.9	2.38	0.31 BW63
10W9331	BW4	413	7.0	23	126	13	251	50	84	36	16	0.18	13.2	2.36	0.21 BW4
	TRAY: 283	POS: 36			LOCAL SPECIFICITY: BW62										
10W9332	BW77	558	8.0	13	0	317	228	84	0	96	100	0.13	9.2	5.72	0.84 BW77
10W9332	BW57	545	7.4	20	1	297	227	83	4	93	96	0.15	12.3	5.92	0.79 BW57
10W9332	BW62	524	7.9	153	1	144	226	84	0	48	100	0.56	161.7	7.81	0.78 BW62
10W9332	BW76	370	6.6	17	0	127	226	71	0	88	100	0.27	28.0	5.64	0.78 BW76
10W9332	BW75	353	8.0	43	1	84	225	73	2	66	98	0.49	83.2	6.44	0.76 BW75
10W9332	B15.3	309	7.0	8	1	76	224	59	11	90	89	0.24	17.8	3.96	0.62 B15.3
10W9332	BW63	300	7.3	23	2	53	222	59	8	69	92	0.46	64.1	4.69	0.61 BW63
10W9332	BW46	275	6.6	46	42	7	180	52	47	13	53	0.57	90.6	1.67	0.18 BW46

References

1. Albert ED, Mickey MR, McNicholas AC, Terasaki PI. Seven new HL-A specificities and their distribution in three races. In: Terasaki PI (ed): Histocompatibility Testing 1970. Munksgaard, Copenhagen, 1970;221-230.
2. Alonso A, Ollier W, Doyle P, Williams E, Festenstein H. Family investigation demonstrating the association of HLA-Bw62 with HLA-Bw4. Tissue Antigens 1983;22:32-36.
3. Alonso A, Doyle P, Williams E, Festenstein H. Further splits of HLA-B15. Ninth Workshop Newsletter No. 5, 1984.
4. Alonso A, Doyle P, Williams E, Gill D, Ollier W, Jaraquemada D, et al. HLA-B15 heterogeneity in different populations. Tissue Antigens 1985;25:33-37.
5. Cambon-Thomsen A, Chandanayingyong D, Thomsen M, Hammond MG. Antigen Report: HLA-Bw63 and Other Bw4-Associated Variants of B15. In: Albert ED, et al. (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, Heidelberg, 1984;171-174.
6. Campell EM, Taljaard DG, Du Toit ED. B15 Kemp/B15 short Thai. Ninth Workshop Newsletter No. 2, 1983.
7. Chan SH, Naito S. Antigen Report: HLA-Bw46. In: Albert ED, et al. (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, Heidelberg, 1984;153-154.
8. Chan SH. Antigen Report: Bw46. In: Aizawa M (ed): HLA in Asia-Oceania 1986. Hokkaido University Press, Sapporo, Japan, 1986;106-107.
9. Chandanayingyong D. Very probable new Thai variant of B15. Ninth Workshop Newsletter No. 2, 1983.
10. Chandanayingyong D, Cambon-Thomsen A, Hammond MG. Antigen Report: HLA-Bw62 and other Bw6-associated variants of B15. In: Albert ED, et al. (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, Heidelberg, 1984;169-171.
11. Chiewsilp P, Chandanayingyong D, Sujirachato K. B15 short Thai. Ninth Workshop Newsletter No. 2, 1983.
12. Coates E, Stratton A, Dewar PJ. Definition of Bw62, Bw63, and B15.3. Ninth Workshop Newsletter No. 4, 1983.
13. Danilovs J, Pollock C. Joint report: 8w66. In: Terasaki PI (eds): Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, 1980;477-479.
14. Dejour G, Fauchet R, Jalais E, Bouhallier O, Genetet B. B15.3 (Chinese) specificity. Ninth Workshop Newsletter No. 3, 1983.
15. Dick HB. HLA-A, B and C serology and antigen reports. In: Boder WF, et al. (eds): Histocompatibility Testing 1977. Munksgaard, Copenhagen, 1977;157.
16. Fan LA, Choo SY, Beatty PG, Hansen JA. HLA-Bw62 variants identified by cytotoxic lymphocytes and isoelectric focusing gel electrophoresis. Tenth Workshop Newsletter No. 3, 1987.
17. Hammond MG. HLA-B15 complex in South African Indians. Ninth Workshop Newsletter No. 8, 1984.
18. Joysey VC, Roger JH, Bland C, et al. Further studies on a Malay population. In: Kissmeyer-Nielsen F (ed): Histocompatibility Testing 1975. Munksgaard, Copenhagen, 1975;251.
19. Kennedy LJ, Marsh SGE, Bodmer JG. Some preliminary thoughts on our results using the core typing sets. Tenth Workshop Newsletter No. 1, 1987.
20. Loon J, Bernoco D, Terasaki P, Takemura S. Report of the 72nd International Cell Exchange. UCLA Tissue Typing Laboratory, Los Angeles, 1981.
21. Loon J, Terasaki P, Takemura S. Report of the 73rd Cell and 1st Serum Exchange. UCLA Tissue Typing Laboratory, Los Angeles, 1981.
22. Parham P, et al. In: Dupont B. (ed): Immunobiology of HLA, Volume I: Histocompatibility Testing 1987. New York: Springer-Verlag, 1989.
23. Richiardi P, Castagneto M, D'Amaro J, et al. Four new HLA allelic factors subtypic to HLA-12 and w15. Their correlation with w4 and w6. J Immunogenet 1974;1:323.
24. Saueracker G, Christiansen FT, Dawkins RL. Antigen Report: ABC B15. In: Simons MJ, Tait BD (eds): Proceedings of the Second Asia and Oceania Histocompatibility Workshop Conference. Immunopublishing, Australia, 1981;80.
25. Shiraki T, Akasa T, Asami M, Ueda Y, Morishima Y, Hasegawa I. A study of a new HLA-B locus antigen TS-1 with 9th workshop sera. Ninth Workshop Newsletter No. 3, 1983.
26. Sierp G, Albert ED, Cambon-Thomsen A, Ohayon E. Data analysis of the HLA-typing for "Provinces Francaises," genetique des populations humaines. Colloque INSERM 1986;142:177-196.
27. Singal DP, Lung P. Bw62. In: Terasaki PI (ed): Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, 1980;462-464.
28. Taljaard DJ, Martell RW, du Toit ED. HLA Bw62, Bw63 and other B15 variants found in Cape Town. Tenth Workshop Newsletter No. 6, 1987.
29. Thorsby E, Kissmeyer-Nielsen F. New HL-A alleles. Identification by planned immunization. Vox Sang, Basel, 1970;18:138-148.
30. Thorsby E, Kissmeyer-Nielsen F, Svejgaard A. New alleles of the HLA system: Serological and genetic studies. In: Terasaki PI (ed): Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angles, 1980;137.
31. Troup GM. Joint Report: Bw63. In: Terasaki PI (ed): Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, 1980;465-486.
32. Zhao TM, Lee TD, Bu KJ, Zhang GL, Rubinstein P. Definition of the HLA-B antigen SH7, identical to TS-1 in the Chinese population. Ninth Workshop Newsletter No. 7, 1984.
33. Zhao TM, Shiraki T. Antigen report: HLA-Bw62, Bw63, Bw70, Bw62.1 and B15 variants. In: Aizawa M (ed): HLA in Asia-Oceania 1986. Hokkaido University Press, Sapporo, Japan, 1986;93-95.
34. Zhao TM, Lee TD. Equivalence of SH7, B15SL1 and other Bw6-associated variants of B15. Tenth Workshop Newsletter No. 4, 1987.

Antigen Society #12 Report (Bw54, Bw55, Bw56 and Bw42)

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History

The antigen Bw54 was officially designated as a split of Bw22 at the Seventh International Histocompatibility Workshop. This antigen was primarily defined in the Japanese population by several laboratories independently, and designated as J-1 by Juji et al. (1), as SAP-1 by Nakayama et al. (2) and as SN-1 by Saito et al. (3). Splits of Bw22 in Caucasians were recognized mainly by the association with either Cw1 or Cw3. The antigens Bw55 and Bw56, splits of Bw22 in Caucasians that were primarily called BT22 (4) and Te22 (5), Da30 (6) and AA-AJ (7), were confirmed at the Eighth International Workshop in 1980. The antigen Bw55 is associated with Cw3 in Caucasians and with Cw1 in Japanese. The antigen Bw56 is associated with Cw1 in Caucasians and with Cw4 in Japanese. A new Bw22 antigen as a split of Bw56 was primarily described by Tokunaga in 1983 at the Eighth Japanese Histocompatibility Workshop, but was not clearly defined. The antigen Bw42, previously called MWA (8), was defined at the Fifth International Workshop in 1972. This antigen was primarily found in Negroid populations.

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Serology

At this Workshop, 43 antisera and 3 extra antisera were submitted for this society set. Many of the sera were polyspecific (B7+Bw42, B7+Bw55, Bw42+Bw55 and so on). Several sera, however, were confirmed as monospecific for Bw55 and B7. The following sera formed clusters, identifying the Bw55 specificity (4592, 4590, 4595, 4581, and 4578), and the B7 (4607 and 4613). Other sera were polyspecific. The monospecific sera for Bw54, Bw56, and Bw42 were not obtained in this society set. Twenty-one sera (4602, 4608, 4605, 4610, 4586, 4593, 4600, 4601, 4612, 4614, 4615, 4582, 4583, 4613, 4607, 4578, 4581, 4590, 4592, 4588, and 4589) had R values greater than 0.7 with Bw22 antigen group. The reactivities of these sera are given in Table 1. The reaction patterns of these antigens are shown in Table 2. It seems that four sera (4584, 4593, 4617, and 4614) could split Bw56 into two distinct portions (long

Table 1. Analysis of the Correlation Between Serum Activity and Antigenic Specificity of the Sera

Serum Identity	Specificities	R value	% False pos	% False neg	Extras
4582	Bw22	0.89	6.7	2.6	
4583	Bw22	0.77	3.5	22.8	
4592	Bw55	0.82	3.4	8.8	Bw42
4590	Bw55	0.82	2.9	12.1	Bw42
4595	Bw55	0.76	4.2	15.5	Bw42
4581	Bw55	0.75	7.1	5.2	Bw54,Bw67
4578	Bw55	0.85	3.9	7.1	Bw42
4607	B7	0.87	0.9	15.9	
4613	B7	0.82	3.8	10.0	Bw42
4588	Bw55+w42	0.82	5.6	7.9	
4589	Bw55+w42	0.76	1.7	27.8	
4593	Bw22+w42	0.78	5.4	14.8	
4586	Bw22+w42	0.77	9.2	49.0	B7
4602	B7+w42+w55	0.84	7.7	7.5	Bw56
4608	B7+w42+w55	0.83	5.6	11.3	
4610	B7+w42+w55	0.79	4.1	16.8	Bw56
4614	B7+w42+w55	0.75	3.1	25.6	Bw56,Bw67
4606	B7+w42+w55+56	0.91	4.9	3.4	Bw54
4615	B7+w42	0.88	2.1	10.7	
4612	B7+w42	0.87	6.0	1.9	
4601	B7+w42	0.84	7.7	2.0	Bw55
4600	B7+w42	0.77	3.7	22.1	

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Table 2. Reaction Pattern of the Sera

	Serum No.																				
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6
	9	9	9	7	8	8	8	8	9	8	1	1	0	1	0	1	1	0	0	0	1
HLA	0	5	2	8	1	3	2	6	3	4	7	4	6	0	8	5	2	1	0	7	3
Bw54	–	–	–	–	*	+	+	+	–	–	–	–	–	*	–	–	–	–	–	–	–
Bw55	+	+	+	+	+	*	+	+	+	+	+	*	+	+	+	–	–	–	–	–	–
Bw56	–	–	–	+	–	–	+	+	*	*	*	*	+	+	–	–	–	–	–	–	–
Bw42	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–
B7	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+

pattern and short pattern. Table 3). Most of the Japanese are positive for the cluster sera, but all Caucasians except one are negative. Two families were available for the segregation of Bw56 short, but the family having Bw56 long was not found. There were a few false positives or false negatives, but the segregations were confirmed in these families. The cells of family 1 were the same cells that were used at the Eighth Japanese Workshop. No distinctive patterns suggestive of a split were detected in the 52 positive cells of B7, 20 positive cells of Bw42, 57 positive cells of Bw54, and 48 positive cells of Bw55 (Table 4).

A cluster of sera appears to split the Bw56 antigen into two portions, Bw56.1 and Bw56.2. The "short" Bw56.1 antigen, seen predominantly in Caucasians, reacts only with the sera 4578, 4582, 4605, 4606, and 4610, but does not react with the sera 4584, 4617, 4614, and 4593. The "long" Bw56.1 + Bw56.2 antigen seen predominantly in Japanese reacts with both groups of the tested sera.

In the Core serum set, Bw54 can be defined by three sera, and one serum of Bw42 monospecific sera was found from 30 sera submitted as containing Bw54, Bw55, Bw56, and/or Bw42. The reactivities of these sera are given in Table 5. Sera 303, 304, and 305 as Bw54, and 320 as Bw42 were confirmed as monospecific sera respectively. There were sera in the Core set showing concomitant specificities of Bw56, B35, and Bw62 but not with Bw55 or Bw54. This may be of some significance in that antigen Bw56 is different from the other Bw22 complex antigen in its epitope.

Table 3. The Reaction Pattern of Bw56 Antigen

					Serum No.									
					4	4	4	4	4	4	4	4	4	
					5	5	6	6	6	5	6	6	5	
					7	8	0	0	1	8	1	1	9	
Lab	ID	Ethnic	C	Locus	8	2	5	6	0	4	7	4	3	
TSU	412	Jap	4	–	+	+	+	+	+	+	+	+	+	Long
TSU	401	Jap	1	3	+	+	+	+	+	+	+	+	+	pattern
KSH	6015	Jap	4	–	+	+	+	+	+	+	+	+	+	
KSH	6021	Jap	4	–	+	+	+	+	+	+	+	+	+	
TOK	2515	Jap	1	3	+	+	+	+	+	+	+	+	+	
PAK	122	Jap	1	3	+	+	+	+	+	+	+	–	+	
SEK	3697	Jap	1	7	+	+	+	+	+	+	0	+	+	
MRV	846	Cau	1	7	–	+	+	+	+	–	+	+	+	
NEU	8	Cau	1	7	+	+	+	+	+	–	+	–	–	Short
NEU	9	Cau	1	6	+	+	+	+	+	–	–	–	–	pattern
NEU	12	Cau	1	4	+	+	+	+	+	–	–	–	–	
NEU	53	Cau	1	–	+	+	+	+	+	–	–	–	–	
NEU	4	?	1	–	+	+	+	+	+	–	–	–	–	
TOK	1072	Jap	1	7	+	+	+	0	+	–	–	–	–	Family 1 F
TOK	1853	Jap	1	5	+	+	+	+	+	–	–	–	–	M
TOK	1854	Jap	1	3	+	+	+	+	+	–	–	–	–	C
NEU	1	Cau	1	4	+	+	+	+	+	–	–	–	–	Family 2 F
NEU	2	Cau	1	7	–	–	+	+	+	–	–	–	–	M
NEU	3	Cau	1	1	+	+	+	+	+	–	–	–	–	C1
NEU	4	Cau	1	7	+	+	+	+	+	–	–	–	–	C2
NEU	5	Cau	1	4	+	+	+	+	0	–	–	–	–	C3
NEU	6	Cau	1	7	+	–	–	+	+	–	–	–	–	C4

Table 4. Pattern Analysis in Antigens of Antigen Society #12 Positive Cells

	Serum No.												
	4	4	4	4	4	4	4	4	4	4	4		
B7	6	6	6	6	6	6	6	6	6	6	6		
N=52	0	0	1	0	1	1	0	0	1	0	1		
	2	8	0	6	5	2	1	0	4	7	3	n=	Freq.
pattern 1	+	+	+	+	+	+	+	+	+	+	+	40	76.9%
pattern 2	+	+	+	+	+	+	+	+	+	−	+	3	5.8
pattern 3	+	−	+	+	+	+	+	+	+	−	+	2	3.8

	Serum No.												
	4	4	4	4	4	4	4	4	4	4	4		
Bw42	5	5	6	6	6	6	6	6	6	6	6		
N=20	9	8	0	0	1	0	1	1	0	0	1		
	3	6	2	8	0	6	5	2	1	0	4	n=	Freq.
pattern 1	+	+	+	+	+	+	+	+	+	+	+	9	45.0%
pattern 2	−	−	+	+	+	+	+	+	+	+	+	2	10.0
pattern 3	+	−	+	+	+	+	+	+	+	+	+	2	10.0
pattern 4	+	−	+	+	+	+	−	+	+	+	+	2	10.0

	Serum No.						
	4	4	4	4	4		
Bw54	5	5	5	5	6		
N=57	7	8	8	8	1		
	9	2	3	6	0	n=	Freq.
pattern 1	−	+	+	+	+	17	29.8%
pattern 2	−	+	+	+	−	17	29.8
pattern 3	+	+	+	+	+	11	20.4
pattern 4	−	−	−	−	−	7	13.0

	Serum No.														
	4	4	4	4	4	4	4	4	4	4	4	4	4		
Bw55	5	5	5	5	5	5	5	5	5	6	6	6	6		
N=48	9	9	9	8	7	7	8	9	8	0	0	1	0		
	2	0	5	1	8	9	2	3	6	2	8	0	6	n=	Freq.
pattern 1	+	+	+	+	+	+	+	+	+	+	+	+	+	28	58.3%

	Serum No.										
	4	4	4	4	4	4	4	4	4		
Bw56	5	5	5	5	5	5	6	6	6		
N=13	7	7	8	8	9	8	0	1	0		
	8	9	0	2	3	6	0	0	6	n=	Freq.
pattern 1	+	+	+	+	+	+	+	+	+	3	23.1%
pattern 2	+	−	+	+	−	−	−	+	+	2	15.4
pattern 3	+	−	−	+	−	−	−	0	+	2	15.4

Table 5. Analysis of Correlation Between Serum Activity and Antigenic Specificity of the Sera of Core Serum Set

Serum Identity	Specificities	++	+−	−+	−−	Total	R value
303	Bw54	41	1	20	312	374	0.78
304	Bw54	39	4	14	319	376	0.78
303	Bw54	33	9	10	320	372	0.74
320	Bw42	33	3	15	318	369	0.76
325	B7+Bw42	76	13	13	260	362	0.80
324	B7+Bw42	89	3	20	256	368	0.84

Conclusion

The following sera formed clusters defining Bw54 (10W303, 304, and 305), Bw55 (10W4578, 4581, 4590, 4592, and 4595), broad Bw22 (10W296, 4582, and 4583), and Bw42 (10W320). Bw54 and Bw42 could be defined well with the tenth Core serum set and Bw55 with the sera of Antigen Society set 12. Bw56, however, was not defined as a monospecific serum. It was possible to detect Bw55 and Bw56 based on reactivity with several polyspecific sera of the Core serum set containing Bw22 antigen group. We can split Bw56 into two portions, Bw56.1 and Bw56.2, by a cluster of sera. Most Japanese belong to Bw56.1+56.2 (long Bw56). Caucasian, Bw56.1 (short Bw56). The (short) Bw56.1 was seen in a Caucasian family, a Japanese family, and a random Caucasian population. The report by E.L. Milford et al. on The Serologic Exercises of the 10th International Histocompatibility Workshop gives additional detail on the serology of Antigen Society #12.

References

1. Juji T, Hagino Y, Tamaru M, et al. A possible new Japanese specific HL-A antigen (J-1). Jap Exp Med 1973;43:447.
2. Nakayama E, Itakura K, Yakura H, et al. Sa-1, a possible new HL-A specificity found in the Japanese population. Vox Sang, Basel, 1974;27:134.
3. Saito S, Naito S, Toyoda K, et al. A study on HLA system in Japanese. Tissue Antigens 1975;5:217.
4. Batchelor JR, Chapman BA. Genetic background and transplantation antigens. J Clin Path 1967;30:415.
5. Albert ED, Mickey MR, McNicolas, et al. In: Terasaki PI (ed): Histocompatibility Testing 1970. Munksgaard, Copenhagen, 1970:221.
6. Dausset J, Colombani J, Legrand L, et al. Population and family studies in a French population with special reference to non-HLA antibodies. In: Dausset J, Colombani J (eds): Histocompatibility Testing 1972. Munksgaard, Copenhagen, 1973;107.
7. Thorsby E, Lidholm A, Sandberg L, et al. The HL-A system. Further evidence of antigenic heterogeneity. Vox Sang, Basel, 1971;21:68.

Antigen Society #13 Report (B7, B27, Bw47, Bw73)

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HLA-B7 and HLA-B27 are well defined by monospecific Tenth International Workshop sera listed in Table 1 (Core sera) and Table 2 (Antigen Society 13 sera). There are no monospecific Workshop sera to define BW47, but this specificity is easily defined using B27 + Bw47 sera and B40 + B13 + Bw47 sera (Tables 1 and 2). Bw73 was originally described by Mayr and Kirnbauer as "ka" in 1977 (1), and was given WHO nomenclature after the Ninth Workshop, based on the reactivity of three sera (9w245, 246, 247), which also reacted with other B7-CREG (cross reactive group) antigens (2). In the Tenth Workshop there are five Core sera plus seven Antigen Society 13 sera that define Bw73 in the absence of other B7-CREG antigens. In addition, there are two Core sera, 10w336 (9w440) and 10w338, that were submitted as monospecific Bw73 sera. Although these two sera were not strictly monospecific, having extra reactivity toward Bw46 and Cw7, they were not reactive with other B7-CREG antigens. The Cw7 reactivity in these two sera does not appear to be an artifact of Cw7 linkage with B7 and Bw73. A "new" B7-like antigen, pot, previously described by Reekers et al. (3), is identified by three Core sera and five sera from Antigen Society 13. These eight sera are positive with other B7-CREG antigens, so assignment of B pot is possible only in the absence of other B7-CREG antigens.

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Table 1. Best Core Sera

	Percent Inclusion					
Serum	B7	B27	Bw47	Bw73	B pot	Others
276	96					
278	89					
335	86					
275	95					Bw42(65)
325	98					Bw42(83)
280		96				
282		98				
283		88				
292		91	92			
288		94	56			
368			90			Bw60(94),Bw61(92), B13(89)
353			99			Bw60(98),Bw61(98), B13(96),Bw48(91)
375			97			Bw60(98),Bw61(98), B13(97),Bw48(95)
370			87			Bw60(95),Bw61(94), B13(90)
339	98	89		68		Bw42(65),Bw67(50)
341	98	97		76		
343	98	95		59		Bw48(68)
340	98	92		77	100	Bw42(93),Bw48(77), Bw60(69),Bw55(74)
333	96	21		86	67	Bw42(47)
336				70		Bw46(55),Cw7(32)
338				70		Bw46(23),Cw7(31)
381	97	90			100	Bw42(81),Bw48(78), Bw67(57)

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13. Yang SY, Chang A, Olivero R, Relias V, Yunis EJ. IEF patterns of HLA-B13 antigens from Orientals and Caucasians. Immunogenetics 1986;21:125-134.
14. Kamura H, Kohara S, Shimizu K, Akaza T. Antigen report: B13, B40(Bw60,Bw61), Bw41, B247, Bw48, Bw73, FU. In: Aizawa M (ed): HLA in Asia-Oceania. Hokkaido University Press, Sapporo, Japan, 1986;120-122.
15. Mervart H, Taylor C, Ting A. Antigen report: HLA-Bw41. In: Albert ED, Baur MP, Mayr WR (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, 1984;149.
16. Mueller-Eckhardt G, Schrender I, Mayr WR, Westphal E, Mueller-Eckhardt C. A new Bw6-associated B locus antigen "RI." Ninth Workshop Newsletter No. 5, pp 24-26.
17. Reekers P, Beucken vander M, Andrien M, Beelen J, Mueller-Eckhardt G, Schreuder GMTh. HLA-BPot: A new HLA antigen within the B7 cross-reactive group. Tissue Antigens 1986;28:182-189.
18. Watson K, Ness D, Gantan Z, Perkins H, Grumet FC. ST40: A new Bw40 subtype identified by alloantisera and a monoclonal antibody. Hum Immunol 1982;5:170-179.

Antigen Society #15 Report (Bw4 and Bw6)

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During the Tenth Workshop, 27 anti-sera in the Antigen Society and 22 anti-sera in the Core serology serum set were provided for the serologic analysis of the supertypic HLA-B locus specificities Bw4 and Bw6. The serum sets included 12 monoclonal antibodies with Bw4 or Bw6 specificity. For each of the submitted sera, Q score, R values, % extra, and missed reactions, as well as "tail" antigens derived by 2x2 comparison of their reactivity on Caucasians, Negroes, and Orientals, are shown in Tables 1 and 2.

The Bw4 specificity was best defined with the alloantisera 493, 495, 498 and the monoclonals 2064, 2065, 2102 of the Core serum set, as well as the alloantisera 4750, 4751, 4752, 4758, 4759, 17017II MUE of the Antigen Society with highly concordant positive reaction patterns in 87% of the Bw4 positive cells in all analyzed different ethnic groups. Among the anti-Bw6 anti-sera reagents, 4765, 4766, 4768, 4769 and the monoclonal antibody 2BC WES of the Antigen Society, as well as the sera 499, 500 and the monoclonals 2106, 2107, showed the highest Q scores and R values in all analyzed races. Pattern analysis revealed that in 96% of Bw6 positive cells of all ethnic groups there was a positive reaction with at least six of the nine best Bw6 sera.

Three of the anti-Bw4 alloantisera (4750, 4751, 4752) appeared to be "monospecific" for HLA-Bw4 in all ethnic subpopulations. As in previous Workshops, a larger group (4758, 4759, 17017II MUE, 271MUC, 493, 498, 2064, 2065, 2102) of anti-Bw4 alloantisera and monoclonals were found to recognize also determinants on HLA-A locus antigens, in particular A23, A24, A32, A2. In tail and pattern analysis most of these anti-Bw4 sera showed extra-reactions with A24, A23, A32 in Caucasians (Table 3), whereas in Negroes and Orientals only the monoclonals 2064 and 2065 detected these HLA-A locus antigens frequently. Two anti-Bw4 alloantisera (495, 260 MUC) revealed extra-reactivity with subgroups of the Bw6-associated HLA-B locus antigens B50 and B35 in Caucasians. Monoclonal antibodies 2101 and 2198 appeared to have Bw4 reactivity, but were frequently negative with Bw4 and Bw6 positive cells. In serum-to-serum correlations, 16 of the 25 submitted anti-Bw4 anti-sera were included in one cluster with three serum subgroups of highest correlations (Table 4).

In contrast, all anti-Bw6 anti-sera except the alloantiserum 4764 were operationally monospecific and had high correlations among each other (Table 4). Serum

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Table 1. Tenth Workshop Core Sera With Anti-Bw4, Bw6 Reactivity

10th WS No.	Origin	Specificity	Q score			R value			% extras			% misses			Other Specificities
			C	N	O	C	N	O	C	N	O	C	N	O	
493	LAW	Bw4	4.1	5.4	3.8	0.77	0.83	0.79	4	3	4	4	4	6	A32
495	GAN	Bw4	5.3	5.2	6.4	0.79	0.84	0.90	4	5	3	3	1	2	B50
498	ILE	Bw4	5.6	5.8	7.0	0.83	0.88	0.92	5	3	3	2	2	1	A32
496	MBC	Bw4	3.7	3.8	4.4	0.70	0.67	0.76	3	2	2	12	14	9	
497	DPT	Bw4	0.8	0.7	0.4	0.30	0.26	0.23	1	3	3	43	55	41	
2057*	MUC	Bw4	4.2	4.0	3.1	0.63	0.73	0.67	0	20	2	20	11	13	A32, A23, A24
2064*	TSU	Bw4	8.3	5.7	5.5	0.97	0.89	0.83	0	2	1	1	3	6	A32, A23, A24
2065*	TSU	Bw4	7.3	5.2	5.6	0.94	0.86	0.83	1	4	1	1	3	6	A32, A23, A24
2102*	GEL	Bw4	8.6	5.4	4.7	0.92	0.86	0.81	3	2	7	0	1	2	A32
2098*	WAN	Bw4	1.9	1.6	1.3	0.55	0.47	0.48	2	0	4	23	28	25	
2101*	MUC	Bw4	1.1	0.4	0.3	0.41	0.27	0.21	1	11	1	35	53	45	
499	BRN	Bw6	6.0	5.2	3.3	0.81	0.80	0.62	0	9	2	7	7	11	
500	MUE	Bw6	5.9	5.0	2.5	0.83	0.81	0.56	5	4	1	0	1	7	
507	FAU	Bw6	2.9	3.4	2.3	0.58	0.64	0.51	4	3	2	12	8	13	
509	ROO	Bw6	1.3	1.9	1.2	0.40	0.56	0.41	12	8	8	6	2	2	
501	RIC	Bw6	3.5	3.4	2.4	0.59	0.59	0.52	1	1	2	18	16	14	
504	BER	Bw6	4.1	3.1	1.6	0.70	0.55	0.37	1	2	2	12	17	28	
502	DPT	Bw6	3.6	2.4	1.8	0.68	0.69	0.45	6	4	6	3	4	7	
2106*	KRE	Bw6	6.0	3.6	2.1	0.86	0.72	0.50	2	2	3	2	8	12	
2107*	SFR	Bw6	5.6	4.5	2.4	0.89	0.80	0.54	1	3	4	2	4	8	
2103*	GEL	Bw6	1.3	0.2	–	0.42	0.07	–	5	11	–	21	22	–	
2105*	THP	Bw6	0.1	0.1	–	0.07	0.13	–	13	–	–	23	27	–	
427	HAM	B44	7.2	5.3	7.4	0.81	0.77	0.87	30	4	2	0	1	0	Bw4
166	EAE	B51	4.6	1.7	5.1	0.66	0.46	0.71	3	1	2	0	10	0	Bw4

*Monoclonal antibody; C Caucasians (N=410); N Negros (N=589); O Orientals (N=1001)

Table 2. Tenth Workshop Antigen Society Sera With Anti-Bw6, -Bw6 Reactivity

10th WS No.	Origin	Specificity	Q score		R value		% extras		% misses		Other Specificities
			C	N	C	N	C	N	C	N	
4750	YAG	Bw4	4.7	1.6	0.76	0.57	1	6	11	16	
4751	BRN	Bw4	3.5	3.4	0.74	0.78	5	6	8	3	
4752	ROO	Bw4	8.2	1.3	0.89	0.51	2	8	3	16	
4753	BRU	Bw4	2.4	2.4	0.62	0.26	6	7	13	32	
4755	ROO	Bw4	3.9	1.2	0.64	0.49	0	5	21	22	
4756	KWC	Bw4	2.8	1.9	0.58	0.58	20	18	1	3	
4757	MBC	Bw4	1.5	1.4	0.43	0.53	6	16	21	6	
4758	CEP	Bw4	5.4	1.7	0.84	0.59	2	16	3	3	A32, A23, A24
4759	DPT	Bw4	4.5	1.7	0.73	0.50	6	24	2	1	A32, A23, A24
4760	DPT	Bw4	4.1	3.6	0.67	0.68	15	16	1	0	
17023II#	MUE	Bw4	1.4	–	0.51	–	12	–	5	–	A23, A24
17017II#	MUE	Bw4	3.7	–	0.74	–	6	–	3	–	A23, A24
260#	MUC	Bw4	4.4	–	0.71	–	14	–	0	–	B35
271#	MUC	Bw4	1.6	–	0.53		16	–	6	–	A2
4761	OHA	Bw6	2.7	1.4	0.53	0.40	2	10	22	12	
4762	BAC	Bw6	2.7	1.0	0.59	0.42	3	5	16	22	
4763	BAC	Bw6	0.4	0.7	0.15	0.33	2	4	60	33	
4764	ROO	Bw6	3.5	1.7	0.71	0.55	1	6	7	11	A3
4765	BRU	Bw6	7.3	3.0	0.93	0.74	1	6	1	2	
4766	BRU	Bw6	5.4	2.2	0.84	0.64	4	7	2	5	
4767	MAC	Bw6	7.4	2.5	0.94	0.67	1	6	1	5	
4768	PNW	Bw6	6.3	3.6	0.87	0.78	2	6	3	1	
4769	ROO	Bw6	6.6	2.6	0.81	0.70	1	16	7	3	
4770	HFP	Bw6	4.6	2.2	0.78	0.61	1	5	9	10	
4771	ROO	Bw6	5.4	1.9	0.79	0.59	2	6	6	8	
307#	MUC	Bw6	3.4	–	0.67	–	3	–	10	–	
2BC4*#	WES	Bw6	4.9	–	0.80	–	4	–	3	–	

= Local assignment; * = monoclonal antibody; C = Caucasians (N=585); N = Negroes (N=141)

Table 3. Serologic Patterns of Selected Anti-Bw4 Sera on A23⁺, A24⁺ or A32+, Bw4⁻ Cells

		10th WS Sera							
	%	493	498	2064	2065	2105	2057	4758	4759
In 50 Caucasians	32.0	–	–	+	+	+/–	+/–	+	+
	26.0	+	+/–	+	+	+/–	+/–	+	+
	12.0	+	+	+	+	+	+	+	+
	12.0	–	+	+	+	+/–	+/–	+	+
	6.0	–	–	+	+/–	–	+	–	+
	4.0	+/–	–	+	+	–	–	–	+
	2.0	+	+	+	+	+	+	–	–
	2.0	+	+	+	+	–	+	–	+
	2.0	–	–	–	–	+	+	–	+
	2.0	–	–	+	+	–	+	–	+
In 161 Negroes and Orientals	56.5	–	–	+	+	–	+	nt	nt
	8.6	–	–	–	–	–	–	nt	nt
	7.4	–	–	+	+	–	–	nt	nt
	7.4	+/–	–	+	+	+	+	nt	nt
	6.2	+	+/–	+	+	–	+	nt	nt
	4.9	–	–	–	–	–	+	nt	nt
	4.8	–	–	+	–	–	–	nt	nt
	1.8	–	+	+	+	–	+	nt	nt
	1.8	+/–	+/–	+/–	+/–	–	+/–	nt	nt

Table 4. Internal Correlations of Selected Anti-Bw4 and Bw6 Sera

a) Anti-Bw4 Sera											b) Anti-Bw6 Sera		
493													
495	.8												
498	.8	.8											
2102	.7	.7	.8								499		
2064	.7	.7	.7	.7							500	.7	
2065	.7	.7	.7	.7	.9						2106	.7	.8
4756	.6	.6	.6	.6	.6	.7					2107	.7	.8 .9
4760	.7	.6	.7	.7	.7	.7	.8						
4758	.6	.6	.6	.6	.8	.8	.8	.8					
4759	.5	.5	.5	.5	.7	.7	.7	.8	.8				
4750	.6	.6	.7	.6	.6	.6	.6	.7	.7	.6			
4752	.6	.6	.7	.7	.6	.6	.7	.7	.7	.6	.8		
4751	.7	.7	.7	.6	.6	.6	.7	.7	.7	.6	.8	.8	

4764 showed extra-reactions with A3 in Caucasians. Two monoclonal antibodies 2106 and 2107 were excellent Bw6 reagents in all races. A further monoclonal antibody 2103 with Bw6 reactivity missed many Bw6 positive cells.

In summary, inclusion analysis of the Bw4 and Bw6 specificities revealed no significant deviation with previous reports. The anti-Bw4 sera submitted to the Tenth Workshop were more heterogenous in their reactivity with HLA-A locus antigens than the anti-Bw6 anti-sera and showed differences in this reaction in various ethnic groups. All anti-Bw4 monoclonal reagents with high performance in this serologic analysis also were found to cross-react with HLA-A locus antigens and thus did not lead to a further differentiation of the HLA-Bw4 specificity.

Antigen Society #16 Report (Cw1, Cw3, Cw9, Cw10, Cw11)

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History

HLA-Cw1 and HLA-Cw3 were first described in the Fourth and the Fifth International Histocompatibility Workshops, respectively (1,2). The three subtypic factors of Cw3, namely Cw3.1, Cw3.2, and Cw3.3, were demonstrated in the Ninth International Histocompatibility Workshop (3). The expression of Cw1 and Cw3 on the same haplotype in Orientals was observed by Payne et al. in 1975 (4) and was later found to be strongly associated with Bw46. CwB had been proposed as a new C locus antigen for this Cw1-Cw3 co-segregating phenotype in the Second Asia and Oceania Histocompatibility (AOH) Workshop (5). However, CX46 was more recently used to designate this C locus antigen in the Third AOH Workshop (6). In the present Workshop, the new assignment of HLA specificities is given by the WHO nomenclature committee (7). This included antigens in C locus, i.e., Cw9 (Cw3.1), Cw10 (Cw3.2), and Cw11 (CX46, Cw1+3, Cw1X3, C-Bangkok, CSH1).

Linkage Disequilibrium

There are strong associations of Cw1 with B27 in Caucasoids; with Bw54, Bw55, and Bw59 in Japanese, and with Bw54, Bw55, and B40 in Chinese and Thais (Table 1). Further strong associations were observed for Cw9 with Bw55 and Bw62; Cw10 with Bw62, Bw60 in Caucasians; Cw9 with B35, Bw55; Cw10 with B40; Cw11 with Bw46 in Japanese; and Cw9 with B15; Cw10 with Bw58 and B40; Cw11 with Bw46 in Chinese and Thais.

Serology

Cw1. Eight sera were submitted as anti-Cw1, six from the Core set and two from the Antigen Society (Table 2 and Table 3). The Cw1 antigen was well defined by the Tenth Workshop anti-sera: 508, 512, 513, 514, 259, 510, and 4773. Pattern analysis among Caucasians, Japanese, and Thais indicated that they were strongly correlated with one another.

Cw3, Cw9, Cw10, and Cw11. Twenty-nine sera were submitted as anti-Cw3, 10 from the Core set and 19 from the Antigen Society (Table 2 and Table 3). There appeared to be seven possible subtypes of Cw3, all of which were identified by patterns. It was confirmed that Cw3 could be divided into Cw9 and Cw10 by the sera 524, 525, 526, 527, 529, 530, 260, 528, 9064, 532, 4777, 4778, 4779, 4783, 4785, 4786, 4787, 4788, 4780, 4781, 4793, and 9035 (Table 4). Cw9 reacted with all of the above anti-Cw3 sera, while Cw10 gave negative reactions with sera 9064 and 532.

Only a few Thai individuals were found to be Cw9 short (Cw9S). Approximately 45 cells of several ethnic origins appeared to have the Cw9S pattern as displayed in the CXS Workshop data base. Cw9S can be differen-

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SD locus? In: Kissmeyer-Nielsen F (ed): Histocompatibility Testing 1975. Munksgaard, Copenhagen, 1975; p 343.
5. Chandanayingyong D. Bw46 report. In: Simons MJ, Tait BD (eds): Proceedings of the Second Asia and Oceania Histocompatibility Workshop Conference. Melbourne, 1981; p 127.
6. Shimbo M, Mitani T, Ikeda H, Sekiguchi S: Analysis of "CX46" specificity. In: Aizawa W (ed): Proceedings of the Third Asia and Oceania Histocompatibility Workshop Conference. Sapporo, Japan, p 52.
7. Bodmer WF, Albert E, Bodmer JG, Dupont B, Mach B, Mayr W, et al. Nomenclature for factors of the HLA system.
8. Sun Y, Shimbo M, Mitani T, Ikeda H, Sekiguchi S. HLA-Cw1, Cw3 Report. In: Aizawa M (ed): Proceedings of the Third Asia and Oceania Histocompatibility Workshop Conference. Sapporo, Japan, p 52.

Antigen Society #17 Report (Cw5 and Cw8)

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As in the previous Workshops, the definition of Cw5 and Cw8 was rather difficult because of the cross-reactivity between Cw5 and Cw8 and because of the strong linkage disequilibrium between Cw5 and B44 as well as between Cw5 and B14.

Cw5 and Cw8 were nearly absent in non-Caucasian populations; therefore, the reactivity of the anti-sera used to define these factors was analyzed by taking into account only the cells of Caucasians tested in Antigen Society 17 (n = 164).

The best sera for the definition of Cw5 and Cw8 are listed in Table 1. Serum 10w552 contains, besides an anti-Cw5 + Cw8, a strong anti-B44.

There was no good indication for the existence of splits of Cw5 or Cw8.

In spite of the fact that one good anti-Cw5 (serum 10w551) and one anti-Cw8 of reasonable quality (serum 10w554) have been found, difficulties remain with regard to the definition of Cw5 and Cw8.

Table 1. Reactivity of the 10w Anti-Cw5 and Cw8 Sera

10w serum	antigen	ave.str	%fp	%fn	r	Qsc
551 #	Cw5	7.5	0	0	0.98	8.96
550 #	Cw5	5.3	0	30	0.79	5.63
554 #	Cw8	6.3	25	10	0.78	5.21
9004 #	Cw5	7.2	41	0	0.72	5.91
	Cw8	2.5	29	77	0.35	1.51
553 #	Cw5	5.1	37	26	0.61	4.58
	Cw8	8.0	10	0	0.90	7.48
4794 ##	Cw5	3.0	60	65	0.28	1.01
	Cw8	6.4	55	10	0.59	5.21

serum of the Core set
serum of Antigen Society 17
ave.str = average strength
%fp = percentage of false positive reactions
%fn = percentage of false negative reactions
r = correlation coefficient
Qsc = quality score

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Antigen Society #18 Report (Cw4 and Cw6)

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HLA-Cw4 (formerly T4, RH315) and HLA-Cw6 (formerly T7) obtained the formal HLA designation after the Sixth and Seventh International Histocompatibility Workshops respectively (1,2). Since then, it has been observed that some troubles arise in identifying Cw6 when Cw4 is present: The difficulty is due to the fact that while bispecific Cw4 + Cw6 and monospecific Cw4 sera are comparatively frequent, Cw6 sera not recognizing Cw4 are quite rare (3,4).

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Table 1. Core Set Sera Analysis: Blacks

	STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
533 BEN ENG CLB105												
CW4	90	99	7.5	96	357	137	5	3	212	7.735	0.948	320.593
RES	14	CW6 = 20%		A1 = 10%		A30 = 10%		B27 = 10%		BW57 = 10%		
536 US5 CRO Martinez2.21.83												
CW4	76	99	6.6	87	357	122	19	3	213	6.023	0.867	268.529
RES	14	A2 = 10%		BW46 = 10%		B− = 10%		CW1 = 10%		A11 = 10%		
537 UKI BRS 5956.CBT												
CW4	93	95	7.5	94	356	134	8	11	203	5.806	0.884	278.261
+ B7	47	97	2.1	19	214	6	26	5	177	0.544	0.262	14.708
+ BW57	78	99	2.0	14	182	3	18	2	159	0.694	0.262	12.534
OR	92	99	6.0	73	356	143	52	2	159	4.752	0.726	187.595
RES	14	CW6 = 20%		A3 = 10%		AW19 = 10%		BW65 = 10%		BW70 = 10%		
539 EAE ZAR MZ467												
CW4	75	93	6.7	90	337	121	14	14	188	4.475	0.822	227.680
+ BW50	33	94	5.3	75	202	3	1	11	187	3.669	0.380	29.240
OR	74	94	6.6	89	337	124	15	11	187	4.588	0.835	235.124
RES	52	CW6 = 10%		A2 = 9%		B7 = 7%		CW2 = 7%				
541 NCY MRV 22166.0												
CW4	76	100	7.0	94	355	133	9	1	212	7.457	0.936	311.197
RES	33											
543 US8 JLE Billing												
CW4	78	77	7.0	92	349	127	11	49	162	2.895	0.669	156.128
CW6	55	98	6.2	88	211	45	6	4	156	5.839	0.859	155.515
OR	72	98	6.7	91	349	172	17	4	156	6.045	0.877	268.406
RES	27	A30 = 11%		BW42 = 11%								
545 US5 TER TER.C4610B												
CW4	93	70	7.7	98	358	139	3	65	151	3.668	0.666	158.673
CW6	51	88	6.0	87	216	45	7	20	144	3.728	0.686	101.511
+ CW5	54	90	5.6	71	164	5	2	15	142	3.021	0.380	23.650
* TEC10	33	90	0.0	0	157	0	0	15	142	12.532	0.106	1.751
OR	82	90	7.2	94	358	189	12	15	142	5.122	0.842	253.736
RES	33	A2 = 7%		CW2 = 7%		A1 = 6%		A28 = 6%				
540 FRA DDC Pierson												
CW4	95	70	7.6	96	356	134	6	64	152	2.648	0.646	148.446
CW6	86	90	7.1	90	216	47	5	17	147	3.382	0.741	118.589
OR	93	90	7.4	94	356	181	11	17	147	4.070	0.837	249.426
RES	84	A30 = 7%		CW3 = 7%		B7 = 6%						
547 GER MUE MUE23928												
CW6	74	92	6.5	84	359	53	10	23	273	3.939	0.706	178.731
RES	42	CW4 = 17%		BW53 = 12%		AW36 = 7%		B35 = 7%		A2 = 6%		
558 FRA DDC Devinat												
CW7	71	78	4.9	61	358	43	27	64	224	0.777	0.338	40.946
+ B39	38	79	4.3	71	388	5	2	59	222	1.493	0.187	10.087
+ AW34	44	81	3.3	42	281	11	15	48	207	0.484	0.170	8.117
+ BW60	45	82	4.7	67	255	4	2	44	205	1.489	0.193	9.461
+ A24	47	84	3.1	43	249	6	8	38	197	0.661	0.167	6.942
+ A26	65	86	3.3	35	235	7	13	31	184	0.620	0.162	6.164
+ BW4	77	96	2.2	18	215	29	129	2	55	0.259	0.178	6.794
OR	67	96	3.1	35	358	105	196	2	55	0.705	0.246	21.607
RES	43	A30 = 22%		A29 = 11%		B45 = 11%		BW72 = 11%		CW6 = 11%		
205 GER BRA 918B												
B35	78	77	5.7	74	360	48	17	67	228	1.825	0.419	63.304
+ B39	6	79	3.7	70	295	7	3	60	225	1.254	0.211	13.181
+ AW34	33	82	3.4	48	285	12	13	48	212	0.585	0.207	12.204
+ BW70	24	85	2.9	42	260	13	18	35	194	0.452	0.225	13.122
+ A1	33	87	2.8	35	229	9	17	26	177	0.498	0.197	8.851
+ BW71	38	89	3.5	42	203	5	7	21	170	1.173	0.224	10.204
+ A29	9	91	2.5	36	191	4	7	17	163	0.942	0.211	8.542
OR	51	91	4.0	54	360	98	82	17	163	1.199	0.479	82.771
RES	24	CW4 = 12%		A2 = 10%		BW53 = 7%		A30 = 6%				

Table 1. *Continued*

		STK	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
192	US5 PNW Juli												
	B35	64	92	4.8	62	354	40	24	24	266	1.804	0.539	102.800
+	AW34	20	93	2.0	23	290	6	20	18	246	0.386	0.177	9.082
+	CW4	45	97	1.8	15	264	13	72	5	174	0.314	0.230	13.918
*	BW53	20	98	1.3	7	179	1	14	4	160	0.283	0.105	1.989
	OR	55	97	2.9	34	354	59	116	5	174	0.946	0.398	56.067
	RES	23	CW3 = 17%		A28 = 12%		A30 = 8%		BW70 = 8%		BW71 = 8%		
191	US2 MBC BC.CY.CHAD												
	BW53	70	79	6.3	85	358	73	13	56	216	2.297	0.569	115.706
	B35	75	96	6.2	82	272	47	10	9	206	3.866	0.780	165.463
	OR	73	96	6.3	84	358	120	23	9	206	4.214	0.809	234.089
	RES	43	CW4 = 10%		CW6 = 7%		BW70 = 7%						
562	ITI PUR CNTS.105												
	CW4	76	99	6.4	84	357	120	23	2	212	5.200	0.852	259.267
	RES	20	A26 = 20%		A30 = 20%		BW42 = 20%		BW58 = 20%		CW3 = 20%		
535	EAE MYR Furlinger												
	CW4	82	98	7.2	94	322	118	7	3	194	6.365	0.928	277.584
	RES	71	BW53 = 22%		CW6 = 22%		A2 = 11%		A11 = 11%		BW61 = 11%		
347	US5 MIT B5612.4												
	B7	93	71	7.9	100	354	42	0	90	222	4.185	0.471	78.543
	BW42	64	82	7.0	98	312	40	1	50	221	4.588	0.584	106.483
	CW4	47	97	3.1	38	271	46	75	4	146	1.338	0.448	54.432
*	BW67	33	97	0.0	0	150	0	0	4	146	17.600	0.213	6.813
	OR	68	97	4.9	63	354	128	76	4	146	2.750	0.610	131.694
	RES	27	A2 = 11%		BW73 = 11%		A1 = 11%		B8 = 11%		B45 = 6%		
196	FRA BET E1218												
+	CW4	98	79	7.8	97	359	139	4	45	171	4.519	0.744	198.518
	BW53	58	85	6.1	83	216	15	3	30	168	3.227	0.458	45.308
	B35	61	91	5.5	74	198	14	5	16	163	2.766	0.525	54.574
+	B51	27	93	3.4	50	179	4	4	12	159	2.054	0.318	18.149
+	CW2	26	97	2.0	19	171	8	34	4	125	0.375	0.267	12.219
	OR	87	97	6.2	78	359	180	50	4	125	4.795	0.717	184.646
	RES	9	A1 = 10%		A30 = 10%		B7 = 10%		BW71 = 10%				
214	US2 WOL NW14002												
	CW4	95	73	7.4	93	358	133	10	59	156	3.033	0.640	146.695
	BW53	62	79	7.1	100	215	17	0	42	156	3.709	0.468	47.054
	B35	84	86	6.9	89	198	17	2	25	154	3.253	0.536	56.781
	B51	58	90	6.5	100	179	8	0	17	154	5.065	0.522	48.828
+	BW58	33	92	4.8	75	171	3	1	14	153	2.799	0.337	19.390
	BW70	20	94	2.1	22	167	6	21	8	132	0.356	0.225	8.430
*	BW52	20	95	3.5	50	140	1	1	7	131	3.186	0.263	9.703
*	TE79	33	94	0.0	0	140	0	0	8	132	14.100	0.151	3.189
	OR	87	94	6.6	84	358	184	34	8	132	3.938	0.766	209.840
	RES	37	CW3 = 10%		BW71 = 8%								
538	BEN BOU 64307 6/7/84												
	CW4	63	98	5.9	79	349	111	30	4	204	4.781	0.797	221.594
	RES	43	A30 = 10%		B35 = 10%		BW42 = 10%		CW7 = 10%		A23 = 10%		
204	ITI CON CA181												
	CW4	88	91	7.0	89	356	125	16	19	196	4.098	0.791	222.621
+	A32	33	92	4.0	60	215	3	2	16	194	1.909	0.286	17.627
	OR	87	92	6.9	88	356	128	18	16	194	4.068	0.798	226.503
	RES	37	A2 = 7%		CW7 = 7%								
534	UKI FES Langlais												
	CW4	49	100	4.3	56	355	79	62	1	213	2.872	0.646	148.193
	RES	33											
2101	GER MUC TU109												
*	BW4	52	95	2.7	29	347	70	175	5	97	0.533	0.258	23.043
	RES	23	A24 = 8%		B7 = 8%		A2 = 8%		B45 = 8%		BW70 = 8%		
2103	UKI GEL 103.1.51												
*	BW6	76	35	5.6	72	351	199	78	48	26	0.104	0.063	1.145
	RES	68	CW4 = 13%		BW53 = 11%		AW33 = 6%						

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Table 2. Core Set Sera Analysis: Caucasians

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
533	BEN ENG CLB105												
	CW4	90	100	7.8	100	382	98	0	1	283	10.199	0.986	371.724
	RES	33											
536	US5 CRO Martinez2.21.83												
	CW4	85	100	7.3	95	382	93	5	1	283	8.857	0.952	346.275
	RES	33											
537	UKI BRS 5956.CBT												
	CW4	91	98	7.7	99	381	96	1	7	277	9.334	0.940	336.847
	RES	38	CW6 = 15%		A24 = 12%		B13 = 8%		A1 = 8%		BW60 = 8%		
539	EAE ZAR MZ467												
	CW4	75	97	7.2	98	378	95	2	9	272	6.798	0.920	320.215
+	BW50	54	99	3.5	45	281	5	6	4	266	2.192	0.482	65.314
	OR	74	99	6.8	93	378	100	8	4	266	6.246	0.916	317.000
	RES	27	A1 = 12%		A24 = 12%		BW57 = 12%		CW6 = 12%		A2 = 6%		
541	NCY MRV 22166.0												
	CW4	71	99	6.5	88	382	86	12	2	282	7.280	0.897	307.135
	RES	20	A2 = 17%		A31 = 17%		BW50 = 17%		B44 = 17%		CW6 = 17%		
543	US8 JLE Billing												
	CW4	78	71	7.2	98	381	95	2	81	203	3.447	0.603	138.355
	CW6	74	99	6.9	94	284	78	5	3	198	8.465	0.924	242.343
	OR	77	99	7.1	96	381	173	7	3	198	8.152	0.943	338.464
	RES	78	A2 = 13%		A3 = 7%		B7 = 7%		B13 = 7%		CW2 = 7%		
545	US5 TER TER.C4610B												
	CW4	90	64	7.7	99	382	97	1	101	183	3.794	0.550	115.722
	CW6	74	89	7.0	95	284	79	4	22	179	5.229	0.794	179.039
+	B5	64	91	4.0	50	201	4	4	18	175	1.317	0.263	13.880
*	TEC10	33	91	0.0	0	193	0	0	18	175	13.381	0.097	1.833
	OR	83	91	7.2	95	382	180	9	18	175	5.196	0.855	279.357
	RES	44	A2 = 11%		CW7 = 8%		B7 = 7%		CW5 = 7%				
540	FRA DDC Pierson												
	CW4	98	69	8.0	100	382	98	0	89	195	4.191	0.596	135.617
	CW6	92	97	7.8	100	284	83	0	6	195	8.886	0.943	252.745
	OR	96	97	7.9	100	382	181	0	6	195	9.758	0.964	354.972
	RES	33	A2 = 7%		A23 = 7%		B49 = 7%		B51 = 7%		A1 = 7%		
547	GER MUE MUE23928												
	CW6	79	94	6.8	90	380	81	9	16	274	4.278	0.818	254.447
	RES	61	B35 = 17%		CW4 = 17%		A3 = 11%		A2 = 7%				
558	FRA DDC Devinat												
	CW7	81	68	6.8	89	381	119	15	78	169	1.840	0.544	112.608
+	CW7L	81	71	7.6	100	247	9	0	69	169	2.850	0.278	19.101
	OR	81	71	6.9	90	381	128	15	69	169	2.092	0.583	129.543
	RES	56	CW4 =9%		B35 = 7%		A3 = 6%		A1 = 6%		A2 = 6%		
205	GER BRA 918B												
	B35	95	95	7.9	100	382	78	0	16	288	8.081	0.880	295.958
+	CW7L	33	96	2.7	30	304	3	7	13	281	1.207	0.221	14.901
+	B39	56	96	2.4	21	294	3	11	10	270	0.799	0.202	12.054
	OR	92	96	6.6	82	382	84	18	10	270	4.504	0.804	246.907
	RES	30	CW7 = 9%		A1 = 7%		CW6 = 7%						
192	US5 PNW Juli												
	B35	73	95	6.9	94	382	73	5	14	290	6.377	0.849	275.409
+	B51	20	97	1.7	15	304	6	33	8	257	0.275	0.204	12.629
*	BW53	60	97	2.0	14	265	1	6	7	251	0.958	0.150	5.944
	OR	69	97	5.2	68	382	79	38	8	257	2.998	0.704	189.591
	RES	26	A1 = 10%		CW7 = 10%		B8 = 8%		CW6 = 8%		A24 = 8%		
191	US2 MBC BC.CY.CHAD												
	B35	61	96	5.9	81	382	63	15	13	291	4.412	0.767	224.624
	BW53	69	97	5.4	71	304	5	2	8	289	4.329	0.503	76.985
+	B5	11	98	3.1	38	297	3	5	5	284	3.053	0.370	40.642
+	CW4	33	99	1.8	16	289	3	16	2	268	0.888	0.295	25.173
	OR	59	99	5.0	66	382	74	38	2	268	4.263	0.740	209.121
	RES	14	A2 = 18%		B44 = 18%		CW5 = 18%		B51 = 9%		CW2 = 9%		

Table 2. *Continued*

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
562	ITI PUR CNTS.105												
	CW4	86	100	6.3	78	376	76	21	1	278	5.818	0.840	265.000
	RES	33											
535	EAE MYR Furlinger												
	CW4	79	99	6.9	93	373	87	7	3	276	6.511	0.922	316.906
	RES	14	A26 = 10%		A11 = 10%		B8 = 10%		B39 = 10%		CW7L = 10%		
347	US5 MIT B5612.4												
	B7	96	80	7.9	100	374	74	0	60	240	5.861	0.660	162.918
	CW4	45	97	4.2	59	300	53	37	7	203	2.455	0.631	119.551
+	BW60	27	98	2.0	22	210	4	14	3	189	0.810	0.328	22.570
*	BW67	33	98	0.0	0	192	0	0	3	189	19.330	0.243	11.321
*	BW42	60	99	8.0	100	192	1	0	2	189	9.370	0.524	52.690
	OR	74	98	5.5	72	374	131	51	3	189	3.604	0.730	199.194
	RES	56	A11 = 12%		A2 = 12%		CW7 = 12%		A25 = 6%		B51 = 6%		
196	FRA BET E1218												
+	CW4	99	95	7.9	99	382	97	1	13	271	7.207	0.905	312.595
	B35	45	97	7.0	100	284	4	0	9	271	6.542	0.526	78.619
*	BW53	71	97	8.0	100	280	2	0	7	271	7.075	0.447	56.037
	OR	97	97	7.9	99	382	101	1	9	271	7.518	0.930	330.413
	RES	62	A2 = 10%		CW7 = 10%		A24 = 6%		B5 = 6%		A11 = 6%		
214	US2 WOL NW14002												
	CW4	96	87	7.7	97	382	95	3	38	246	4.843	0.761	221.408
	B51	57	94	5.4	71	284	24	10	14	236	3.635	0.613	106.827
+	B5	79	98	6.8	89	250	8	1	6	235	4.877	0.679	115.309
	B35	78	99	8.0	100	241	3	0	3	235	7.421	0.654	103.234
*	BW52	33	99	1.3	0	238	0	12	3	223	1.611	0.041	0.395
*	BW53	71	100	8.0	100	238	2	0	1	235	9.444	0.718	122.600
*	BW70	33	99	1.0	0	238	0	6	3	229	0.674	0.074	1.308
*	TE79	33	99	0.0	0	238	0	0	3	235	19.862	0.244	14.195
	OR	89	99	7.1	90	382	130	14	3	235	6.219	0.900	309.756
	RES	56	CW6 = 18%		A29 = 12%		BW53 = 12%		A25 = 6%		A30 = 6%		
538	BEN BOU 64307 6/7/84												
	CW4	62	98	5.9	83	382	81	17	6	278	4.709	0.833	265.101
	RES	45	A24 = 10%										
204	ITI CON CA181												
	CW4	87	99	7.4	95	380	93	5	4	278	6.754	0.932	329.811
	RES	33	A2 = 12%		B7 = 12%		A24 = 12%		B27 = 12%		CW2 = 12%		
534	UKI FES Langlais												
	CW4	56	100	4.3	57	380	55	42	1	282	3.419	0.688	179.707
	RES	33											
2101	GER MUC TU109												
+	CW6	66	86	4.5	56	359	48	37	37	237	1.131	0.428	65.637
+	B5	71	89	6.3	82	274	9	2	28	235	3.186	0.405	44.866
*	BW4	71	98	2.1	17	263	26	124	2	111	0.443	0.244	15.600
	OR	69	98	3.1	34	359	83	163	2	111	1.104	0.345	42.716
	RES	43	CW8 = 17%		A3 = 8%		AW33 = 8%		BW64 = 8%		B35 = 8%		
2103	UKI GEL 103.1.51												
	BW6	74	81	5.3	68	360	205	97	11	47	1.220	0.364	47.776
+	CW4	38	88	3.8	50	58	5	5	6	42	1.078	0.355	7.324
	OR	73	88	5.3	67	360	210	102	6	42	1.537	0.377	51.144
	RES	47	B44 = 14%		A2 = 10%		A32 = 7%		CW5 = 7%		CW7 = 7%		

Table 3. Core Set Sera Analysis: Orientals

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
533	BEN ENG CLB105												
	CW4	86	99	7.1	91	586	50	5	5	526	7.209	0.891	465.682
	RES	9	A26 = 12%		BW61 = 12%		A24 = 12%		CW1 = 12%		CW3 = 8%		
536	US5 CRO Martinez2.21.83												
	CW4	79	92	6.8	87	567	47	7	42	471	3.936	0.632	226.767
+	BW46	40	95	2.8	31	513	20	44	22	427	0.856	0.319	52.070
+	CW1	57	100	2.0	18	449	22	103	0	324	0.812	0.361	58.538
	OR	66	100	3.3	37	567	89	154	0	324	2.015	0.495	139.153
	RES	33											
537	UK1 BRS 5956.CBT												
	CW4	92	96	7.1	87	584	48	7	20	509	6.259	0.755	332.629
	RES	22	A24 = 13%		A26 = 10%		CW1 = 9%		A2 = 7%		BW61 = 7%		
539	EAE ZAR MZ467												
	CW4	77	99	6.5	85	572	47	8	5	512	5.973	0.858	421.570
	RES	27	A11 = 16%		CW6 = 11%		A2 = 11%						
541	NCY MRV 22166.0												
	CW4	78	99	6.7	87	584	48	7	4	525	6.345	0.879	451.257
	RES	14	A24 = 17%		A2 = 8%		B38 = 8%		BW54 = 8%		CW1 = 8%		
543	US8 JLE Billing												
	CW4	78	97	6.7	89	579	49	6	18	506	4.829	0.779	351.478
	CW6	54	100	5.1	67	524	16	8	2	498	4.198	0.747	292.310
	OR	73	100	6.2	82	579	65	14	2	498	5.560	0.872	440.527
	RES	43	A32 = 10%		B44 = 10%		A2 = 10%		BW63 = 10%		CW3 = 10%		
545	US5 TER TER.C4610B												
	CW4	83	94	7.2	93	583	51	4	31	497	5.281	0.725	306.637
	CW6	54	97	5.0	67	528	16	8	15	489	3.068	0.560	165.657
+	BW59	45	98	2.3	22	504	4	14	11	475	0.713	0.232	27.045
*	TEC10	20	98	6.0	100	486	1	0	10	475	8.491	0.300	43.878
	OR	75	98	5.7	73	583	71	26	11	475	3.673	0.756	332.831
	RES	36	A24 = 16%		CW3 = 11%		A11 = 7%		B7 = 7%		AW33 = 7%		
540	FRA DDC Pierson												
	CW4	93	94	7.7	98	584	54	1	31	498	6.096	0.759	336.583
	CW6	69	99	7.1	100	529	24	0	7	498	8.153	0.859	390.401
	OR	86	99	7.5	99	584	78	1	7	498	8.264	0.938	513.480
	RES	41	A24 = 17%		BW62 = 10%		AW33 = 7%		B44 = 7%		A26 = 7%		
547	GER MUE MUE23928												
	CW6	63	96	4.1	52	561	12	11	20	518	1.957	0.415	96.718
+	CW4	60	98	2.4	22	538	11	40	9	478	0.753	0.308	50.953
+	CW1, 3	11	99	2.0	27	487	3	8	6	470	1.402	0.304	44.989
	OR	56	99	2.8	31	561	26	59	6	470	1.183	0.451	114.130
	RES	20	A2 = 10%		AW33 = 10%		A11 = 10%		B44 = 7%		CW1 = 7%		
558	FRA DDC Devinat												
	CW7	73	89	6.1	81	586	112	27	50	397	2.682	0.657	253.217
+	A11	52	94	2.7	29	447	30	74	20	323	0.492	0.308	42.402
+	B7	64	95	3.4	40	343	4	6	16	317	1.377	0.264	23.962
	OR	69	95	4.6	58	586	146	107	16	317	1.944	0.584	199.587
	RES	31	A2 = 12%		A24 = 10%		CW1 = 8%		CW3 = 6%				
205	GER BRA 918B												
	B35	69	85	6.4	87	580	60	9	76	435	3.088	0.548	174.250
+	BW54	47	88	2.7	31	511	21	46	55	389	0.263	0.182	16.883
+	B27	69	89	4.9	62	444	5	3	50	386	1.747	0.210	19.635
+	B15	56	89	4.8	60	436	3	2	47	384	1.792	0.172	12.831
+	CW8	60	90	2.8	30	431	6	14	41	370	0.479	0.143	8.797
+	TS1	33	91	3.4	43	411	3	4	38	366	1.162	0.156	10.021
+	CW4	24	92	2.2	23	404	9	30	29	336	0.256	0.159	10.173
+	BW58	33	93	2.6	38	365	3	5	26	331	1.004	0.178	11.532
	OR	60	93	3.9	49	580	110	113	26	331	1.178	0.481	134.119
	RES	27	A2 = 11%		A24 = 10%		CW3 = 8%		A11 = 6%				

Table 3. *Continued*

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
192	US5 PNW Juli												
	B35	58	95	6.1	85	562	58	10	24	470	4.072	0.738	306.432
+	B15S-												
	LI	60	96	6.3	86	494	6	1	18	469	6.522	0.446	98.270
+	CW8	9	97	1.8	21	487	4	15	14	454	0.658	0.199	19.269
*	BW53	33	97	1.0	0	468	0	1	14	453	4.704	0.081	3.079
	OR	55	97	5.2	72	562	68	26	14	454	3.344	0.729	298.730
	RES	29	A2 = 12%		A24 = 10%		CW3 = 10%		BW62 = 7%		BW52 = 6%		
191	US2 MBC BC.CY.CHAD												
	B35	68	96	6.0	81	572	55	13	21	483	3.781	0.727	302.233
+	B51	28	98	2.1	20	504	11	43	10	440	0.563	0.284	40.536
*	BW53	60	98	8.0	100	450	1	0	9	440	8.689	0.314	44.355
	OR	61	98	4.3	54	572	66	56	10	440	2.138	0.623	221.938
	RES	39	A24 = 19%		A2 = 6%		BW60 = 6%		BW52 = 6%		CW3 = 6%		
562	ITI PUR CNTS.105												
	CW4	86	97	7.0	89	565	47	6	13	499	5.679	0.808	369.230
+	BW62	38	98	1.5	8	512	5	55	8	444	0.157	0.142	10.304
	OR	81	98	4.0	46	565	52	61	8	444	2.027	0.571	184.480
	RES	26	A24 = 13%		A31 = 10%		A2 = 10%		B44 = 6%		CW3 = 6%		
535	EAE MYR Furlinger												
	CW4	90	98	6.9	87	575	47	7	12	509	5.276	0.808	375.230
	RES	52	A24 = 12%		A2 = 8%		CW3 = 8%		B44 = 6%		A26 = 6%		
347	US5 MIT B5612.4												
	B7	89	88	7.6	98	583	61	1	63	458	5.320	0.646	243.489
	CW4	73	93	4.4	54	521	28	24	35	434	1.709	0.426	94.381
	BW67	7	94	2.7	46	469	6	7	29	427	1.277	0.256	30.617
+	BW56	11	94	3.2	60	456	3	2	26	425	2.396	0.241	26.517
+	BW48	38	95	2.0	16	451	5	26	21	399	0.460	0.131	7.709
+	BW55	27	96	1.9	15	420	4	22	17	377	0.731	0.135	7.621
+	B40	27	96	1.8	14	394	4	25	13	352	0.403	0.144	8.135
+	BW60	23	97	1.4	8	365	5	55	8	297	0.166	0.121	5.366
*	BW42	33	97	1.0	0	305	0	1	8	296	4.502	0.106	3.423
	OR	69	97	3.6	42	583	116	162	8	297	1.545	0.475	131.515
	RES	26	A2 = 15%		CW3 = 12%		A26 = 8%		BW61 = 8%				
196	FRA BET E1218												
+	CW4	96	84	7.7	96	575	53	2	81	439	4.411	0.559	179.453
	B35	73	92	6.0	77	520	43	13	38	426	2.969	0.583	176.826
+	B51	14	95	2.5	31	464	17	37	21	389	0.856	0.310	44.522
+	B27	33	96	3.1	43	410	3	4	18	385	1.596	0.240	23.697
*	BW53	20	96	6.0	100	403	1	0	17	385	7.650	0.234	22.111
	OR	74	96	5.3	67	575	116	56	18	385	2.640	0.679	265.296
	RES	38	A24 = 13%		BW60 = 8%								
214	US2 WOL NW14002												
	CW4	91	77	7.6	96	584	53	2	123	406	3.386	0.463	124.928
	B35	69	84	5.8	78	529	47	13	76	393	1.887	0.464	114.004
	B51	77	90	5.0	62	469	33	20	43	373	1.681	0.445	92.706
+	B15S-												
	LI	54	91	5.6	71	416	5	2	38	371	3.492	0.265	29.216
	BW52	35	94	2.5	28	409	17	44	21	327	0.547	0.269	29.700
+	B5	56	95	5.8	75	348	3	1	18	326	3.860	0.316	34.757
*	BW53	20	95	6.0	100	344	1	0	17	326	8.618	0.233	18.619
*	BW70	33	95	1.0	0	344	0	3	18	323	1.272	0.033	0.381
*	TE79	33	95	0.0	0	344	0	0	18	326	17.346	0.105	3.806
	OR	75	95	5.2	66	584	158	82	18	326	2.354	0.647	244.739
	RES	49	BW62 = 11%		CW3 = 11%		A2 = 9%		A24 = 9%		BW46 = 6%		
538	BEN BOU 64307 6/7/84												
	CW4	69	98	5.8	76	579	41	13	10	515	4.866	0.753	328.587
+	BW60	38	99	1.3	7	525	5	69	5	446	0.171	0.149	11.731
	OR	66	99	3.2	36	579	46	82	5	446	1.801	0.507	148.884
	RES	8	A24 = 18%		A2 = 9%		B7 = 9%		CW1 = 9%				

Table 3. *Continued*

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
204	ITI CON	CA181											
	CW4	79	93	6.5	85	580	46	8	36	490	4.437	0.649	244.548
+	CW3.												
	2	4	95	1.9	22	526	10	35	26	455	0.451	0.191	19.276
+	B27	56	95	4.2	50	481	3	3	23	452	2.411	0.234	26.434
	OR	65	95	4.4	56	580	59	46	23	452	2.162	0.565	185.191
	RES	14	A24 = 8%		CW1 = 8%		BW61 = 8%		A11 = 8%		CW3 = 8%		
534	UKI FES	Langlais											
	CW4	61	99	5.1	67	581	37	18	6	520	4.597	0.733	312.372
	RES	8	A2 = 13%		BW61 = 9%		A24 = 9%						
2101	GER MUC	TU109											
+	B27	87	91	6.4	75	570	6	2	50	512	3.761	0.263	39.364
	BW4	37	98	1.9	16	562	43	224	7	288	0.352	0.239	31.971
	OR	45	98	2.0	18	570	49	226	7	288	0.409	0.257	37.626
	RES	41	A26 = 11%		BW60 = 9%		A24 = 9%		A31 = 9%		A2 = 6%		
2103	UKI GEL	103.1.51											
*	BW6	75	17	6.6	88	573	450	60	52	11	0.064	0.058	1.944
	RES	79	A24 = 14%		A2 = 8%		B44 = 7%		AW33 = 7%		B51 = 7%		

Core Set Serology

Results of the Core set sera analysis in the three main ethnic groups (Blacks, Caucasians, Orientals) are reported in Tables 1, 2, and 3.

The analysis includes the sera submitted as recognizing Cw4 and/or Cw6 as well as the sera whose reactions have been observed to be associated with these antigens in the previous central data analysis.

The high number and the good quality of the sera submitted allow a very good definition of Cw4. The situation regarding definition of the Cw6 antigen with the Tenth Workshop Core set sera is not satisfactory: when Cw4 is absent, Cw6 may be assigned because of the positivity of the cluster of Cw4+Cw6 sera (10W 543, 10W540, and 10W545): serum 10W547, the only Cw6 monospecific serum, is negative in 14% of Cw4-ve, Cw6+ve cells and positive in 15% of Cw4+ve,Cw6−ve cells. This problem is clearly shown in Table 4, in which the reaction patterns of some selected sera in the three main ethnic groups are reported.

Antigen Society Serology

The results of the Antigen Society sera analysis are reported in Tables 5 and 6: Analysis has not been performed in Orientals because of the very low number (18) of cells tested. Among the 15 sera submitted, two recognize both Cw4 and Cw6, ten Cw4, and two Cw6. The reaction patterns of some selected sera are shown in Table 7: The problems in the definition of Cw6 appear to be similar to those observed with Core set sera.

Table 4. Frequency (%) of the Reaction Patterns of Selected Core Set Sera (Patterns Observed in Only One Cell Have Not Been Reported)

Phenotype		Tenth W No.							Population		
Cw4	Cw6	5	5	5	5	5	5	5	Black	Cauc	Ori
		4	4	4	4	3	4	3			
		3	0	5	7	3	1	5			
		a	a	a	b	c	c	c			
+	−								(N=118)	(N=73)	(N=56)
		+	+	+	−	+	+	+	63	75	57
		+	+	+	+	+	+	+	14	4	16
		+	+	+	−	+	−	+	−	8	−
		+	+	+	−	+	+	−	−	4	7
		+	+	+	+	+	−	+	−	3	−
		−	+	+	−	+	+	+	−	−	5
		+	+	−	−	+	−	+	−	−	4
		+	+	+	+	+	+	−	2	−	−
		+	−	+	+	+	+	+	2	−	−
−	+								(N=54)	(N=84)	(N=26)
		+	+	+	+	−	−	−	50	74	46
		+	+	+	−	−	−	−	9	8	15
		−	+	+	+	−	−	−	−	6	−
		+	+	−	+	−	−	−	11	5	8
		−	+	+	−	−	−	−	−	−	8
		+	−	+	+	−	−	−	11	−	−
−	−								(N=174)	(n=198)	(n=498)
		−	−	−	−	−	−	−	98	87	91
		−	−	+	−	−	−	−	6	8	2
		−	+	−	−	−	−	−	6	−	1
		−			+		−	−	2	−	1
		−						−	2	1	−

a = Cw4+Cw6; b = Cw6; c = Cw4

Table 5. Antigen Society Sera Analysis: Blacks

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
4795	US5 MIT C5902.4												
	CW4	66	88	5.2	66	200	19	10	20	151	1.642	0.474	44.873
+	A1	7	91	3.4	55	171	6	5	14	146	1.818	0.351	21.108
+	CW6	57	97	2.4	24	160	10	32	4	114	0.569	0.314	15.813
+	BW42	45	100	1.6	11	118	4	33	0	81	0.312	0.261	8.068
	OR	53	100	2.9	33	200	39	80	0	81	1.129	0.398	31.699
	RES	33											
4796	NCY KAP CWR.PLA.241												
+	B35	65	94	7.4	100	200	7	0	11	182	5.736	0.587	68.853
+	A26	9	96	1.7	17	193	4	20	7	162	0.576	0.189	6.897
+	A23	33	97	1.8	14	169	3	19	4	143	0.487	0.197	6.541
*	CW4	71	98	1.7	10	147	2	19	2	124	0.280	0.185	5.035
	OR	42	97	2.5	26	200	14	39	4	143	0.733	0.361	26.064
	RES	82	A29 = 13%		B44 = 13%		BW42 = 13%		B13 = 13%		BW70 = 13%		
4797	US5 MIT D4155.1												
	CW4	28	95	4.3	62	200	18	11	9	162	2.239	0.578	66.768
+	B35	56	96	7.3	100	171	3	0	6	162	6.478	0.539	49.628
+	A26	45	99	2.1	19	168	4	17	2	145	0.700	0.317	16.882
	OR	36	99	3.6	47	200	25	28	2	145	1.869	0.583	68.019
	RES	14	A2 = 17%		A29 = 17%		B13 = 17%		B39 = 17%		CW2 = 17%		
4798	GER GAZ T.1758												
	CW4	97	89	8.0	100	200	29	0	19	152	6.247	0.722	104.182
+	A1	33	94	5.3	82	171	9	2	10	150	3.559	0.577	56.976
	OR	82	94	7.3	95	200	38	2	10	150	4.961	0.820	134.457
	RES	57	A30 = 12%		A26 = 8%		CW6 = 8%		A29 = 8%				

Table 5. *Continued*

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
4799	FRA BET 1268												
	CW4	43	91	5.0	79	200	23	6	16	155	2.681	0.613	75.254
+	A23	39	96	3.0	40	171	10	15	6	140	1.770	0.432	31.873
	OR	42	96	4.1	61	200	33	21	6	140	2.861	0.631	79.623
	RES	7	A30 = 14%		A26 = 7%		B7 = 7%		B15 = 7%		CW3 = 7%		
4800	US5 PNW BORT												
	CW4	30	99	4.1	59	200	17	12	2	169	2.647	0.677	91.727
	RES	33											
4801	ITI MTT F10113												
	CW4	75	92	7.1	93	199	26	2	14	157	5.001	0.723	104.112
+	A1	26	96	4.6	73	171	8	3	6	154	3.264	0.603	62.195
+	A30	7	100	1.4	10	160	6	53	0	101	0.287	0.246	9.655
	OR	54	100	3.4	41	199	40	58	0	101	1.643	0.501	50.044
	RES	33											
4802	UK1 CXH Lopian												
	CW4	90	95	7.9	100	200	29	0	8	163	7.126	0.850	144.433
+	B44	9	97	1.8	16	171	4	21	4	142	0.763	0.229	8.987
+	A29	8?	100	3.0	29	146	4	10	0	132	1.645	0.495	35.750
	OR	82	100	4.6	54	200	37	31	0	132	2.909	0.655	85.790
	RES	33											
4803	EAE ZAR MZ435												
	CW4	49	87	4.7	67	198	18	9	22	149	2.104	0.455	41.073
+	B13	26	91	5.4	80	171	8	2	14	147	3.447	0.491	41.229
+	AW68	5	95	2.1	24	161	8	26	6	121	0.476	0.273	11.987
+	B45	9	98	1.8	27	127	4	11	2	110	1.376	0.377	18.054
	OR	29	98	3.2	44	198	38	48	2	110	1.505	0.516	52.774
	RES	14	A30 = 33%		B44 = 33%		BW58 = 33%		B15 = 33%		CW3 = 33%		
4804	FRA PRR SCH1423												
	CW4	88	93	6.8	85	198	23	4	12	159	4.510	0.692	94.920
+	A1	7	96	3.4	55	171	6	5	6	154	2.738	0.483	39.914
+	B44	45	99	1.9	17	160	4	19	2	135	0.592	0.296	14.013
	OR	68	99	4.3	54	198	33	28	2	135	2.580	0.629	78.248
	RES	14	A30 = 20%		A26 = 20%		B7 = 20%		B15 = 20%		CW3 = 20%		
4805	BEN DPT SMEETS.80												
	CW4	96	91	8.0	100	198	27	0	16	155	6.668	0.742	109.106
+	A1	7	94	3.4	55	171	6	5	10	150	2.680	0.407	28.279
+	A30	47	98	1.7	14	160	8	51	2	99	0.268	0.225	8.092
	OR	74	98	3.6	42	198	41	56	2	99	1.232	0.481	45.896
	RES	71	AW68 = 20%		A29 = 20%		BW57 = 20%		BW70 = 20%		CW6 = 20%		
4803	EAE ZAR MZ435												
	CW4	49	87	4.7	67	198	18	9	22	149	2.104	0.455	41.073
+	B13	26	91	5.4	80	171	8	2	14	147	3.447	0.491	41.229
+	AW68	5	95	2.1	24	161	8	26	6	121	0.476	0.273	11.987
+	B45	9	98	1.8	27	127	4	11	2	110	1.376	0.377	18.054
	OR	29	98	3.2	44	198	38	48	2	110	1.505	0.516	52.774
	RES	14	A30 = 33%		B44 = 33%		BW58 = 33%		B15 = 33%		CW3 = 33%		
4804	FRA PRR SCH1423												
	CW4	88	93	6.8	85	198	23	4	12	159	4.510	0.692	94.920
+	A1	7	96	3.4	55	171	6	5	6	154	2.738	0.483	39.914
+	B44	45	99	1.9	17	160	4	19	2	135	0.592	0.296	14.013
	OR	68	99	4.3	54	198	33	28	2	135	2.580	0.629	78.248
	RES	14	A30 = 20%		A26 = 20%		B7 = 20%		B15 = 20%		CW3 = 20%		
4805	BEN DPT SMEETS.80												
	CW4	96	91	8.0	100	198	27	0	16	155	6.668	0.742	109.106
+	A1	7	94	3.4	55	171	6	5	10	150	2.680	0.407	28.279
+	A30	47	98	1.7	14	160	8	51	2	99	0.268	0.225	8.092
	OR	74	98	3.6	42	198	41	56	2	99	1.232	0.481	45.896
	RES	71	AW68 = 20%		A29 = 20%		BW57 = 20%		BW70 = 20%		CW6 = 20%		

Table 5. *Continued*

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
4806	UKI GEL VAU20802												
	CW4	84	94	7.6	100	200	29	0	10	161	7.504	0.823	135.468
+	A1	33	98	3.4	55	171	6	5	4	156	2.434	0.535	48.926
+	B44	9	100	1.5	17	160	4	19	0	137	0.870	0.378	22.824
	OR	68	100	1.6	62	200	39	24	0	137	3.343	0.716	102.621
	RES	33											
4807	BEN ROD VR61809												
	CW4	74	80	6.6	86	198	25	4	34	135	2.377	0.504	50.332
	CW6	45	93	4.0	52	169	26	24	8	111	1.278	0.509	43.761
	OR	60	93	5.0	65	198	51	28	8	111	2.014	0.612	74.275
	RES	26	A30 = 12%		BW58 = 12%		A1 = 6%		B7 = 6%		BW65 = 6%		
4808	US5 SIN 13782												
	CW6	67	91	4.9	62	200	32	20	14	134	1.778	0.537	57.701
+	B39	45	93	5.0	67	148	4	2	10	132	2.674	0.400	23.722
	OR	65	91	4.9	62	200	36	22	10	132	2.069	0.587	68.854
	RES	22	BW58 = 14%		B44 = 14%		A30 = 14%		AW68 = 10%		BW70 = 10%		
4809	UKI AST DB5424												
	CW6	71	80	6.3	83	200	43	9	30	118	1.937	0.563	63.284
+	B39	33	83	6.7	100	148	6	0	24	118	4.012	0.394	23.028
+	CW4	74	88	3.6	40	142	10	15	14	103	0.655	0.285	11.560
+	A3	45	90	3.0	33	117	4	8	10	95	0.621	0.232	6.310
	OR	67	90	5.2	66	200	63	32	10	95	1.718	0.583	67.961
	RES	39	A30 = 18%		BW70 = 12%		CW2 = 12%		A26 = 6%		B7 = 6%		

Table 6. Antigen Society Sera Analysis: Caucasians

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
4795	US5 MIT C5902.4												
	CW4	61	95	5.3	71	348	63	26	14	245	2.931	0.682	162.059
+	BW57	20	97	2.7	29	259	6	15	8	230	1.224	0.310	24.927
+	B37	33	98	2.5	23	238	3	10	5	220	1.163	0.278	18.445
	OR	56	98	4.5	59	348	72	51	5	220	2.595	0.644	144.328
	RES	38	A1 = 11%		CW6 = 11%		A24 = 7%		B44 = 7%		CW3 = 7%		
4796	NCY KAP CWR.PLA.241												
+	B35	61	97	5.1	69	346	47	21	9	269	3.132	0.705	171.945
+	BW53	54	99	5.1	71	278	5	2	4	267	3.922	0.602	100.862
+	B51	33	100	1.5	8	271	3	35	1	232	0.466	0.217	12.817
*	CW4	33	100	1.2	0	233	0	13	1	219	1.073	0.075	1.294
	OR	59	100	4.9	19	316	55	58	1	232	2.470	0.609	128.438
	RES	60	A2 = 20%		A11 = 20%		B7 = 20%		B44 = 20%		CW7 = 20%		
4797	US5 MIT D4155.1												
	CW4	74	92	6.3	83	349	74	15	22	238	3.634	0.724	182.982
+	B51	27	97	3.2	44	260	15	19	7	219	1.530	0.493	63.114
+	B35	33	98	5.3	100	226	3	0	4	219	7.817	0.610	84.090
	OR	65	98	5.4	73	349	92	34	4	219	3.610	0.761	202.141
	RES	64	A2 = 14%		A24 = 9%		B7 = 9%		CW7 = 9%		A29 = 9%		
4798	GER GAZ T.1758												
	CW4	93	91	7.7	98	348	87	2	24	235	5.534	0.823	235.494
+	B51	35	96	3.3	41	259	14	20	10	215	2.071	0.426	46.979
+	B35	78	97	8.0	100	225	3	0	7	215	6.424	0.516	59.796
	OR	86	97	6.5	83	348	104	22	7	215	4.322	0.813	230.287
	RES	65	CW7 = 11%		A2 = 8%		B7 = 8%		CW6 = 8%				
4799	FRA BET 1268												
	CW4	61	98	5.8	80	347	71	18	4	254	4.841	0.824	235.405
	RES	33	A2 = 13%		A24 = 13%		B51 = 13%		B7 = 7%		B44 = 7%		
4800	US5 PNW BORT												
	CW4	54	98	5.6	81	349	72	17	4	256	4.633	0.832	241.471
	RES	33	CW6 = 12%		B44 = 12%		A26 = 6%		A30 = 6%		B13 = 6%		

Table 6. *Continued*

		STR	SPEC	AVE	INCL	N	TP	FN	FP	TN	Q	R	CHI²
4801	ITI MTT F10113												
	CW4	80	91	6.5	84	346	74	14	22	236	3.747	0.730	184.366
+	B35	56	93	7.3	100	258	3	0	19	236	5.469	0.346	30.928
+	CW3	47	95	2.4	24	255	8	25	11	211	0.443	0.251	16.061
	OR	77	95	5.5	69	346	85	39	11	211	2.768	0.677	158.442
	RES	36	CW7 = 14%		A2 = 12%		B7 = 10%		A1 = 9%				
4802	UKI CXH Lopian												
	CW4	86	99	7.4	96	349	85	4	3	257	6.945	0.940	308.401
	RES	60	A2 = 20%		A24 = 20%		B7 = 20%		B44 = 20%		CW7 = 20%		
4803	EAE ZAR MZ435												
	CW4	78	90	5.7	72	347	63	24	26	234	2.275	0.615	131.455
+	CW6	58	97	2.5	26	260	20	58	6	176	0.616	0.338	29.718
	OR	74	97	4.2	50	347	83	82	6	176	1.746	0.534	98.832
	RES	47	B7 = 12%		A2 = 9%		CW2 = 6%		CW7 = 6%		A24 = 6%		
4804	FRA PRR SCII1423												
	CW4	87	87	7.4	97	348	86	3	34	225	4.568	0.761	201.685
+	A23	85	88	4.9	56	259	5	4	29	221	1.326	0.244	15.371
	OR	88	88	7.2	93	348	91	7	29	221	3.854	0.764	203.098
	RES	74	A1 = 9%		A2 = 9%		CW6 = 9%						
4805	BEN DPT SMEETS.80												
	CW4	94	95	7.7	98	319	80	2	11	226	7.739	0.892	254.021
+	B49	11	96	1.8	27	237	3	8	8	218	1.005	0.254	15.317
+	CW5	11	97	1.4	10	226	3	27	5	191	0.232	0.150	5.057
	OR	87	97	5.6	70	319	86	37	5	191	3.259	0.721	165.869
	RES	38	CW6 = 15%		A1 = 12%		A2 = 12%		A24 = 8%		A29 = 8%		
4806	UKI GEL VAU20802												
	CW4	92	98	7.7	99	349	88	1	6	254	8.136	0.942	309.646
	RES	23	CW6 = 16%		A2 = 12%		B44 = 12%		A1 = 8%		CW5 = 8%		
4807	BEN ROO VR61809												
	CW4	66	76	6.6	92	346	81	7	62	196	2.584	0.597	123.490
	CW6	51	98	5.3	76	258	58	18	4	178	4.322	0.783	158.309
	OR	60	98	6.0	85	346	139	25	4	178	4.915	0.832	239.641
	RES	64	A2 = 15%		CW7 = 15%		B7 = 10%		A24 = 10%				
4808	US5 SIN 13782												
	CW6	38	98	3.5	44	340	37	47	6	250	2.321	0.537	97.973
	RES	54	A3 = 12%		CW4 = 12%		A2 = 12%		B51 = 8%		CW7 = 8%		
4809	UKI AST DB[illegible]												
	CW6	73	91	6.0	80	350	69	17	25	239	2.788	0.683	163.163
+	AW68	20	92	3.2	46	264	6	7	19	232	1.324	0.291	22.344
+	BW73	33	93	3.2	50	251	3	3	17	229	1.955	0.263	17.384
	OR	67	93	5.5	74	350	78	27	17	229	2.537	0.696	169.558
	RES	60	A2 = 13%		CW4 = 11%		B35 = 9%		A3 = 7%		B51 = 7%		

When there are taken into account the cells typed both with the Core set and the Antigen Society set sera (Table 8) the definition of Cw6 appears improved, although in some Cw4−ve,Cw6+ve cells the Cw6 sera react as in the Cw4+ve,Cw6−ve cells: This improvement is clearly due to the increase in the number of the monospecific Cw6 sera.

Segregation Data

Some interesting segregation data (S.A. Blacks and Cape colored) have been kindly supplied by Dr. E. Du Toit. In Table 9 the segregation pattern of some Core set sera in family Dut 228 is shown. The four children receive from the father the pattern 10W543+, 10W540+, 10W545+: 10W537 and 10W539 segregate together and in repulsion with 10W547 (the monospecific Cw6 serum); in this family the sera 10W537 and 10W539 segregate with the haplotype Aw68,Cw6,Bw72. Similar segregation patterns have been observed in families Dut 10 and Dut 20, associated respectively with B15K and with Bw57.

Population Data

In Cw4−ve unrelated Black population 10W537 and 10W534 are associated (r=0.54); there are eight cells 10W537+,10W539+: seven of them are Cw6+ve, and five out of them are Bw57+ve. Among the 10 cells Cw4-, Cw6-, Bw57+ none has been observed to be

Table 7. Frequency (×100) of the Reactivity Patterns of Selected Antigen Society Sera (Patterns Observed in Only One Cell Have Not Been Reported)

Phenotype		Tenth W No.						Population	
Cw4	Cw6	4	4	4	4	4	4	Black	Caue
		8	8	8	8	8	8		
		0	0	0	0	0	0		
		7	9	8	2	5	6		
		a	b	b	c	c	c		
+	−							(N=25)	(N=66)
		+	−	−	+	+	+	36	70
		+	+	−	+	+	+	40	5
		−	−	−	+	+	+	16	−
		+	−	+	+	+	+	8	−
−	+							(N=50)	(N=67)
		+	+	−	−	−	−	12	30
		+	+	+	−	−	−	24	30
		−	+	−	−	−	−	12	7
		−	−	−	−	−	−	4	6
		+	−	−	−	−	−	−	4
		−	+	+	−	−	−	20	3
−	−							(N=119)	(N=158)
		−	−	−	−	−	−	75	91
		−	+	−	−	−	−	7	3
		−	−	−	−	+	−	−	3

a = Cw4 + Cw6; b = Cw6; c = Cw4

10W537+, 10W539+; among the 38 cells Cw4−, Cw6+, Bw57−, only two are positive with both sera.

These family and population data may suggest that 10W537 and 10W539 recognize a split of Cw6 or, perhaps, a new HLA-C antigen cross-reacting with Cw4 and Cw6 and in linkage disequilibrium with Bw57 in Blacks.

Table 9. Segregation Patterns of Some Core Set Sera in Family Dut 228

	Tenth W No.									
	5	5	5	5	5	5	5	5	5	
	4	4	4	4	3	4	3	3	3	
	3	0	5	7	3	1	5	7	9	
F	+	+	+	+	−	−	−	+	+	ab
M	−	−	−	−	−	−	−	−	−	cd
C1	+	+	+	−	−	−	−	+	+	ac
C2	+	+	+	−	−	−	−	+	+	ad
C3	+	+	+	+	−	−	−	−	−	bd
C4	+	+	+	+	0	−	−	−	−	bd

Haplotypes: a = Aw68, Cw6, Bw72; b = A3, Cw6, Bw58; c = A30, Cw2, Bw71; d = Aw34, Cw7, B8

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References

1. Nomenclature for Factors of the HLA System. In: Kissmeyer-Nielsen F (ed): Histocompatibility Testing 1975. Munksgaard, Copenhagen, 1975; p 5.
2. Nomenclature for Factors of the HLA System 1977. In: Bodmer WF, et al. (eds): Histocompatibility Testing 1977. Munksgaard, Copenhagen; 1977; p 14.
3. Tiilikainen A, Cw6. In: Terasaki PI (ed): Histocompatibility Testing 1980. UCLA, Los Angeles, 1980; p 496.
4. Scholz S, Wakisaka A. HLA-Cw6. In: Albert ED, et al. (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, Heidelberg, 1984; p 181.

Table 8. Frequency (×100) of the Reaction Patterns of Selected Core and Antigen Society Sera (Patterns Observed in Only One Cell Have Not Been Reported)

Phenotype		Tenth W No.													Population	
Cw4	Cw6	5	5	5	4	5	4	4	4	5	5	5	4	4	Black	Cauc
		4	4	4	8	4	8	8	8	3	4	3	8	8		
		3	0	5	0	7	0	0	0	3	1	5	0	0		
					7		9	8	6				2	5		
		a	a	a	a	b	b	b	c	c	c	c	c	c		
+	−														(N=13)	(N=63)
		+	+	+	+	−	−	−	+	+	+	+	+	+	15	67
		+	+	+	+	−	+	−	+	+	+	+	+	+	8	6
		+	+	+	+	−	−	−	+	+	−	+	+	+	−	6
		+	+	+	+	−	−	−	+	+	+		+	+	−	3
		+	+	+	+	+	−	−	+	+	−	+	+	+	−	3
−	+														(N=26)	(N=67)
		+	+	+	+	+	+	+	−	−	−	−	−	−	4	29
		+	+	+	+	+	+	−	−	−	−	−	−	−	4	23
		+	+	+	+	−	+	−	−	−	−	−	−	−	−	6
		+	+	+	−	+	+	−	−	−	−	−	−	−	−	4
		+	+	+	+	+	+	+	−	−	−	−	−	+	8	3
		−	+	+	−	+	−	−	−	−	−	−	−	−	−	3
		+	+	+	−	−	−	−	−	−	−	−	−	−	−	3
		+		+		+	+	+	−	−	−	−	−	−	4	3
		+	+	+	+	+	+	+	+	−	−	−	−	−	−	3
		+	+	−	+	+	+	+	−	−	−	−	−	−	15	−
		+	+	−	−	+	+	+	−	−	−	−	−	−	15	−
		+	+	−	+	+	+	−	−	−	−	−	−	−	8	−
−	−														(N=59)	(N=156)
		−	−	−	−	−	−	−	−	−	−		−	−	68	86
		−	−	+	−	−	−	−	−	−	−	−	−	−	−	4
		−	−	+	−	−	+	−	−	−	−	−	−	−	−	3
		−	−	−	−	−	−	−	−	−	−	−	−	+	−	2
		−	+	−	−	−	−	−	−	−	−	−	−	+	−	1
		−	−	−	−	−	+	−	−	−	−	−	−	−	3	−

a = Cw4 + Cw6; b = Cw6; c = Cw4

Conclusion

With some variation of standard techniques, it is possible to achieve excellent serologic typing of LCLs. DR4 remains heterogeneous with one obvious split. Undoubtedly, other splits exist, but the various patterns cannot be classified until additional well characterized LCLs are available.

References

1. Williamson J, Tait B, Richiardi P, et al. Antigen report: HLA-DR4. In: Albert ED, Baur MP, Mayr WR (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, 1984:188–189.
2. Grimsley G, Homes P, Townsend D, Williamson J, Dawkins RL. HLA typing of lymphoblastoid cell lines (LCL). In: Aizawa M (ed): HLA in Asia-Oceania 1986. Sapporo, Japan: Hokkaido University Press, 1986:909–913.

Antigen Society #24 Report (DRw11, DRw12, DRw8)

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HLA-DR5

Groups of sera defining a DR5 specificity correlated with HLA-Dw5 were first observed in the Seventh Workshop. However, sera of excellent quality recognizing a distinct DR5 antigen have been rare. In the Ninth Workshop, it was discovered that most DR5 sera contained antibodies against related DQ-specificities, and shorter patterns (DRw11 and DRw12) were defined.

In the present Workshop no reagents for definition of DR5 have been found. However, DRw11 and DRw12 can be seen quite clearly.

Reporting Laboratories: US4STA,[1] LATLAY,[2] US5SIN,[3] SCASVE,[4] BENBER,[5] JAPDOH[6]
Participating Laboratories: LATCOL,[7] ANZCHI,[8] US3GVY,[9] FRAFAU,[10] LATHAA,[11] SAFHAM,[12] UKIKNT,[13] US8JLE,[14] NCYMRV,[15] BENROO,[16] US1TUL[17]

HLS-DRw11

A large group of sera defining DRw11 was observed. While many of these do have some contaminating antibodies (especially DQw3 and DQw7), enough very good reagents could be selected that reacted in block and established DRw11 typing quite unequivocally (see Tables 1 and 2).

HLA-DRw12

Three reagents of the Workshop (10W, 9999, 9050, and 3068) recognized DRw12 monospecifically. These were three different aliquots of the same monoclonal antibody (DN1), which clustered with high correlation values. Coincident reaction of the replicates provided excellent definition for this antigen in most of the participating laboratories. The continuity of the definition of this

Table 1. Tenth Workshop Sera Used for the Analysis of DRw11, DRw12, and DRw8

HLA Specificity	Core Set Alloantibodies	Monoclonal	Ag. Soc. 24
DRw11	1096, 1103, 1113, 1114	3036	5154, 5157, 5170 5172, 5175, 5181 9059, 9069, 9070 9075
DRw12		9999, 9050, 3068	
DRw8	1085, 1086, 1087, 1089, 1091		5190, 9093
DRw52	1152, 1198, 1199, 1201, 1204, 5384		
DRw52-short		3025, 3062, 3063	9059
DQw1	1153, 1155, 1159	3091	
DQw3	1116, 1176, 1179	3066, 3111	
DQw7		3119, 3120, 3121	9051
DQw4		3101	
DQw5	1136		
DQw6	1141		
IIB3	1217	3086, 3088, 3090 3122	9055, 9056

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Table 2. The Most Common Haplotype Associations With HLA-DRw11, DRw12, and DRw8

Observed HLA-DR/DQ Haplotypes		HLA-DR w11	w12	w8	w13	w14	w52	w52s	HLA-DQ w3	w7(w3)	w4	w1	w6(w1)	w5(w1)	11B3
		1 1 3 9 9 5 5	9 9 3	1 1 1 1 5 9	1 1 3	9 1	1 1 1	3 9	3 3	3 3 3 9	3	1 1	1	1	3 3 1
		0 1 0 0 0 1 1	9 0 0	0 0 0 0 1 0	1 1 0	0 1	1 1 2	0 0	0 1	1 1 1 0	1	1 1	1	1	0 0 2
		9 1 3 5 6 8 5	9 5 6	8 8 8 9 9 9	3 2 2	6 1	9 9 0	6 5	6 1	2 2 1 5	0	5 5	4	3	8 8 1
		6 4 6 9 9 1 4	9 0 8	5 9 7 1 0 3	4 6 1	0 1	9 8 1	3 9	6 1	0 1 9 1	1	3 5	1	6	8 6 7
DRw11	DQw3(w7)	+ + + + + + +	– – –	– – – – – –	– w –	– +	+ + +	+ +	+ +	+ + + +	–	– –	–	–	– – –
DRw11	DQw1	+ + + + + + +	– – –	– – – – – –	– w –	– +	+ + +	+ +	– –	– – – –	–	+ +	+	–	+ + +
DRw12	DQw3(w7)	– – – – – – –	+ + +	– – – – –	– w –	– w	+ + +	– –	+ +	+ + + +	–	– +	–	–	– – –
DRw12	DQw1	– – – – – – –	+ + +	– – – – –	– w +	– w	+ + +	– –	– –	– – – –	–	+ +	–	+	+ + +
DRw8	DQw4	– – – – – – –	– – –	+ + + + +	– – –	– –	+ + +	– –	– –	– – – –	+	– –	–	–	+ + +
DRw8	DQw3(w7)	– – – – – – –	– – –	+ + + + +	– – –	– –	+ + +	– –	+ +	+ + + +	–	– –	–	–	– – –
DRw8	DQw3(w7N)	– – – – – – –	– – –	+ + + + +	– – –	– –	+ + +	– –	+ +	– – – –	–	– –	–	–	+ + +
DRw8	DQw1	– – – – – – –	– – –	+ + + + +	– – –	– –	+ + +	– –	– –	– – – –	–	+ +	?	w	+ + +

w = weak or variable reactions
w7N = DQw7-negative
? = not enough information available

antigen with the previous definition in the Ninth Workshop was demonstrated by typing of the cell line Herluf and several other cells and members of families that had been tested in the previous Workshop. In some laboratories occasional reactivity of DN1 with DRw8 cells was observed. It was brought to our attention that a T-cell clone from M. Thomsen reacted uniquely with DRw12-positive cell lines.

DRw8

DRw8 antigen was defined in this Workshop by several good sera (Table 1). In addition, many other sera on the Antigen Society tray appeared to react with DRw8 cells and to contain antibodies against the new DQw4 specificity.

Haplotype Associations of HLA-DRw11, HLA-DRw12, and DRw8 With DRw52 and DQ Alleles

The haplotype associations are summarized in Table 2. DRw11, DRw12, and DRw8 all reacted with broad DR sera defining DRw52. However, a subset of reagents among those clustering with DRw52 gave shorter patterns, some of which are observed to be negative with DRw12-positive cells and/or DRw8-positive cells.

DQ haplotype associations observed with DRw11 and DRw12 were similar. The most common haplotypes were with DQw3, and these were almost always positive for DQw7. While a few DRw11, DQw3, DQw7-negative cells were present, their number was sufficiently small to likely represent a residual of typings with technical problems.

Other haplotypes observed with DRw11, DRw12, and DRw8 carried DQw1. Such cells reacted either with 10W1141, defining DQw6, or with 10W1136, an antibody correlated with LY1327 (4), reported to define DQw5. Most DRw11 and DRw8, DQw1 cells were positive with 10W1141 (DQw6); the DRw12, DQw1 cells were 10W1141-negative and sometimes reacted with 10W1136, suggesting they were positive for DQw5.

The frequency of haplotype associations with DRw8 and DQ antigens varies in different ethnic groups. In Caucasoids the most common haplotype was DRw8, DQw4. In Orientals, DRw8, DQw1 was more commonly observed. Other haplotypes observed were DRw8, DQw7, and less frequently, DQw3-positive, DQw7-negative.

A cluster of antibodies defining the broad DQ specificity 11B3 reacted with DRw11, DRw12, and DRw8, DQw1-associated cells. Also included were the DRw8, DQw4, and DRw8, DQw3, DQw7-negative haplotypes.

Relationship with DRw13 and DRw14

Cells having DRw11 frequently gave patterns of weak reactivity with sera used to define DRw13. This was also true of cells that were coded as DRw12. DRw14 was defined in the Core set by only one reagent (10W9060). In addition, 10W1111 was a duospecific serum reacting with both DRw11 and DRw14.

References

1. Bodmer JG, Pickbourne P, Richards. Joint Report: 1a Serology. In: Bodmer WF, et al. (eds): Histocompatibility Testing 1977. Munksgaard, Copenhagen, 1978; pp 35–84.
2. Betuel H, Gebuhrer L, Schreuder GMT, Goldmann SF, Arnaiz Villena A, Layrisse Z. Antigen Report: HLA-DR5 and its subtypes HLA-DRw11 and HLA-DRw12. In: Albert ED, Baur M, Mayr W (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, 1984; pp 190–192.
3. Park MS, et al. In: Terasaki P (ed): Histocompatibility Testing 1980. UCLA Tissue Typing Laboratory, Los Angeles, 1980: p 548.
4. Gebuhrer L, Betuel H, Freidel AC, Farre A. A division of HLA-DQw1 associated with DR1, DR1x-DQw1, DR2 short, DRw10, and DRw14. I. Definition of allo-antiserum LY1327. Hum Immunol 1987;18:235–245.

DRw12 specificity could be clearly distinguished from DRw13 (Table 3). DRw12 was predominantly observed with DQw7. Some of these cells were reported as DB6 (D Herluf).

Laboratory DUT originally reported some cells as DR6x12 but the analysis suggests that they could be DRw12 in association with DQw5 (Table 3). This pattern has thus far only been observed in South African blacks.

References

1. Schreuder GMT, Kennedy LJ, Gebuhrer L, Awad J, Betuel H, Degos L, et al. Antigen report: HLA-DRw6 and its subgroups HLA-DRw13 and HLA-DRw14. In: Albert ED, Baur MP, Mayr WR (eds): Histocompatibility Testing 1984. Springer-Verlag, Berlin, Heidelberg, 1984; p 192.
2. Schreuder GMT, Maeda H, Koning F, D'Amaro J. TA10 and 2B3, two new alleles in the HLA-DQ region recognized by monoclonal antibodies. Hum Immunol 1986; 16:127.
3. Gebuhrer L, Betuel H, Freidel AC, Farre A. A division of HLA-DQw1 associated with DR1, DR1x-DQw1, DR2 short, DRw10, and DRw14. I. Definition by alloantiserum LY1327. Hum Immunol 1987;18:235.

Antigen Society #26 Report (DR3, DR7, DQw2): Part 1

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General Introduction

There were 1,019 cells analyzed in the Antigen Society (795 Caucasians, 202 Negroes, 22 Orientals); 1,011 were tested both on Core serology and Antigen Society reagents. The total number of sera and monoclonal antibodies (MAbs) in the antigen group was 182, distributed as shown in Table 1.

The list of these reagents is given in Table 2 with their identity and lab of origin. It must be noted that due to a heterogeneity in the tray layout between the different labs, the central data analysis team cut down the results of 15 anti-DR7 sera from the Antigen Society as indicated between the two horizontal lines in Table 2. The results concerning these sera could not be analyzed in detail.

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Table 1. Type and Number of Serologic Reagents Toward the Specificities Studied in Antigen Group 26 : DR3, DR7, DQw2

Specificity	Core Serum	Core MAb	Antigen Society
DR3 (mainly)	14	5	41
Related to DR3 (DRw52...)	10	3	/
DR7 (mainly)	12	4	59
Related to DR7 (DRw53, DRw9...)	6	2	/
DQw2	12	3	11

Table 2. List and Identification of Serologic Reagents for Antigen Group 26

Origin	10WID Original ID
Order = 1 US6 BRN	1039 CC434.1
Specs: DR3	
Order = 2 UK1 DRK	1043 WILLIAMS.773
Specs: DR3	
Order = 3 SAF HAM	1040 N1165
Specs: DR3	
Order = 4 SAF HAM	1037 N1164
Specs: DR3	
Order = 5 ANZ RSH	1068 SPARKS
Specs: DR7	
Order = 6 ANZ DAW	1070 SIMMONDS.P6.5590
Specs: DR7	
Order = 7 IT1 MRA	1071 PV35
Specs: DR7	
Order = 8 IT2 GAN	1075 CO812
Specs: DR7	
Order = 9 UK1 JOY	1152 HAYES
Specs: DR3 DRw6 DRw8	
Order = 10 NCY MRV	1201 18660
Specs: DRw52	
Order = 11 SAF HAM	1069 N1101
Specs: DR7	
Order = 12 UK1 AST	1072 DB2410
Specs: DR7	
Order = 13 US5 TER	1182 TER.DQ3A
Specs: DQw3	
Order = 14 IT1 MTT	1169 F102406
Specs: DQw2	
Order = 15 FRA FAU	1173 CHEVRIER
Specs: DQw2	
Order = 16 US6 BRN	1168 CC413.1
Specs: DQw2	
Order = 17 FRA FAU	1067 JEGU
Specs: DR7 DRw9	
Order = 18 US8 RUB	1038 NYBC002
Specs: DR3	
Order = 19 NCY MRV	1035 10466
Specs: DR3	
Order = 20 NCY MRV	1034 11552
Specs: DR3	
Order = 21 UK1 FES	1036 ALLEN
Specs: DR3	
Order = 22 US2 BAC	1150 REYNOLDS
Specs: DR3 DRw6	
Order = 23 GER NEU	1077 GO810468B
Specs: DR7	
Order = 24 FRA BET	1079 E915
Specs: DR7	
Order = 25 US6 BRN	1080 CC327.5
Specs: DR7	
Order = 26 GER GOL	1076 924.4
Specs: DR7	
Order = 27 US1 THP	1042 EUINK
Specs: DR3	
Order = 28 NCY GOE	1202 PL855
Specs: DRw52	
Order = 29 US5 SIN	1205 15886
Specs: DRw52	
Order = 30 FRA DDC	5384 P6465
Specs: DRw52	
Order = 31 US8 JLE	1216 H181
Specs: DRw53	
Order = 32 IT2 FER	1213 FE200
Specs: DRw53	
Order = 33 GER BRA	1170 422B
Specs: DQw2	
Order = 34 IT1 MTT	1174 F103295
Specs: DQw2	
Order = 35 US2 MBC	1175 BC.CA.MAGN
Specs: DQw2	
Order = 36 BEN BER	1172 MSD20
Specs: DQw2	
Order = 37 US5 TER	1117 TER.DP1
Specs: DPw1	
Order = 38 NCY YUN	1144 BLOUDETTE
Specs: DR3 DR6	
Order = 39 UK1 TAT	1041 WINTER.SOH.487
Specs: DR3	
Order = 40 FRA PRR	1064 VEL1557
Specs: DRw9	
Order = 41 ANZ DAW	1195 R5.6295
Specs: DR4 DR5	
Order = 42 NCY MRV	1212 24784
Specs: DRw53	
Order = 43 UK1 DRK	1078 OWEN.812
Specs: DR7	
Order = 44 NCY MRV	1167 11028
Specs: DQw2	
Order = 45 FRA FAU	1066 ANTIN
Specs: DR7 DRw9	
Order = 46	9060
Specs: UNK	
Order = 47 FRA JEA	1151 DROZ
Specs: DR3 DRw6	
Order = 48 FRA MYE	1147 LECOINTRE
Specs: DR3 DRw6	
Order = 49 BEN BER	1135 MSD6
Specs: DRw13 DRw14	
Order = 50 IT1 MTT	1180 F101404
Specs: DQw3	
Order = 51 IT2 FER	1186 FE94
Specs: DQw3	
Order = 52 US1 DUQ	1171 QUAGLIER
Specs: DQw2	
Order = 53 UK1 DRK	1208 PRATT.80%
Specs: DR3 DR5 DRw6 DRw52	
Order = 54 IT1 MTT	1200 F102422
Specs: DRw52	
Order = 55 GER WAN	3005 C5C5
Specs: DR1 DR3 DR4 DRw8	
Order = 56 FRA DDC	3010 CHE153
Specs: DR1 DR7	
Order = 57 JAP AIZ	3011 HU30
Specs: DR2 DR1	
Order = 58 GER WAN	3020 M4F11
Specs: DR3 DRw13	
Order = 59 FRA DDC	3023 CHE41.2
Specs: DR3 DRw6	
Order = 60 UK1 FES	3031 JA1
Specs: DR3	
Order = 61 JAP JUJ	3048 PLM3
Specs: DR7 DRw9 DRw12	
Order = 62 UK1 BOD	3049 17.3.3
Specs: DR7	

Origin	10WID Original ID
Order = 63 NCY SFR	3050 SFR16.DR7M
Specs: DR7	
Order = 64 US7 GSC	3051 GSP65.1
Specs: DR7 DRw10	
Order = 65 GER WAN	3062 M4G8
Specs: DRw52	
Order = 66	3105
Specs: DQw2	
Order = 67 IT2 FER	3106 MP1
Specs: DR2 DR3 DR4 DR5 DRw6	
Order = 68 IT2 GAN	3107 XIII358
Specs: DQw2	
Order = 69 US7 GSC	3113 GSP91.1
Specs: DQw3	
Order = 70	3025
Specs: DR3 DR5 DRw6	
Order = 71 GER WAN	3046 C6E2
Specs: DR3 DR4 DR7	
Order = 72 IT1 CEP	1081 TORP1017
Specs: DR7	
Order = 73 FRA PRR	5006 PUY.A.217
Specs: DR7	
Order = 74 UK1 BRS	5210 10726.LCS
Specs: DR3	
Order = 75 NCY MRV	5211 27026
Specs: DR3	
Order = 76 NCY MRV	5212 18835
Specs: DR3	
Order = 77 NCY MRV	5213 20571
Specs: DR3	
Order = 78 NCY MRV	5214 2551.6
Specs: DR3	
Order = 79 US2 MBC	5215 BC.HE.BURJ
Specs: DR3	
Order = 80 US2 MBC	5216 BC.JA.SADO
Specs: DR3	
Order = 81 US2 MBC	5217 BC.VE.BREE
Specs: DR3	
Order = 82 IT1 MIT	5218 F101972
Specs: DR3	
Order = 83 FRA BIG	5219 H12
Specs: DR3	
Order = 84 FRA DDC	5220 P2983
Specs: DR3	
Order = 85 ANZ CRS	5221 PAGE
Specs: DR3	
Order = 86 US7 HAN	5222 SEA1250
Specs: DR3	
Order = 87 ANZ DAW	5223 SLEEPY.F29026
Specs: DR3	
Order = 88 NCY MRV	5224 3953.5
Specs: DR3	
Order = 89 NCY MRV	5225 9134.2
Specs: DR3	
Order = 90 US5 ABS	5226 ABS.17
Specs: DR3	
Order = 91 UK1 MID	5227 BEL553D
Specs: DR3	
Order = 92 NCY CAR	5228 BOS/BAT
Specs: DR3	
Order = 93 FRA FAU	5229 BRIAND
Specs: DR3	

Origin	10WID Original ID
Order = 94 US6 BRN	5230 CC519.2
Specs: DR3	
Order = 95 US6 BRN	5231 CCB1060.1
Specs: DR3	
Order = 96 UK1 JOY	5232 DENNING
Specs: DR3	
Order = 97 EAE RIC	5233 H1195.1
Specs: DR3	
Order = 98 NCY GOE	5234 J18571
Specs: DR3	
Order = 99 EAE KAS	5235 KAS7292
Specs: DR3	
Order = 100 US8 JLE	5236 M325
Specs: DR3	
Order = 101 FRA OHA	5237 MARCHE
Specs: DR3	
Order = 102 BEN BER	5238 MSD8
Specs: DR3	
Order = 103 SAF HAM	5239 N1163
Specs: DR3	
Order = 104 SAF HAM	5240 N1233
Specs: DR3	
Order = 105 US7 DUP	5241 NJ4050
Specs: DR3	
Order = 106 FRA PRR	5242 ROU.A212
Specs: DR3	
Order = 107 US4 SND	5243 SI5L178
Specs: DR3	
Order = 108 US5 TER	5244 TER.DR3
Specs: DR3	
Order = 109 UK1 JOY	5245 WATERHOUSE
Specs: DR3	
Order = 110 US3 PER	5246 WOODARDP0109A
Specs: DR3	
Order = 111 US1 DUQ	5247 Y.CARROLL
Specs: DR3	
Order = 112 UK1 TAT	5248 DURNFORD.
Specs: DR3	SOH.517
Order = 113 UK1 DRK	5249 MARSHALL.1070
Specs: DR3	
Order = 114 US1 DUQ	5250 YURUS
Specs: DR3	
Order = 115 US5 SIN	5251 15452
Specs: DR7	
Order = 116 NCY KAP	5252 CWRP1518
Specs: DR7	
Order = 117 US7 POL	5253 045
Specs: DR7	
Order = 118 NCY MRV	5254 11542
Specs: DR7	
Order = 119 GER GOL	5255 15924.4
Specs: DR7	
Order = 120 EAE SHA	5256 SHA.8
Specs: DR7	
Order = 121 GER GOL	5257 16738
Specs: DR7	
Order = 122 NCY MRV	5258 19330
Specs: DR7	
Order = 123 NCY MRV	5259 20387
Specs: DR7	
Order = 124 NCY MRV	5260 20502
Specs: DR7	
Order = 125 NCY MRV	5261 21420
Specs: DR7	

Origin	10WID Original ID
Order = 126 NCY MRV Specs: DR7	5262 25552
Order = 127 BEN BOU Specs: DR7	5263 64352 9/11/82
Order = 128 NCY MRV Specs: DR7	5264 7128
Order = 129 NCY MRV Specs: DR7	5266 9235
Order = 130 GER BRA Specs: DR7	5267 950
Order = 131 US5 ABS Specs: DR7	5268 ABS.20
Order = 132 IT1 GAB Specs: DR7	5269 AN127
Order = 133 UK1 JOY Specs: DR7	5270 ANDREWS
Order = 134 IT1 MTT Specs: DR7	5271 B08.3
Order = 135 IT1 CEP Specs: DR7	5272 CDS9031
Order = 136 UK1 MID Specs: DR7	5273 BEL213D
Order = 137 UK1 MID Specs: DR7	5274 BEL522D
Order = 138 FRA OHA Specs: DR7	5275 BERNON
Order = 139 EAE RIC Specs: DR7	5276 BH3747
Order = 140 UK1 GEL Specs: DR7	5277 CLA22677
Order = 141 IT2 GAN Specs: DR7	5278 CO868
Order = 142 US5 SWE Specs: DR7	5279 DELMASTRO
Order = 143 FRA FAU Specs: DR7	5280 ESNAULT
Order = 144 IT1 MTT Specs: DR7	5281 F101577
Order = 145 IT2 FER Specs: DR7	5282 FE208
Order = 146 IT2 FER Specs: DR7	5283 FE216
Order = 147 ANZ CRS Specs: DR7	5284 HENNING
Order = 148 US6 GAT Specs: DR7	5285 JH
Order = 149 US4 STA Specs: DR7	5286 KWI
Order = 150 US1 DUQ Specs: DR7	5287 MCCLOSKEY12.84
Order = 151 FRA KRE Specs: DR7	5288 MTC8122
Order = 152 FRA KRE Specs: DR7	5289 MTC8337
Order = 153 SAF HAM Specs: DR7	5290 N1188
Order = 154 SAF HAM Specs: DR7	5291 N1201
Order = 155 SAF HAM Specs: DR7	5292 N1234
Order = 156 SAF HAM Specs: DR7	5293 N945
Order = 157 FRA PRR Specs: DR7	5294 PH11466
Order = 158 UK1 JOY Specs: DR7	5295 RICHARDSON
Order = 159 US8 FOT Specs: DR7	5297 STRATIS
Order = 160 US5 TER Specs: DR7	5298 TER.DR7
Order = 161 FRA MYE Specs: DR7	5299 THOMAS
Order = 162 IT1 CNG Specs: DR7	5300 TSRP170
Order = 163 IT1 CNG Specs: DR7	5301 TSRP307
Order = 164 US3 PER Specs: DR7	5302 ULRICHP0019C
Order = 165 US8 JLE Specs: DR7	5303 W283
Order = 166 UK1 DRK Specs: DR7	5304 JONES. GERRARD.807
Order = 167 UK1 LAW Specs: DR7	5305 WILLIAMS
Order = 168 NCY KAP Specs: DR7	5306 CWR.PLA.1355
Order = 169 NCY KAP Specs: DR7	5307 CWR.NIE.3
Order = 170 NCY KAP Specs: DR7 DRw9 DR3	5308 CWR.PLA.1007
Order = 171 UK1 GEL Specs: DR7	5309 DAY20558
Order = 172 US6 BRN Specs: DR3 DR7	5310 CCB.1045.2
Order = 173 IT2 GAN Specs: DR3 DR7	5311 CO1178
Order = 174 UK1 GLA Specs: DR7 DR3 DQw2	5312 HUTTON
Order = 175 NCY KAP Specs: DR3 DR7 DRw9	5313 CWR.PRIM
Order = 176 US6 BRN Specs: DQw2	5314 CCB.1035.1
Order = 177 US2 BAC Specs: DQw2	5315 M.LARSEN
Order = 178 NCY GOE Specs: DQw2	9104 PL1758
Order = 179 NCY GOE Specs: DQw2	9105 PL2051
Order = 180 NCY GOE Specs: DQw2	9106 PL2282
Order = 181 NCY GOE Specs: DQw2	9107 PL2441
Order = 182 NCY GOE Specs: DQw2	9108 PL2451

Antigen Society #31 Report Part 2: Antigen Society #31 Report

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History

The discovery that alloactivated T lymphocytes express new class II antigens (1,2) stimulated experiments in which activated lymphocytes were studied for the expression of new antigens not detected at the quiescent stage. Other workers have shown that resting T lymphocytes could be subdivided by the use of sera recovered from patients with juvenile rheumatoid arthritis (3) or from alloimmunized volunteers (4,5). Later (6), TCA-TCB system expressed on T gamma-enriched cells was also identified by alloantisera. When PHA-activated lymphocytes were used in the screening of pregnancy sera, it appeared that some sera reacted exclusively with the lectin-activated lymphocytes, but not with the resting T or B lymphocytes separated from the same individual (7). Cross-absorption experiments indicated that, indeed, these determinants were not shared by the resting autologous lymphocytes. In blocking experiments, it was shown that the new determinants are associated with B-2 microglobulin, which classified them into the class-I gene product family. Their absence from thymocytes suggested that they may be the human counterpart of the murine Qa-like gene products (8). Family studies have shown that the reactivity segregated with HLA, which maps it to chromosome VI (7). When the lymphocytes used for screening were assigned their HLA phenotype, linkage disequilibrium with HLA was observed, specifically with locus A gene products. Thus, some sera exhibited linkage disequilibrium with HLA-A3,A10,A2,A9 or HLA-A1 (7-10). It was originally suggested by Gazit et al. to term them HT (human T) because of their similarity with the mouse T-region gene products (8) or later H-A by Fauchet et al. (9) because they were expressed by PHA or alloactivated on T cells and B-cell lines. In biochemical experiments, it was shown that the antigenic determinant which was precipitated by the specific alloantibody was a 41-12 K dimer distinct from the HLA class I 44-12 K antigen (7). Sequential immune precipitation with the w6/32 or HLA-A3 monoclonal antibodies did not remove the antigenic reactivity of this determinant, indicating that it is different from HLA-ABC antigens. Taken together, the reports published so far indicate that PHA activation, alloactivation, or beta interferon stimulation (11) induces the expression of new non-HLA class I antigens.

This system is distinct from HLA for the following reasons. The reactivity is not absorbed by either platelets or resting T or B lymphocytes. Lysostripping with HLA alloantibody does not remove the reactivity (7). There is a linkage disequilibrium with HLA; however, there are cells that do not express the linked HLA antigen, but do react with the serum. The molecular weight of the heavy chain is 41 to 42 K and not 44 K, which is typical for the heavy chain of HLA. To date, all efforts have failed to produce a murine monoclonal antibody that specifically reacts with the PHA-activated lymphocyte and precipitates the antigen.

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Participating Laboratories: IT1MRA,[10] IT2GAN,[11] SAFDUT,[12] NCYYUN[13]

Table 1. Cellular Targets Tested in the Workshop

Name of Target	No. of Cells
1) Peripheral blood leucocytes	70
2) Nylon wool purified T-cells	324
3) Nylon wool purified B-cells	270
4) PHA activated lymphocytes	423
5) Alloactivated lymphocytes	30
6) EBV transformed B cell lines	76
7) Leukemia T-cell lines (HPB, Jurkat, MOLT-4, 8402, PEFR, HSB-2)	6
8) Leukemia lymphoblasts	
T-cell acute lymphoblastic (ALL)	11
Common acute lymphoblastic (C-ALL)	22
Acute Myeloid (AML)	33
Chronic lymphoid (CLL)	15
Unclassified	7

Results

This is the first international HLA workshop in which these novel antigens have been studied. The objectives

Table 2. PHA Activation Protocol

Gazit (8)	Fauchet (9)
PBL + PHA (purified)	PBL + PHA (crude)
3 days	2 days
IL-2	IL-2
4 days	2 days
Harvest + freeze	Harvest + freeze
Cytotoxicity Testing	
1 + 2 hours	1 + 1 hours

Table 3. Patterns of Reactivities of the Alloantibodies

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Peripheral blood lymphocytes	−	+	−	−	−	−	−
T lymphocytes	−	+	−	−	−	−	−
B lymphocytes	−	+	+	−	−	+	+
PHA T cells	−	+	+	+	+	+	−
Allogenic T cells	−	+	+	+	+	+	−
EBV cell lines	−	+	+	+	−	−	−
Number of sera	5	13	25	14	6	1	1
	↓	↓	↓				
Possible interpretation	negative	anti-HLA-A, -B	anti-HLA-DR, -DQ		New Class I markers ?		B cell

were to compare the different protocols of PHA T-cell preparations to define the linkage with the classic HLA antigens and to identify the serologic clusters. Fifteen Laboratories took active part in these studies. The protocol required a study of 20 to 30 unrelated selected cells and two or three families including different lymphocyte targets: PBL or T cells, PHA T cells, B cells, and EBV cell lines. Additional targets were selected as an optional study. They included thymocytes, pathologic cells, leukemia T-cell lines, and leukemia lymphoblasts. Sixty-nine platelets, allosera, and six monoclonal antibodies were submitted by eight laboratories. Classic class I and class II sera were added as control (Fe1-Fa7: HLA-A2, HLA-DR4,Fe3-Fa9: HA10, Fe2-Fa8: negative sera). Table 1 summarizes the lymphocyte targets that were used. Two protocols were followed for the preparation of the activated lymphocytes (Table 2). The difference between Gazit's and Fauchet's protocols lies in the use of crude PHA by Fauchet and length of the tissue culture (a total of 7 days in the first protocol and 4 days in the second). From preliminary experiments (5), it appeared that the crude PHA is superior for the preparation of blasts, but it has not been decided whether short- or long-term culture is superior. In Table 3, the overall pattern of the reactivities is summarized. It is clear that several sera reacted like HLA ABC or DR antibodies. They were subsequently identified and removed from the analysis. The serum by serum (SxS) analysis resulted in 10 clusters, 4 of which were found to be the classic HLA. The remaining six clusters are summarized in Table 4. It is worth mentioning that some sera that reacted as classic HLA antibodies clustered with the corresponding sera, which showed linkage disequilibrium with a particular HLA antigen. In four clusters, there is a significant linkage disequilibrium with HLA, and the r value is given in Table 4. There is

Table 4. PHA Activated Lymphocytes Form Clusters of Reactivity*

Cluster No.	Serum No.	HLA Association	R value	Reacting with EBV	Reacting with Thymocytes	Reacting with T-cell Lines
	4875	A1	0.26	−	+	+
1	4877	A1	−	−	+	−
	4879	A1	0.29	−	−	−
2	4888	A−2	0.45	+	+	−
	4889	A−2	0.38	+	+	+
	4844	A−3	0.39	++	−	+
3	4890	A−3	0.55	+	−	+
	9378(BRAN)	A−3	0.27	+	−	+
	4880	A−10	0.29	+	−	+
4	4881	A−10	0.25		−	−
	4887	A+10	0.22		−	−
	4889	A−10	−		−	−
	4850	−			++	−
5	9379(SCHN)	−			+	+
	9380(SPIE)	−			+	+
	4880	−			+	+
	4838	−			−	+
6	4839	−			−	+
	4840	−			+	+
	4842					
	4843					

*ALL sera listed in this table reacted positively with PHA-activated lymphocytes.

Table 5. The Reactivity of Sera from Cluster 3 with Leukemia Lymphoblasts

Serum Number	Leukemia Type T-ALL (n = 12)	AML (n = 28)	c-ALL (n = 16)	B-CLL (n = 14)
4844	NS	NS	0.027	0.054
4890	NS	0.017	0.027	0.011
BRAN	NS	NS	NS	NS

The number in the table is the p value, which was calculated for the numbers of cells reacting with the sera in the cluster and having HLA-A3. NS = nonsignificant.

a cluster that is associated with HLA-A1, A2, A3, and A10. The sera in the clusters were analyzed by their reactivities with B-cell lines, thymocytes, T cell lines, segregation in families and reactivities with leukemia lymphoblasts (Tables 4 and 5). Two groups of sera from clusters 2 and 3 were found to react with B-cell lines, and cluster 5 reacted with thymocytes (Table 4).

Leukemia T-cell lines were found to react with sera in clusters 3, 5, and 6. The segregation of the reactivities was studied in several families, and clusters 1 to 4 were found to segregate with HLA. ALL, which classified as c-ALL and were HLA-A3-positive, reacted with sera in cluster 3 (HT-3,), i.e., the reactivity was associated with the presence of the HLA-antigen, and not with the leukemia per se.

Discussion

The experiments performed in this Workshop successfully identified and defined six clusters of non-HLA alloantibodies, four of which were in linkage disequilibrium with HLA. Sera in the remaining two clusters also reacted with thymocytes, T-cell lines, and some T-ALL lymphoblasts. Thus, it is possible to divide the clusters into two groups: Qa-like and the TL-like antigens. In family studies, the reactivities of most clusters segregated with HLA. Clusters 2 and 3 also reacted with EBV-transformed B-cell lines, and cluster 5 reacted with T-cell lines and thymocytes. The reactivity of these non-HLA antibodies with human thymocytes extends the findings in early reports (12–14). Common ALL lymphoblasts in HLA-A3-positive individuals reacted with sera in cluster 3.

These results indicated that the serologic aspects of the new system are almost resolved, even though the reproducibility of the tests have not, as yet, been worked out. Unfortunately, the biochemistry experiments failed. Therefore, no biochemistry data were presented in this Workshop. Also, Qa-Tla-like probes were not assayed in the molecular biology experiments of this workshop.

It is hoped that immune precipitation and Southern blot analysis will be studied in future workshops using specific monoclonal antibodies in biochemistry experiments and non-HLA class I probes in DNA experiments.

References

1. Evans RL, et al. J Exp Med 1978;148:1440.
2. DeWolf WC, Schlossman SF, Yunis EJ. J Immunol 1979;122:1780.
3. Strelkauskas AJ, et al. J Immunol 1978;120:1278.
4. Ferrara GB, et al. J Immunol 1979;123:1272.
5. Van Leeuwen A, et al. Tissue Antigens 1979;14:437.
6. Van Leeuwen A, et al. Tissue Antigens 1987;31:33.
7. Gazit E, Terhorst C, Yunis EJ. Nature 1980;284:275.
8. Gazit E, et al. J Immunol 1984;132:165.
9. Fauchet R, et al. Human Immunol 1986;17:3.
10. Mitsuishi Y, et al. Human Immunol 1986;15:175.
11. Gazit E, et al. Human Immunol 1987;18:53.
12. Ferrara GB, et al. Clin Immunol Immunopathol 1982; 222:428.
13. Mahoney RJ, et al. J Immunol 1982;129:263.
14. Park S, et al. Human Immunol 1988;22:151.

Antigen Society #31 Report Part 3: Leukemic Blasts Express New HLA Class I-Like Alloantigens

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New HLA, β2m-associated alloantigens, undetectable on resting T and B cells and platelets, are detected on PHA-activated lymphocytes (PHA-L) using platelet-absorbed alloantisera (1–4). The expression of these new HLA class I-like specificities was examined in newly diagnosed acute leukemias. Bone marrow blasts obtained at onset and peripheral blood lymphocytes during remission were tested by the complement-dependent lymphocytotoxicity technique using platelet-absorbed alloantisera. All leukemia samples (26 cALL, 6 T-ALL, 28 ANLL) were tested with locally selected alloantisera (1), while only a limited number (8 cALL, 3 T-ALL, 19 ANLL) was examined with the alloantisera submitted

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References

1. Ness, D. B., Watson, A. K., and Grumet, F. C. *Hum. Immunol.* 4, 55 (1982).
2. Zhao, T. M. and Lee, T. D. Tenth International Histocompatibility Workshop Newsletter, no. 1, p. 23 (1987).
3. Chandanayingyong, D., Bejrachandra, S., Rungroung, E., *et al.* Tenth International Histocompatibility Workshop Newsletter, no. 1, p. 4 (1987).
4. Alonso, A., Altshuler, R., Batat, S., *et al. Histocompatibility testing 1984* (ed. E. D. Albert, M. P. Baur, and W. R. Mayr), p. 146. Springer-Verlag, Berlin (1984).
5. Charoenwongse, P. and Noppornpunth, V. *Proceedings of the Third Asia and Oceania Histocompatibility Workshop and Conference 1986* (ed. M. Aizawa *et al.*), p. 97. Hokkaido University Press, Sapporo (1986).

W2.19 Antigen Society no. 112: HLA-B40 crossreacting group, HLA-B60, -B61, -B47, -B48, -B13

M. G. HAMMOND, K. TOKUNAGA, M. FOTINO, N. GRUNNET, B. GRAUGAARD, and J. VIVES

Svejgaard *et al.* was the first to describe the B40 antigen in 1970 [1]. The various splits were described subsequently [2–6]. HLA-B13 has been well defined since 1970 [7] but many of the sera show cross-reactivity. We analysed the reactions of the antisera used in both sets of the Eleventh International Histocompatibility Workshop (IHWS) in order to produce reaction patterns that define each antigen.

Results and discussion

There were 12 870 cells typed for this IHWS and the serographs (Figure 1) produced by the IHWS computer programs [W2.3, this volume] analysed 1106 cells that reacted positively with the sera used to define the antigens of the B40 crossreacting group (creg). Consensus was reached among the members of Antigen Society no. 112 (AS-112) as to the reaction pattern that best defined the different antigens. The reaction pattern of the sera used in Eleventh IHWS core sera set 1 is shown in Table 1.

- **B13** was very well defined by three Eleventh IHWS allosera. Serum no. 0344 (MID107) had a Q score of 14.04 but serum 0345 (DDC213) had weak reactivity in some laboratories. Six monoclonal sera were tested but only one had an *r* value greater than 0.9. Previously reported splits [8] of B13 that had been confirmed by isoelectric focusing (IEF) could not be seen in the reaction pattern of the sera used in this IHWS.

- **B60** was detected by 14 Eleventh IHWS sera but only two were monospecific no. 0355 (JUJ204) and no. 0356 (HSE207). However, three other sera were positive with only B60 + B48 and so were very useful because of the low frequency of B48.

- **B61.** There were no operationally monospecific sera so this specificity could only be defined by 'subtraction', i.e. if the B40 sera were positive and the B60 sera negative. The B60 + B48 sera were helpful in this regard. It was not possible to differentiate between B60 homozygotes and B60/B61 heterozygotes. There was no evidence of new splits of B60 or B61 that could not be explained by weak reactions.

- **B47** was defined by positive reactions with Eleventh IHWS sera 0346 (PRR210), 0347 (NOS208), and 0348 (FER206). One of the B27 Eleventh IHWS sera 0342 (SCN204) also reacted with B47 so that it was possible to define B47 in the presence of B60, B61, and B13. Serum 0347 (NOS208) was very useful because there were no extra reactions outside of these four antigens.

- **B48** was well defined by seven sera, but there is only one monospecific Eleventh IHWS serum 0360 (AKA216) to rely on if B60 is present. Three sera reacted only with B60 + B48, but the others were broadly reactive with many other antigens.

- **BFU**. The definition of BFU depends on a negative reaction with Eleventh IHWS serum 0360 (AKA216) and positive reactions with the other B48 sera which means that BFU cannot be distinguished in the presence of B60. This antigen was first described by Kawaga *et al.* [8] in the Fifth Japanese Red Cross HLA Workshop and was well defined in the Tenth IHWS when several B48 sera failed to react with BFU. In this IHWS, BFU was confirmed to be different from B48 by IEF. In addition, family studies were reported in the Tenth IHWS [9]. It was not possible to distinguish between B60 homozygotes and B60/B48 heterozygotes. Analyses of the complete Eleventh IHWS data showed that suggested further splits of B48 could not be confirmed because of the many extra weak reactions.

- **B41**. This was best detected with Eleventh IHWS serum 0401 (LEV201) and four other sera also reacted with B41. There were some cells that reacted with both B42 and B41 sera when there was another B-locus antigen present. This pattern has been reported previously as a split of B41 [10], but it could just as easily be a split of B42. Further studies will be needed to elucidate these anomalous reactions.

The reaction patterns defining the B40 group of antigens with the Eleventh IHWS core sera set 2 are shown in Table 2. Similar conclusions can be drawn except that it was not possible to define BFU with set 2 sera.

The distribution of these antigens varies widely in different populations as can be seen in Table 3. In general, they are all low-frequency antigens with only a few notable exceptions. B61 and B48 have much higher frequencies in Eskimos and B60 and B48 are very high in Taiwanese aborigines.

Conclusions

The antigens in this group could be clearly Eleventh IHWS discriminated except for difficulty with B60 heterozygotes. The Eleventh IHWS sera confirmed earlier reports of the BFU antigen. No other suggested splits could be confirmed by serology.

Table 1. Reaction patterns with Eleventh IHWS sera (set 1)

Eleventh IHWS serum								
No.	Name	B13	B61	B60	B47	B48	BFU	B41
0343	LEP213	+						
0344	MID107	+						
0345	DDC213	+						
0346	PRR210	+	+	+	+			
0347	NOS208	+	+	+	+			
0348	FER206	+	+	+	+			
0349	DUQ209	+	+	+				
0350	TSU207	w	+	+				
0351	SAJ213		+	+				
0352	MYE204		+	+		+	+	+
0353	SAS205		+	+		+	+	+
0354	TSU209		+	+		+	+	+
0355	JUJ204			+				
0356	HSE207			+				
0357	NIT201			+		+	+	
0358	TOK222			+		+	+	
0359	AST107			+		+	+	
0360	AKA216					+		
0401	LEX201							+
0402	CEP204							+
0532	KOL602	+						
0533	DUP502	+						
0534	TER508	+						
0535	MUC604	+						
0536	WES608	+						
0537	GOL503	+						
0538	WES609	+						
		Bw4	Bw6	Bw6	Bw4	Bw6	Bw6	Bw6
No. of cells		140	266	202	17	88	3	47

Table 2. Reaction patterns with Eleventh IHWS sera (set 2)

Eleventh IHWS serum								
No.	Name	B13	B61	B60	B47	B48	BFU	B41
1307	DDC213	w						
1308	LEP213	+						
1309	MID107	+						
1310	PRR210	+	+	+	+			
1311	TOK214	+	+	+	+			
1312	DUQ209	w	+	+				
1313	SAJ212		+	+				
1314	FAU217			+				
1315	DDC221			+				+
1316	AST103		w	+		+	+	+
1317	HAJ115			+		+	+	
1318	AND214					+	+	
1319	KAW206					+	+	
1320	FTW210							+
1321	ENG201							+
		Bw4	Bw6	Bw6	Bw4	Bw6	Bw6	Bw6
No. of cells		91	80	175	16	32		31

Table 3. Frequency distribution in selected populations

Population	Code	B13	B61	B60	B47	B48	BFU	B41
Black South Africa	10200	2.5	0.0	0.0	0.0	1.0	0.0	1.5
Black Zimbabwe	10204	2.0	0.0	0.0	0.0	0.0	0.0	2.0
Black USA	10400	1.8	0.4	2.2	0.0	0.0	0.0	2.7
Denmark	30111	2.3	1.6	9.4	0.4	0.8	0.0	1.9
France	30113	2.9	2.8	3.5	0.2	0.0	0.0	1.6
Italy	30117	3.2	1.3	0.8	0.6	0.2	0.0	1.3
Japan	40101	2.0	11.1	5.5	0.0	2.8	0.5	0.0
Korea	40104	6.1	9.4	4.0	0.0	4.2	0.0	0.0
Chinese	40210	15.5	1.1	3.0	1.0	1.0	0.0	0.0
Eskimo	41102	0.0	30.0	0.8	1.7	8.9	0.0	0.4
Papua New Guinea Highland	20202	8.5	2.0	14.9	0.0	0.0	0.0	0.0
Taiwan Aborigine TAYA (local)		1.9	5.3	30.6	0.0	19.3	NT	0.0

NT, Not tested.

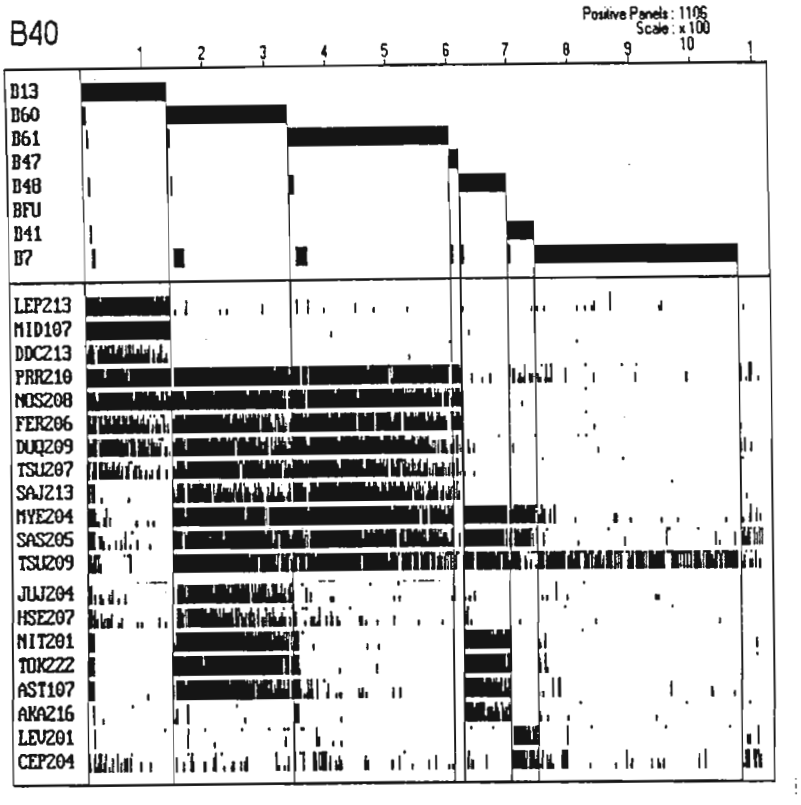

Fig. 1. Serograph of the B40 creg.

References

1. Svejgaard, A., Kissmeyer-Nielsen, F., and Thorsby, E. In *Histocompatibility testing 1970* (ed. P. I. Terasaki), p. 153. Munksgaard, Copenhagen (1970).
2. Thorsby, E. In *Histocompatibility testing 1972* (ed. J. Dausset and J. Colombani), p. 639. Munksgaard, Copenhagen (1973).
3. Bodmer, J. In *Histocompatibility testing 1975* (ed. F. Kissmeyer-Nielsen), p. 50. Munksgaard, Copenhagen (1975).
4. Dick, H. E. and Bodmer, W. F. In *Histocompatibility testing 1977* (ed. W. F. Bodmer, J. R. Batchelor, J. G. Bodmer, H. Festenstein, and P. J. Morris), p. 178. Munksgaard, Copenhagen (1977).
5. Kissmeyer-Nielsen, F., Andersen, H., Hause, M., Kjerbye, K. E., Morgensen, B., and Svejgaard, A., *Tissue Antigens* **1**, 74 (1971).
6. Dawkins, R. L. and Houliston, J. B. In *Histocompatibility testing 1980* (ed. P. I. Terasaki), p. 456. UCLA Tissue Typing Laboratory, Los Angeles (1980).
7. Allen, F. H., Amos, D. B., and Batchelor, J. R. In *Histocompatibility testing 1970* (ed. P. I. Terasaki), p. 49. Munksgaard, Copenhagen (1970).
8. Kawaga *et al. Japanese Blood Program* **9**, 21 (1986). [In Japanese.]
9. Radvany, R. M. *et al.* In *Immunobiology of HLA*, Vol. 1. *Histocompatibility testing 1987* (ed. B. Dupont), p. 209. Springer-Verlag, New York (1989).
10. Mervart, H., *et al.* In *Histocompatibility testing 1984* (ed. E. D. Albert, M. P. Baur, and W. R. Mayr), p. ● ●. Springer-Verlag, Berlin (1984).

polymorphism, such as some form of balanced selection due to heterozygote advantage, as already suggested [8,9]. However, there are indications, from HLA class II analyses, that these factors have been very similar in most population groups, and therefore have not interfered with the differentiation patterns of allelic frequencies throughout the world [Tiercy *et al.*, submitted]. This hypothesis remains to be tested for HLA class I loci as well.

Conclusions

Following the Fifth IHWS directed by J. Dausset in 1972 and devoted to population studies, the Eleventh IHWS held in Yokohama in November 1991 offered the opportunity to gather the largest HLA data set with identical methods in human populations from all over the world. Preliminary analyses of these data show that present HLA genetic differentiations are closely related to historical events and can therefore be used to reconstruct human peopling history. However, an important sampling effort should be made, such as incorporating evenly spaced areas of the world, especially on the African continent where population data are still lacking. Moreover, well defined and large samples should be preferred in order to compute unbiased estimations of allele and haplotype frequencies, and to relate the genetic results with other kinds of information, such as linguistic classifications and archaeological data records. These requirements were not entirely fulfilled in the African continent study reported here. This may explain some of the discrepancies found between HLA class I and class II frequency patterns.

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References

1. Powell, J. R., Levene, H., and Dobzhanski, T. *Evolution* **26**, 553 (1972).
2. Gower, J. C. *Statistician* 17, 13 (1967).
3. Excoffier, L., Pellegrini, B., Sanchez-Mazas, A., Simon, C., and Langaney, A. *Yearbook Phys. Anthropol.* 30, 151 (1987).
4. Sanchez-Mazas, A. Unpublished PhD thesis, University of Geneva (1990).
5. Ehret, C. In *From hunters to farmers. The cause and consequence of food production in Africa* (ed. J. D. Clark and S. A. Brandt), p. 26. University of California Press, Berkeley (1984).
6. Sanchez-Mazas, A., Graven, L., and Pellegrini, B. *Bull. Cercle Genevois Archéol* (In press).
7. Dard, P. Unpublished PhD thesis, University of Geneva (1991).
8. Hughes, A. L. and Nei, M. *Nature* **335**, (1988).
9. Hughes, A. L. and Nei, M. *Proc. natl. Acad. Sci., USA* **86**, (1989).

W5.4 HLA in southern African populations

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The southern African populations tested for the anthropology component of the Eleventh International Histocompatibility Workshop (IHWS) consisted of 103 San (Bushman), 65 Khoi (Hottentots), 101 Zulus, 99 Shona, and 51 Zaireans.

It is believed that the Khoi-San diverged from the Negroid peoples and spread south and west from east or central Africa about 30 000 years ago [1]. The Negroid peoples expanded southwards through central and east Africa between 500 and 1500 years ago. They can be divided into chiefdoms or tribes such as the Shona and Ndebele, resident predominantly in Zimbabwe, and the Zulu, Sotho, and Xhosa in South Africa. The San, Khoi, Shona, and Zulu from southern Africa as well as a population from Zaire in central Africa were selected for the Eleventh IHWS anthropology component.

Table 1. HLA gene frequencies

HLA-	Gene frequencies (%) in population (ethnic code no.) San (10201)	Khoi (10202)	Zulu (10200)	Shona (10204)	Zaire (10206)
	(*n* = 103)	(*n* = 65)	(*n* = 101)	(*n* = 99)	(*n* = 51)
A11	0.0	0.8	0.0	0.5	0.0
A30	21.8	13.2	13.9	30.8	11.8
A43	12.4	17.7	2.0	1.1	1.0
B41	4.0	9.7	1.5	2.0	2.9
B42	1.5	2.3	13.1	6.8	2.0
B53	2.0	0.0	1.0	7.1	9.8
B57	2.5	5.5	2.1	8.1	2.9
B58	36.1	10.6	16.8	14.6	8.4
B70	9.7	18.7	21.7	3.4	7.8
	(*n* = 59)	(*n* = 19)	(*n* = 101)	(*n* = 99)	(*n* = 51)
DR15	1.7	23.9	5.4	8.5	17.1
DR3	1.7	5.4	27.6	10.3	8.4
DR4	44.8	17.3	3.1	2.0	2.0
DR11	5.2	0.0	23.2	23.8	12.4
DR12	1.7	0.0	4.5	4.2	1.0

Results and discussion

In Table 1 we list the gene frequencies of some of the antigens that distinguish African populations and those that show marked differences between the Khoisan and the Negroids. A43 has a high frequency in the Khoisan but a low frequency in the Negroid populations probably caused by recent admixture. B42 has the highest frequency in the southern Zulus and decreases northwards where B53 becomes more dominant. The splits of B17 are typical of African populations. B70 has a high frequency but the IHWS sera were unable to distinguish the splits of this antigen. The DR antigens show clear differences among these populations especially in respect to DR3, DR4, and DR11.

HLA typing with sequence-specific oligonucleotide probes (SSO) and in respect to polymerase chain reaction (PCR)-amplified DNA was done on four of the populations (Table 2). This confirmed the serological results but was also able to subdivide many of the specificities. Again, there were marked differences between the Khoisan and the Negroids. The splits of DRB1 03 (0301, 0302) and DQB1 03 (0301, 0302) had opposite distributions. In addition, several new

Table 2. HLA gene frequencies determined by DNA typing

HLA-	Gene frequencies (%) in population (ethnic code no.) San (10201) (*n* = 108)	Khoi (10202) (*n* = 113)	Zulu (10200) (*n* = 84)	Shona (10204) (*n* = 82)
DRB1				
1501	1.4	27.0	6.5	0.0
02LU	0.0	2.7	0.0	0.0
1503	0.0	0.0	0.0	13.3
0301	1.9	7.9	5.6	7.7
0302	0.4	0.9	18.6	4.4
0401	41.2	14.2	3.0	0.7
0404	4.6	6.6	0.6	0.0
04CT	4.6	0.0	0.0	0.0
0405	0.0	0.5	0.6	3.7
1101	1.4	0.9	21.0	NT
DQA1				
0101	0.9	11.5	5.9	16.7
0201	0.0	0.4	5.3	6.9
03	60.6	25.7	9.1	4.2
0401	5.1	1.8	19.2	6.3
DQB1				
0601	0.0	0.4	0.0	0.0
0602	12.5	26.5	21.5	30.4
0301	4.6	5.8	16.7	9.0
0302	46.8	15.0	2.9	1.2
0402	6.9	8.4	19.6	7.1
DPA1				
01			46.2	30.4
02			40.3	35.0
02A			5.6	
02B			1.1	
DPB1				
0101	23.1	18.1	29.7	26.2
0401	28.2	9.7	9.9	2.8
0402	11.6	31.4	17.9	21.9
1801	0.0	0.9	5.1	11.6
CT1	5.1	1.9		
CT2	12.9	2.8		
CT3	6.9	1.4		
CT4	2.8	0.9		

col.1/? Bold italic/Italic throughout

alleles were detected by this technique—DRB1 02LU, DRB1 04CT, DPA1 02A and 02B, and four new alleles at the DPB1 locus.

Table 3 lists representative haplotypes that exhibit linkage disequilibrium and are typical of these populations.

Table 3. Representative haplotypes showing linkage disequilibrium

San	A30,	Cw4,	B58,	DR13,	DQ1
	A23,	Cw6,	B58,	DR4,	DQ3(8)
	A43,	Cw7,	B7,	DR4,	DQ3(7)
Zulu	A23,	Cw-,	B70,	DR11,	DQ7
	A30,	Cw-,	B42,	DR3,	DQ4
Shona	A30,	Cw-	B45,	DR1,	DQ1
	A30,	Cw6,	B58,	DR15,	DQ1
Zaire	A30,	Cw6,	B58,	DR11,	DQ1
	A28,	Cw4,	B53,	DR3,	DQ2

Acknowledgements

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Reference

1. Botha, M. C., du Toit, E. D., Jenkins, T., van Leeuwen, A., d'Amaro, J., Khan, P. M., van der Steen, D. D., van Rood, J. J., and van der Does, J. A. *Histocompatibility testing 1972* (ed. J. Dausset and J. Colombani), p. ● ●. Munksgaard, Copenhagen (1973).

W5.5 HLA in North American and South American Negroids

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It has been known for some time that African-Americans have many HLA variants and combinations of HLA class I and class II alleles that are rare or not observed in other ethnic groups [1–6]. Among the class I specificities observed in these populations are HLA-A23, A28, A30, A33, A34, A36, A74, B42, B45, B53, B58, B70, B71, and B72. A variety of class II alleles, including subsets of DR8, DR11, DR12, DR13, and DR14, are known to occur in American Negroid subjects and a number of class II combinations (such as DR18, DQ4; DR11, DQ11, DQ1, etc.) are characteristic of this population.

The Eleventh International Histocompatibility (IHWS) data set included corrected data on 348 North American (NA) and 113 South American (SA) Negroid individuals for whom serologic typing was available. DNA typing results were analysed in panels of 124 NA and 42 SA Negroid samples.

Results and discussion

Class I antigens

At the HLA-A locus prevalent alleles were A23, A28, A30, A33, A34, and A36. Among HLA-B locus antigens B42, B45, B53, and B70 were prominent. Cw4 was the highest frequency allele at the HLA-C locus in both populations. The blank alleles in NA and SA Negroid populations were higher than in Caucasian populations. The frequency of A-locus blank genes was 5.1 and 8.5 per cent, respectively, for the B locus 3.0 and 2.8 per cent of genes were blank, and for the C locus 38.0 and 28.4 per cent of blank genes were found. A comparison of frequencies with those in West Africans and NA Caucasoids is shown in Table 1.

Table 3 (*continued*)

Haplotypes					Ethnic groups[‡]						
B	Bf	C4A	C4B	DR	ARM	UKR	URA	UZB	IYE	SAC	MXM
53	S	3	1	4	—	—	—	2.6	—	—	—
52	S	3+2	Q0	15	2.1	—	—	—	—	—	—
57	S	6	1	7	—	—	—	—	2.9	—	—
58	F	3	1	13	—	—	—	—	—	2.0	—
62	S	3	1	4	—	—	—	—	2.2	—	—
63	F	3	2	13	—	—	—	—	—	2.0	—
63	S	3	1	4	—	—	—	—	—	2.0	—

[†]Eight HLA-B blank haplotypes are not listed here.
[‡]ARM, Armenians; UKR, Ukrainians; URA, Uralics; UZB, Uzbeks; IYE, Iyers; SAC, South African Caucasoids; BRA, Brazilians; MXM, Mexican Mestizos.

References

1. Alper, C. A., Boenisch, T., and Watson, L. *J. exp. Med.* 135, 68 (1972).
2. Suzuki, K., Harumoto, T., Ito., S., and Matsumoto, H. *Electrophoresis 8*, 481 (1987).
3. Mauff, G., Alper, C. A., Dawkins, R., Doxiadis, G., Giles, C. M., Hauptmann, G., Rittner, C., and Schneider, P. M. *Complement Inflamm.* 7, 261 (1990).
4. Sim, E. and Cross, J. *Biochem. J.* 239, 763 (1986).
5. O'Neill, G. J. *Vox Sang.* 47, 362 (1984).
6. Giles, C. M., Fielder, A. H. L., Lord, D. K., Robson, T., and O'Neill, G. J. *Immunogenetics* 26, 309 (1987).
7. Gorgi, Y., Amold, D., Uring-Lambert, B., Bardi, R., Ayed, G., and Hauptmann, G. *Tissue Antigens* 35, 217 (1990).

W11.6 Complement polymorphism in African Blacks

M. G. HAMMOND, A. MARCELLI and J. C. POIRIER

Only two African Black populations were tested for Bf and C4 as part of the Eleventh International Histocompatibility Workshop (IHWS). They were not tested for C2. The two populations tested were 100 Zulus from southern Africa (SAF-HAM) and 101 people from Mali in West Africa (FRA-DDC). Bf typing was by immunofixation after agarose gel electrophoresis [1]. C4 typing was by electrophoresis of samples pre-treated with carboxypeptidase B and neuraminidase type VI and immunofixation with anti-C4

Results and discussion

The gene frequencies are shown in Table 1. BfF and BfS07 have a higher frequency in West Africans. C4A1 has a high frequency in West Africa while C4A6 has a higher frequency in southern Africa. The frequency of C4AQ0 seems high in southern Africa, but this frequency is an estimate based on the frequency of heterozygotes. C4B3 has a higher frequency in southern Africa.

The joint occurrence of alleles at different loci are referred to as complotypes and the common complotypes are often a reflection of the high frequency of some alleles. Linkage disequilibrium between alleles is of greater interest as an indication of selective pressures in the population.

Table 2 lists those combinations of antigens at three loci that show linkage disequilibrium in the two populations studied here. There are noticeable differences in the complotype distribution in the two populations and some complotypes are only present in one or the other population.

The strong negative linkage disequilibrium between C4A3 and C4B3 (Table 3) is present irrespective of the Bf allele and seems specific for Africa, because in those populations where C4B3 is present there is no linkage disequilibrium. The complotype consisting of the most

Table 1. Gene frequencies of Bf and C4 polymorphisms

	South Africa Zulu ($n = 100$)	West Africa Mali ($n = 101$)
Bf		
F	0.605	0.712
F1	0.060	0.024
F085	0.010	0.0
S	0.305	0.202
S07	0.020	0.059
C4A		
1	0.005	0.113
2	0.015	0.039
3	0.580	0.638
4	0.005	0.034
5	0.005	0.019
6	0.057	0.019
Other	0.0	0.196
Q0	0.333	0.133
C4B		
1	0.458	0.722
2	0.128	0.084
3	0.235	0.099
4	0.0	0.0
5	0.0	0.0
Q0	0.179	0.094

Table 2. Complotypes showing positive linkage disequilibrium (LD)

			South Africa		West Africa	
Bf	C4A	C4B	Frequency (%)	LD	Frequency (%)	LD
S	A3	B1	15.5	7.4	18.0	7.7
F	Q0	B3	13.9	9.1	4.7	3.8
S	A3	B2	5.9	3.6	1.3	0.1
F	Q0	B2	5.0	2.4		
F1	A3	BQ0	4.2	3.6		
F	A6	B3	2.2	2.0		
F	A3	Q0	7.2	0.8	13.0	6.5
F	A1	B1			9.3	4.6
S07	A3	B1			6.6	2.4
S	Q0	B1			5.3	1.9
F	A5	B2			1.9	1.8
S07	A2	B1			1.7	1.6

Table 3. Complotypes showing negative linkage disequilibrium (LD)

			South Africa		West Africa	
Bf	C4A	C4B	Frequency (%)	LD	Frequency (%)	LD
F	A3	B1	13.7	−2.6	19.1	−6.4
F	A3	B3	4.6	−3.8		
S	A3	B3	2.0	−2.0		

Table 4. Linkage disequilibrium with HLA-B and HLA-DR

HLA-B	Bf	C4A	C4B	HLA-DR
South Africa				
7	S	A3	B1	15
8	S	A3	BQ0	10
42	F	AQ0	B3	3
44	F	A6	B1	11
58	S07	A3	B1	14
70	F1	A3	B1	11
West Africa				
18	S	A3	B1	13
42	F	A3	B1	3
45	S07	A3	B1	—
49	F	AQ0	B1	4
51	F	A3	B1	7
53	—	AQ0	BQ0	1

frequent alleles at each locus (BfF, C4A3, C4B1) shows significant negative linkage disequilibrium in Africa and also in Mexico and Italy but not in other European populations. In Oriental populations there is significant positive disequllibrium.

The linkage disequilibrium with HLA-B and HLA-DR antigens (Table 4) is also different except for the well known B42, DR3 (0302) association. Of interest is the C4A6 disequilibrium with B44 and DR11 in southern Africa.

It would be interesting to investigate the role of C4 null genes in the pathogenesis of malaria because of the marked linkage disequilibrium between C4Q0, and B53 in the light of the recently described association of B53 with protection from severe malaria [3].

Conclusions

Bf gene frequencies in Africa are quite different from those in Caucasian and Oriental populations. The most frequent C4 alleles in African populations are also the most frequent in other populations but some alleles (e.g. C4A1, C4B3) that are rare in other populations have a higher frequency in African Blacks.

References

1. Mauff, G., Hummel, K., and Pulverer, G. *Z. Immunitätsforsch.* **150**, 327 (1975).
2. Sim, E. and Cross, S. J. *Biochem. J.* **239**, 763 (1986).
3. Hill, A. V. S., Allsopp, C. E. M., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J., and Greenwood, B M. *Nature* **352**, 595 (1991).

W11.7 Complement polymorphism in North and South American Negroids

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The two Negroid populations studied in this report are a North American group of 75 from Baltimore, Maryland, USA obtained by Dr Wilma Bias of The Johns Hopkins University School of Medicine and a South American group of 158 from Guapi, Colombia on the Pacific coast of South America obtained by Dr Shunro Sonoda, Kagoshima University, Japan. Both groups were obtained as normal controls and/or for the anthropology component of the Eleventh International Histocompatibility Workshop (IHWS).

C4 phenotypes were determined by classical electrophoretic techniques [1,2] using EDTA samples treated with neuraminidase and carboxypeptidase B, a glycine-barbital buffer system, followed by immunofixation. Haemolytic overlay was used to confirm questionable C4 phenotypes.

The comparison of complement types in these populations yields the following similarities and differences.

Results and discussion

Table 1 shows the frequencies of Bf and C4 alleles in North and South American Negroids. Only two alleles differ significantly: C4A*1 and C4A*Q0. C4A*1 was present in 10 per cent of the North American Negroid population but in less than 1 per cent of the South American sample. Of the data collected for the IHWS, the highest population frequency for C4A*1 was

Table 1. Allele frequencies of factor B (Bf), C4A, and C4B in North and South American Negroid populations (ethnic codes 10400 and 10600, respectively)

	Allele frequency (%)		
Locus	N. American Negroid ($n=75$)	S. American Negroid ($n=158$)	p
Bf			
F	50.7	51.6	NS
F1	2.6	1.9	NS
S	46.7	45.9	NS
S07	0.0	0.6	NS
Other	0.0	0.0	NS
C4A			
A1	9.9	0.3	0.00005
A2	3.3	2.2	NS
A3	78.9	79.9	NS
A4	0.7	3.7	NS
A5	2.0	0.0	NS
A6	2.2	0.3	NS
A3+2	0.7	0.3	NS
Other	0.0	0.3	NS
Q0	2.5	17.2	0.00001
C4B			
B1	74.5	79.5	NS
B2	14.0	8.2	NS
B3	2.6	3.8	NS
B4	0.0	1.6	NS
B5	0.0	0.0	NS
Other	0.0	0.7	NS
Q0	8.9	6.3	NS

NS, Non-significant.

On behalf of Wilma Bias and Shunro Sonoda.

p230 Paulsen G, Markussen G, Acton RT, Tiercy JM, Hammond MG and Fauchet R. **RFLP Standardization Report for DR Beta/Hind III**: In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p598-600.

p234 Paulsen G, Markussen G, Barger BO, Fauchet R, Hammond MG and Tiercy JM. **RFLP Standardization Report for DP Beta/HindIII** In: Dupont B (ed). *Immunobiology of HLA, Volume 1: Histocompatibility Testing 1987.* New York: Springer-Verlag, 1989. p662-663.

p237 Hammond MG. **Correlation between serology and DNA typing.** In: Chandanayingyong D (ed). *Proceedings of the Annual Scientific Meeting of ASEATTA* Dept of Transfusion Medicine, Mahidol University, Bangkok 1991.

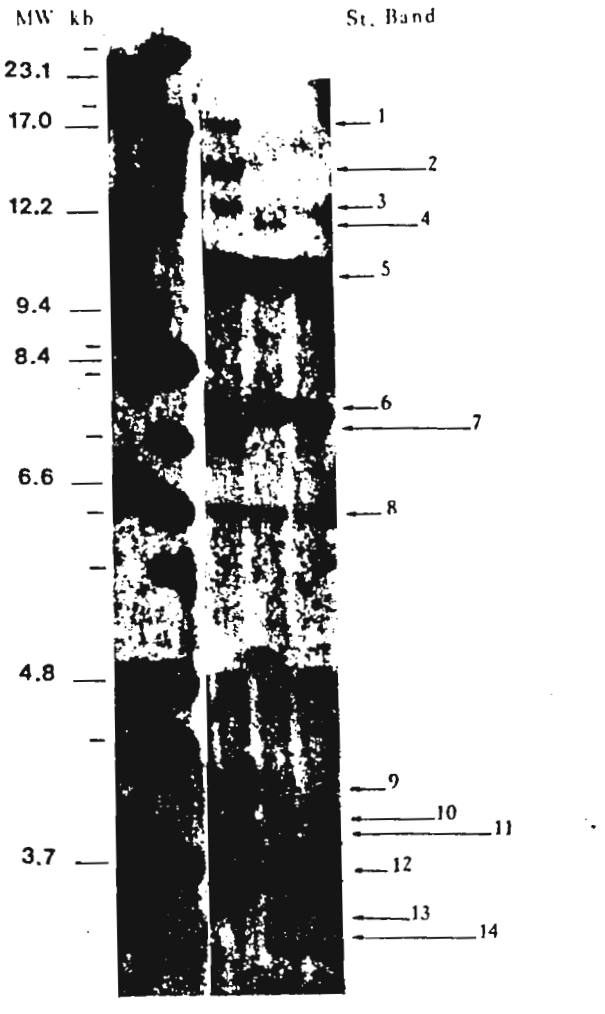

Figure 1.

RFLP Standardization Report for DP Beta/HindIII

G. Paulsen,[1] G. Markussen,[1] B.O. Barger,[3] R. Fauchet,[2] M.G. Hammond,[5] and J.M. Tiercy[4]

Thirty-two bands were identified by RFLP in the DP beta/HID system as shown in Table 1 and in Figure 1. The distribution among core cell lines of 16 bands with high DPB specificity is shown in Table 2. A cross-hybridization table for the HLA class II/HID system can be found in the DR Beta/HID report.

Participating Laboratories: SCATSB,[1] FRAFAU,[2] US5UAB,[3] FRAJEA,[4] SAFHAM[5]

Table 1. Standard Bands in the DRBeta/HID System

Band	kb	Locus[a]	Frequency[b]	% Faint[c]	56[d]	32[d]
1	9.66	1 2 2 0	0.029	0.000		
2	9.65	1 0 2 0	0.014	0.000		
3	8.44	1 0 2 0	0.257	0.000		
4	7.17	1 2 0 0	0.300	0.000		
5	6.94	2 3 1 0	1.000	1.000		
6	6.10	1 0 0 0	0.071	0.000		
7	3.84	1 0 0 0	1.000	1.000		
8	3.43	1 2 1 0	0.071	0.000		
9	3.32	1 0 2 0	0.143	0.000		
10	3.13	1 0 0 0	0.300	0.000		
11	3.02	1 2 3 0	0.071	0.000		
12	2.96	1 0 2 0	0.500	0.000		
13	2.89	1 0 1 0	0.529	0.000	s	
14	2.85	1 0 2 0	0.171	0.000	s	
15	2.70	1 0 0 0	0.057	0.000		
16	2.60	1 2 0 0	0.129	0.000		
17	2.55	1 0 0 0	0.057	0.000		
18	2.51	1 3 2 0	0.429	0.000		
19	2.49	1 3 2 0	0.443	0.000		
20	2.36	1 0 0 0	1.000	0.861		
21	1.83	1 2 0 0	0.029	0.000		
22	1.72	1 0 0 0	0.014	0.000		
23	1.68	1 2 1 0	0.343	0.000		
24	1.63	1 2 1 0	0.414	0.000		
25	1.58	1 2 1 0	0.171	0.000		
26	1.44	1 2 1 0	0.300	0.000		
27	1.30	2 1 2 0	0.086	1.000		
28	1.15	2 1 2 0	1.000	1.000		

[a]Locus assignment in the order DRB, DQB, DPB, DOB. 1 indicates high specificity; 2 and 3 indicate lower specificity.
[b]Band frequency in the core cell lines.
[c]Frequency of faint bands
[d]Discrepancies in faint bands (f) or strong bands (s) of the hidden duplicates (WS. ID. 9056 and 9032).

Table 3. Cross-Hybridization Table—Enzyme: HID—Probe: HLA Class II Beta

DRB	kb	DQB	kb	DPB	kb
		1	12.80	1	13.07
		2	11.42	3	11.66
1	9.66	3	9.76	4	9.76
2	9.65			5	9.64
3	8.44			6	8.59
4	7.17	7	7.24		
5	6.94	8	7.08	7	7.05
		10	5.48	9	5.43
		11	5.13	10	5.06
8	3.43	15	3.47	14	3.50
		16	3.41	15	3.42
9	3.32			16	3.37
		17	3.30	17	3.31
		19	3.20	18	3.42
11	3.02	20	3.09	19	3.09
12	2.96			20	2.99
13	2.89			21	2.90
14	2.85			22	2.83
16	2.60	25	2.62		
18	2.51	26	2.52	23	2.51
19	2.49	27	2.50	24	2.50
21	1.83	28	1.79		
23	1.68	29	1.70	27	1.68
24	1.63	30	1.66	28	1.65
25	1.58	31	1.61	29	1.60
26	1.44	32	1.51	30	1.48
27	1.30	33	1.35	31	1.32
28	1.15	34	1.19	32	1.17

Underline denotes primary locus assignment.

Table 2. Distribution of Bands With High DR Beta Specificity in the DRB/HID System

		Band number																								
		01	02	03	04	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
WS ID	DR																									
9106	7	2	2		2		8			2			1						2	8				1		1
9052	7	2			2		8			2			1					2		8				1		1
9047	7			2	2		8			2			1					2		8				1		1
9048	7			2	2		8			2			1					2		8				1		1
9050	7			2	2		8			2			1					2		8				1		1
9051	7			2	2		8			2			1					2		8				1		1
9104	11			2	2		8			2			1					2		8				1		1
9007	4/16			2	2		8		1	2			1					2		2				1		1
9025	4			2	2		8			2			1					2		8					1	1
9026	4			2	2		8			2			1					2		8					1	1
9028	4			2	2		8			2			1					2		8					1	1
9029	4			2	2		8			2			1					2		8					1	1
9030	4			2	2		8			2			1					2		8					1	1
9031	4			2	2		8			2			1					2		8					1	1
9033	4			2	2		8			2			1					2		8					1	1
9034	4			2	2		8			2			1					2		8					1	1
9091	4			2	2		8			2			1					2		8					1	1
9092	4			2	2		8			2			1					2		8					1	1
9107	4			2	2		8			2			1					2		8					1	1

Table 2. *Continued*

		Band number																								
WS ID	DR	01	02	03	04	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
9032	4			2	2		8			2			1						2	8					1	1
9032	4			2	2		8			2			1						2	8					1	1
9075	9				2		8			2			1					2		8		2	1			1
9002	1					2	8	1			3									8						1
9003	1					2	8	1			3									8						
9004	1					2	8	1			3									8						
9005	1					2	8	1			3									8						
9006	1					2	8	1			3									8						
9010	15						8		3									2		2			1			
9011	15						8		3									2		2			1			
9082	15						8		3									2		2			1			
9017	15						8		3									2		2				1		
9013	15						8		3									2		2				1		
9014	15						8		3									2		2				1		
9008	15						8		3									2		2				1		
9009	2						8		1									2		2				2		
9016	16						8		1									2		2				2		
9019	3						8					3	3				2		2	8			1			
9020	3						8					3	2				2		2	8			1			
9018	3						8					3	2				2		2	8				1		
9022	3						8					3		2		2			2	8				1		
9023	3						8					3		2		2			2	8				1		
9088	3						8					3		2		2			2	8				1		
9021	3						8					3		2					2	8				1		
9036	11						8					3	3			2			2	8			1			
9037	11						8					3	2			2			2	8			1			
9039	11						8					3	3			1			2	8			1			
9042	11						8					3	2			2			2	8			1			
9043	11						8					3	3			2			2	8			1			
9060	13						8					3	3			2			2	8			1			
9105	11						8					3	3			1			2	8				1		
9045	11/12						8					3	2				2		2	8	1			1		
9038	12						8					3	3						2	8	2		1			
9040	11						8					3	2						2	8			1			
9054	14						8					3	3						2	8			1			
9061	14						8					3	3						2	8			1			
9057	14						8					3	3						2	8			1			
9058	13						8					3		2					2	8			1			
9062	13						8					3		2					2	8			1			
9065	13						8					3		2					2	8			1			
9064	14						8					3		2					2	8				1		
9055	6						8					3		2	2				2	8			1			
9059	13						8					3		2	2				2	8			1			
9063	13						8					3		2	2				2	8			1			
9056	13/14						8					3		2	2				2	8			1			
9056	13/14						8					3	2		2				2	8			1			
9066	8						8					3								8				1		
9067	8						8					3								8				1		
9068	8						8					3								8				1		
9069	8						8					3								8				1		
9070	8						8					3								8				1		
9071	8						8					3								8				1		
9072	8						8					3								8				1		
		01	02	03	04	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26

Bands designation: 1-3 = increasing intensity; 8 = faint.

distributed among the participating laboratories. The "variant" analyzed by FAU and TSB has been included in the report.

The DRw53 association of two fragments in accordance with bands 3 and 4 in the present report has previously been described. A fragment corresponding to standard band 3 was associated with a HLA restriction element, which seemed to be more narrow than the serologically determined DRw53 specificity (1). This is in agreement with the present standardization where band 3 was missing in two of seven HLA-DR7 core cell lines.

Reference

1. Paulsen G, Qvigstad E, Gaudernack G, Rask L, Winchester R, Thorsby E. Identification, at the genomic level, of an HLA-DR restriction element for cloned antigen-specific T4 cells. J Exp Med 1985;161:1569.

RFLP Standardization Report for DR Beta/MsPI

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DRbeta/MspI blots were received from four laboratories. In spite of the standardized technique, the overall intensity of the blots and the number of fragments detected were somewhat variable. Marker bands were remeasured where necessary and the data were pooled as follows:

1. Faint bands were listed as standard fragments when they were identified by at least two of the four laboratories.
2. Faint bands seen by only one laboratory, sometimes in only one lane, were not included. This was seen most often with bands of high kb and may have been due to incomplete digestion.
3. Discrepancies between blots were resolved in favor of the majority, or in favor of the positive identification when two labs recorded a band and two did not.
4. Faint bands in particular were sometimes resolved as a doublet in one lab's blots, but appeared as a singlet in others. Such bands were considered as two standard fragments when they could be consistently resolved on the blots from at least two labs; otherwise they were considered as one band.
5. Because of small differences in measurement from blot to blot and from lab to lab, the standardized molecular sizes of cross-hybridizing bands were not always exactly the same. In addition, the pattern of positives and negatives of the same band with different cross-hybridizing probes was not always identical. Therefore, cross-hybridizing bands were identified by overlaying the pairs of autoradiographs of blots hybridized with the relevant probes.

The DRB/MspI system identified 27 fragments on the core cell lines, as shown in Table 1 and the Figure 1. Nine of these were uniquely seen with the DRB probe, and eight additional ones were strongest with DRB; one (fragment 6) was equally strong with DRB and DQB. In Figure 1, the DRB-unique or "dominant" fragments are indicated by a dot next to the fragment number.

Table 2 shows all cross-hybridizing bands for MspI with the class II beta probes; the "primary" assignment for each fragment is underlined (e.g., DRB fragment 1 was strongest with the DQB probe and was also seen with the DRB probe, but not with the DPB or DOB probes). It should be noted that the intensity of many fragments varied from lane to lane within a single blot in patterns that were not due to loading of different amounts of DNA in different lanes; hence, for example, a fragment with the strongest hybridization to the DRB probe would still be faint for certain lanes and might have a stronger signal with the DQB probe in certain lanes. Fragments detected with only one probe are not shown in Table 2.

Discrepancies for the "hidden duplicates" (9056 and 9032) were due to faint bands or to difficulties in reading fainter blots where a fragment was not identified by all four labs. As mentioned above, some of the fragments could not be identified or doublets resolved on all blots, largely due to relatively dark background in some blots. Fragments 14a, 21a, and 22a (see Figure 1) were clear on one lab's blots, very faint on a second lab's, and could not be identified on the other two. Fragment 27 was classified by the computer analysis as freq 0.000,% faint 0.000, although this fragment was identified by two labs in exactly the same manner as Fragments 23, 24, 25, and 26; it has the same presence/absence pattern as Fragment 26.

Participating Laboratories: US2BAC,[1] FRAARN,[2] JAPKSH,[3] GERMUC[4]

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Table 1. *Continued*

WS ID No.	Cell	Fragment No. 1	2	3	5	6	9	10	11	12	13	14	15	16	17	18	19	20
9067	BTB																8	
9068	BM9																8	
9071	OLGA																8	
9070	LUY													8				8
9072	SPACH	8																
9066	TAB089																	
9069	MADURA																	

1 = Single intensity; 2 = double intensity; 8 = faint.

RFLP Standardization Report for DR Beta/HindIII

G. Paulsen,[1] G. Markussen,[1] R.T. Acton,[2] J.M. Tiercy,[3] M.G. Hammond,[4] and R. Fauchet[2]

Twenty-eight bands were identified by RFLP in the DRB/HID system, as shown in Table 1 and Figure 1. The distribution among core cell lines of 25 bands with high DRB specificity is shown in Table 2.

Participating Laboratories: SCATSB,[1] US5UAB,[2] FRAFAU,[3] SAFHAM,[4] FRAJEA[3]

A cross-hybridization table for the HLA class II/HID systems is indicated in Table 3. Bands 13 and 14 had almost identical migration, but were decided to be two distinct fragments. The same decision was made for bands 18 and 19. This made the band number assignment of these bands difficult.

Regarding WS. ID. 9037 (RFLP gel load number 26), DNA from two different cell lines seems to have been

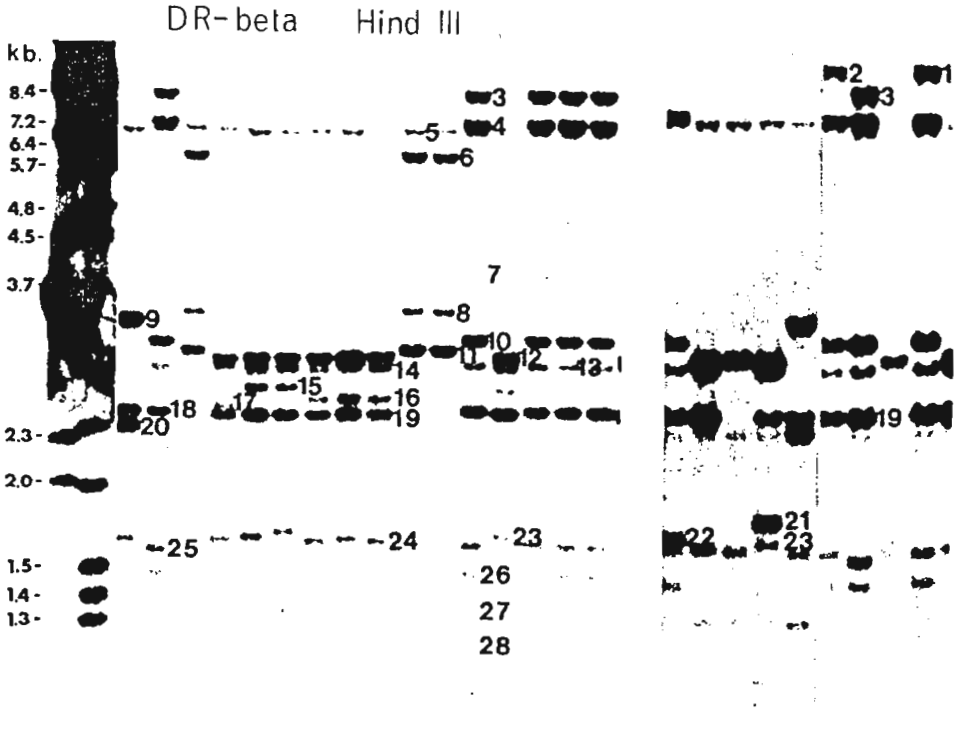

Figure 1. RFLP of 25 DNA samples run in 0.9% agarose gel. Representative core bands are shown on the right. The WS. ID. of the core cell lines is shown from the left following marker 1 and marker 2: 9013, 9033, 9004, 9018, 9055, 9063, 9022, 9043, 9023, 9006, 9005, 9050, 9056, 9104, 9051, 9047, open lane, 9075, 9061, 9067, 9038, 9008, 9106, 9032, 9072, and 9052.

Immunobiology of HLA
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Table 1. Standard Bands in the DP Beta/HID System

Band	kb	Locus[a]	Frequency[b]	% Faint[c]	56[d]	32[d]
1	13.07	0 1 2 0	0.086	1.000		
2	12.57	0 0 1 0	1.000	1.000		
3	11.66	0 1 2 0	0.086	1.000		
4	9.76	1 2 2 0	0.014	1.000		
5	9.64	1 0 2 0	0.014	1.000		
6	8.59	1 0 2 0	0.257	1.000		
7	7.05	2 3 1 0	1.000	0.000		
8	5.47	0 0 1 0	1.000	0.000		
9	5.43	0 2 1 0	0.586	0.000		s
10	5.06	0 2 1 0	0.543	0.000		
11	4.36	0 0 1 0	1.000	1.000		
12	4.10	0 0 1 0	0.014	1.000		
13	4.00	0 0 1 0	0.986	1.000		
14	3.50	1 2 1 0	0.071	0.000		
15	3.42	0 1 2 0	0.171	1.000		
16	3.37	1 0 2 0	0.114	0.000		
17	3.31	0 1 2 0	0.643	1.000		
18	3.24	0 1 2 0	1.000	1.000		
19	3.09	1 2 3 0	0.071	1.000		
20	2.99	1 0 2 0	0.514	1.000		
21	2.90	1 0 1 0	0.514	1.000	f	
22	2.83	1 0 2 0	0.171	0.769	f	
23	2.51	1 3 2 0	0.385	1.000		
24	2.50	1 3 2 0	0.442	1.000		
25	1.86	0 0 1 0	0.986	0.000		
26	1.81	0 0 1 0	0.014	0.000		
27	1.68	1 2 1 0	0.342	0.000		
28	1.65	1 2 1 0	0.414	0.000		
29	1.60	1 2 1 0	0.171	0.000		
30	1.48	1 2 1 0	0.300	0.000		
31	1.32	2 1 2 0	1.000	1.000		
32	1.17	2 1 2 0	1.000	1.000		

[a]Locus assignment in the order DRB, DQB, DPB, DOB; 1 indicates high, 2 and 3 lower specificity.
[b]Band frequency in the core cell lines.
[c]Frequency of faint bands.
[d]Discrepancies in faint bands (f) or strong bands (s) of the hidden duplicates (WS ID 9056 and 9032).

Table 2. Distribution of Bands With High DPB Specificity in the DP Beta/HID System

			Band No.															
WS ID	DR	DP	02	07	08	09	10	11	12	13	14	21	25	26	27	28	29	30
9006	1	1	8	2	2	2		8		8	2		2					
9004	1	4	8	2	2	2		8		8	2		2					
9005	1	–	8	2	2	2		8		8	2		2					
9002	1	4	8	2	2	2		8		8	2		2					
9058	13	1	8	2	2	2		8		8			2		1			
9040	11	3	8	2	2	2		8		8		8	2		1			
9082	15	4	8	2	2	2		8		8			2		1			
9065	13	4	8	2	2	2		8		8			2		1			
9055	6	5	8	2	2	2		8		8			2		1			
9010	15	–	8	2	2	2		8		8			2		1			
9037	11	–	8	2	2	2		8		8		8	2		1			
9042	11	–	8	2	2	2		8		8		8	2		1			
9054	14	–	8	2	2	2		8		8		8	2		1			
9021	3	1	8	2	2	2		8		8			2			1		
9023	3	1	8	2	2	2		8		8			2			1		

Table 2. *Continued*

			Band No.															
WS ID	DR	DP	02	07	08	09	10	11	12	13	14	21	25	26	27	28	29	30
9014	15	4	8	2	2	2		8		8			2		1			
9067	8	4	8	2	2	2		8		8			2		1			
9069	8	4	8	2	2	1		8		8			2		1			
9088	3	1,4	8	2	2	2		8		8			2		1			
9070	8	1,4	8	2	2	2		8		8			2		1			
9016	16	–	8	2	2	2		8		8			2		1			
9051	7	4	8	2	2	2		8		8		8	2		1			1
9052	7	4	8	2	2	2		8		8		8	2		1			1
9025	4	4	8	2	2	1		8		8		8	2			1	1	1
9028	4	4	8	2	2	2		8		8		8	2			1	1	1
9031	4	4	8	2	2	2		8		8		8	2			1	1	1
9107	4	5	8	2	2	2		8		8		8	2			1	1	1
9091	4	–	8	2	2	2		8		8		8	2			1	1	1
9092	4	–	8	2	2	2		8		8		8	2			1	1	1
9064	14	–	8	2	2	2		8		8			2		1			
9057	14	4	8	2	2	2		8		8		8	2	1				
9072	8		8	2	2	1		8		8		1	2		1			
9062	13	4	8	2	2	1	2	8		8			2	1				
9011	15	2,4	8	2	2	2	2	8		8			2	1				
9045	11/12	2,4	8	2	2	1	2	8		8		8	2		1			
9009	2	3,4	8	2	2	1	2	8		8			2		1			
9013	15	–	8	2	2	2	2	8		8			2		1			
9007	4/16	3,4	8	2	2	2	2	8		8		8	2		1			1
9106	7	–	8	2	2	1	2	8		8		8	2		1			1
9032	4	2	8	2	2	1	2	8		8		8	2			1	1	
9032	4	2	8	2	2		2	8		8		8	2			1	1	
9020	3	2	8	2	2		2	8		8		8	2	1				
9036	11	2	8	2	2		2	8		8		8	2	1				
9038	12	2	8	2	2		2	8		8		8	2	1				
9039	11	2	8	2	2		2	8		8		8	2	1				
9063	13	2	8	2	2		2	8		8			2	1				
9059	13	3	8	2	2		2	8		8			2	1				
9061	14	4	8	2	2		2	8		8		8	2	1				
9019	3	–	8	2	2		2	8		8		8	2	1				
9043	11	–	8	2	2		2	8		8		8	2	1				
9056	13/14		8	2	2		2	8		8			2	1				
9056	13/14		8	2	2		2	8		8		8	2	1				
9075	9	4	8	2	2		2	8		8		8	2	1				1
9068	8	2	8	2	2		2	8		8			2		1			
9018	3	3	8	2	2		2	8		8		8	2		1			
9022	3	3	8	2	2		2	8		8			2		1			
9105	11	2	8	2	2		2	8	8			8	2		1			
9071	8	3	8	2	2		2	8		8			2		1			
9008	15	2,4	8	2	2		2	8		8			2		1			
9017	15	2,4	8	2	2		2	8		8			2		1			
9066	8	–	8	2	2		2	8		8			2		1			
9104	11		8	2	2		2	8		8		8	2		1			1
9047	7	–	8	2	2		2	8		8		8	2		1			1
9048	7	–	8	2	2		2	8		8		8	2		1			1
9050	7	2	8	2	2		2	8		8		8	2		1			1
9029	4	2	8	2	2		2	8		8		8	2			1	1	
9030	4	3	8	2	2		2	8		8		8	2			1	1	
9026	4	4	8	2	2		2	8		8		8	2			1	1	
8033	4	4	8	2	2		2	8		8		8	2			1	1	
9034	4	–	8	2	2		2	8		8		8	2			1	1	
9003	1	–	8	2	2		2	8		8	2		2					
9060	13	–	8	2	2		2	8		8		8		1	1			
			02	07	08	09	10	11	12	13	14	21	25	26	27	28	29	30

Bands designation: 1–2 = increasing intensity, 8 = faint.

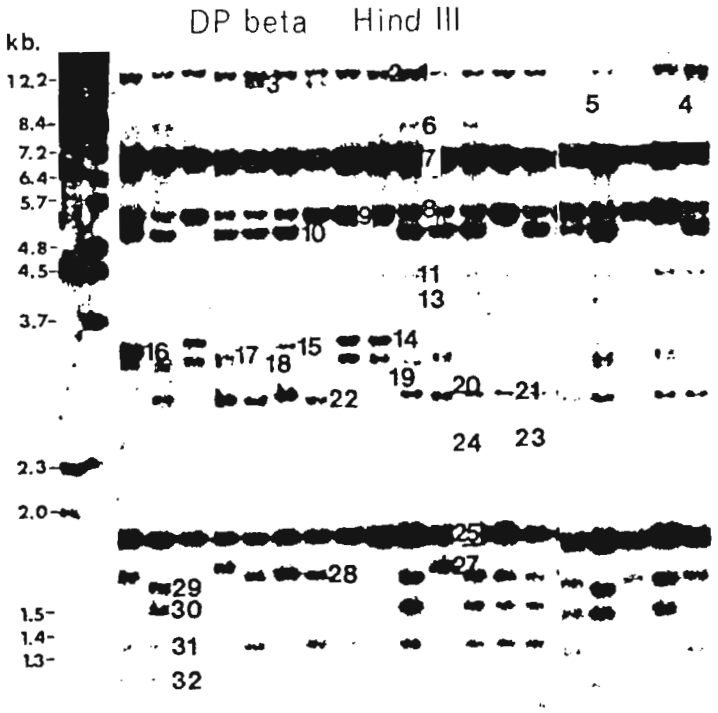

Figure 1. RFLP in the DP beta/HID system of 19 DNA samples run in 0.9% agarose gel. Representative core bands are shown in the figure numbered on their right side. The WS ID of the core cell lines are from the left following marker 1 and marker 2: 9013, 9033, 9004, 9063, 9022, 9043, 9023, 9006, 9005, 9050, 9056, 9104, 9051, 9047, 9106, 9032, 9072, 9052, and 9020.

RFLP Standardization Report for DP Beta/MspI

M. Segall,[1]* L. Schluender,[1]* A. Arnaiz-Villena,[2] N. Kashiwagi,[3] and C. Muller[4]

DP beta/MspI blots were received from four laboratories and were analyzed as detailed in the report on DR Beta/MspI. The DP beta/MspI system identified 20 fragments on the core cell lines (Figs. 1 and 2). Nine of these fragments were unique to DPB, and three were cross-hybridizing but strongest with DPB, as shown in Table 2 of the report on DR Beta/MspI. Unique fragments are are not shown in that table. DPB-dominant or unique fragments are indicated in Figures 1 and 2 with a dot next to the fragment number.

Discrepancies for the "hidden duplicates" (9056 and 9032) were found in bands identified by only two or three of the participating laboratories and generally involved faint bands. Fragments 17 and 18, which were both unique to DPB, were separable on one lab's blots and are certainly a doublet, but could not always be clearly distinguished on the blots of other labs.

Presence/absence data for the polymorphic DPB fragments are shown in Table 1; the cells are arranged in order by DPw specificity. Fragments 2 and 3 both were positive in 6/8 DPw1 (both were negative with cell #9022); fragment 2 also was positive with 1/2 DPw5. Fragment 13 was positive with 16/16 DPw2 and 1 DPw4.

Participating Laboratories: US2BAC,[1] FRAARN,[2] JAPKSH,[3] GERMUC[4]

*This is publication #496 from the Immunobiology Research Center, University of Minnesota, Minneapolis, Minnesota 55455, USA. Supported in part by Juvenile Diabetes Foundation Grant #186175 and March of Dimes Birth Defects Foundation Grant #6-496.

CORRELATION BETWEEN SEROLOGY AND DNA TYPING

M.G. Hammond.[1]

The Eleventh International Histocompatibility Workshop included a protocol for testing DNA using sequence specific oligi-nucleotide probes (SSOP). We were able to test 92 African Blacks by this technique as well as the standard serological method.

There was very close agreement by these two methods but the discrepancies need to be studied further. There were nine cells in the DR 1, 2, 10 group and two differences.

There was almost total agreement in the DR53 group of DR4, 7 and 9. Only one sample tested positive with SSOP's for DR4 and negative by serology. The DR52 group of DR 3, 5, 6 showed several discrepancies especially the splits of DR 6 (DR 13 and DR 14). It seems probable that the serological definition of the narrow antigens is not clear cut.

In the DQ group of specificities, the most recently defined antigen - DQ4 - also had many discrepancies. There were four cells positive by DNA testing and negative by serology and six cells where the opposite occurred.

[1] Natal Institute of Immunology,
Durban, South Africa

CORRELATION BETWEEN SEROLOGY AND DNA TYPING

	AFRICAN		BLACK	
Specificity	+ +	Sero + DNA -	Sero - DNA +	- -
DR 3	37	2	1	48
DR 5(11)	40	0	2	46
DR 5(12)	8	1	0	79
DR 6(13)	22	2	1	63
DR 6(14)	3	2	0	83
DR 8	4	0	0	84
DR 52	80	0	0	8
DR 4	5	0	1	82
DR 7	12	0	0	76
DR 9	3	0	0	85
DR 53	21	0	0	67
DR 1	3	1	0	84
DR 2(15)	11	0	0	77
DR 2(16)	0	0	0	88
DR 10	3	0	1	84
DQ 1	59	1	1	27
DQ 2	25	1	1	61
DQ 3(7)	26	1	2	59
DQ 3(8)	5	1	0	82
DQ 4	26	6	4	52

DR1

SEROLOGY									D N A									
									1 2	2 5 7			8 8					
6	6	6	6	6	6	6			0 8	8 7 0			6 6					
0	0	0	0	0	0	0	D	D	0 0	0 0 0			0 0					
3	4	5	6	7	8	9	R	R	1 1	1 1 1			1 2			DR	DRB	
+	+	+	+	+	+	+	1		+ +	+	+	+	-	+		0101		3 cells
+	+	+	+	+	+	+	1	13	- -	nd						-	1302	1 cell

DR10

SEROLOGY												D N A					
												1 3					
6	6	6	6	6	6	6	8	8	8	8	8	0 7					
1	1	1	1	1	1	1	1	1	2	3	4	D D D	0 0				
0	1	2	3	4	5	6	7	8	0	9	1	R R R	8 8		DRB	DRB	
w	+	+	+	+	+	w	w	+	+	+	+	10	+ +		1001		3 cells
-	-	-	-	+	-	-	-	-	-	+	+	9 -	+ +		1001	0901	1 cell

DR3

SEROLOGY										D N A			
										2 7			
6	6	6	6	6	6	6				8 0			
2	3	3	3	3	3	3	D	D	D	0 0			
9	0	1	2	3	4	5	R	R	R	7 4	DRB	DRB	
+	+	+	+	+	+	+	3			+ +	0301		9 cells
+	+	+	+	+	+	+	3			- +	0302		28 cells
+	+	+	-	+	+	+	3	13		- -	-	1302	1 cell
+	+	+	+	w	+	+	3	11		- -	-	1101	1 cell
-	-	-	-	-	-	-		11	13	- +	0302	1101	1 cell

DR4

SEROLOGY												D N A																	
												1	2	3	5	5	7	7	7	8	8								
7	7	7	7	7	7	7	7	7	7	7		0	8	7	7	7	0	0	0	6	6								
1	1	1	1	1	1	2	2	2	2	2	D	D	D	0	1	0	0	0	0	0	0	0	0						
4	5	6	7	8	9	0	1	2	3	4	R	R	R	4	0	8	1	2	1	5	6	1	3	DRB		DRB			
+	+	-	+	+	+	+	w	+	+	+	4			+	+	+	+	-	-	+	-	+	-	0401					3 cel
+	+	+	-	+	-	+	+	+	+	+	4	11		+	+	+	+	-	+	-	+	-	+	0404		1101			1 cel
+	+	-	+	+	+	+	w	+	+	+	4	-		+	+	+	-	+	+	-	-	+	-	0405		-			1 cel
-	-	+	-	+	-	-	-	+	+	+		11	14	+	+	+	+	-	-	+	-	+	-	0401		1101			1 cel

DR5

SEROLOGY																	DNA													
640	641	642	643	644	645	646	647	648	649	650	651	652	653	DR	DR	DR	5703	7002	8601	7007	8603	1005	2802	8602	5705	7010	3712	DRB	DRB	
+	+	+	+	+	+	+	+	-	-	-	w	w	w	11			+	+	+	-	-	-	-	-	-	-	-	1101		32 cells
+	+	+	+	+	+	+	+	-	-	-	-	w	-	11			+	-	+	+	+	-	-	-	-	+	-	1102		4 cells
+	+	+	+	+	+	+	+	-	-	-	w	-	w	11	3		+	-	-	-	-	-	-	-	-	-	-	1103	301	1 cell
+	+	+	+	+	+	+	+	-	-	-	+	+	+	11	-		+	+	+	-	+	-	-	-	-	-	-	1104	802	1 cell
+	+	+	+	+	+	+	+	+	+	+	+	+	+	11	12		+	+	+	-	-	+	+	+	+	+	-	1101	1201	2 cells
+	-	-	-	-	-	-	-	+	+	+	+	+	+		12		-	-	w	-	-	+	+	+	+	+	-	1201		6 cells
+	-	-	+	+	-	-	-	-	-	-	-	-	-		13	15	+	+	+	-	-	-	-	-	-	-	-	1101	201	1 cell
+	-	-	-	-	-	-	-	+	+	+	+	+	+		12	3	+	+	+	-	w	-	-	-	-	-	-	1101	301	1 cell

DR6

SEROLOGY																		DNA														
636	637	638	639	640	659	660	701	702	819	826	827	828	829	832	834	DR	DR	3703	5701	5702	7027	8601	8603	3712	5704	7003	7009	DR	DR	DR	DR	
-	-	w	+	+	+	-	+	+	-	+	w	-	+	+	+	13		+	+	-	+	-	+	-	-	-	-	1301				6 cells
-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	13	11	+	+	-	-	+	-	-	-	-	-			1101	0302	1 cell
-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	13	15	-	+	-	-	+	-	-	-	-	-			1101	1501	1 cell
-	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	13	-	+	+	-	+	+	+	-	-	-	-	1301	1302			1 cell
+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	14	15	+	+	-	+	-	+	-	-	-	-	1301		1501		1 cell
w	w	+	+	+	+	w	+	+	w	+	+	w	+	+	+	13		+	+	-	+	+	-	-	-	-	-		1302			14 cells
-	-	+	+	+	+	-	+	+	-	-	-	-	+	+	+	13	11	-	-	+	-	+	-	-	-	-	-		1303		1101	1 cell
+	+	w	-	-	+	+	-	-	+	-	-	-	+	-	-	14		-	+	-	-	+	+	+	+	-	+	1401				2 cell
+	+	-	+	+	+	+	+	+	+	+	-	-	w	+	+	14	11	+	+	-	+	+	+	-	-	-	-		1402	1102		1 cell
+	+	+	+	+	-	+	+	-	+	+	-	-	-	+	-	14	11	-	-	-	-	+	-	-	-	+	-			0401	1101	1 cell

DQ1

SEROLOGY														D		N		A															
														2	3	3	4	5	5	5	5	2	2	7	2	2	7						
7	7	7	7	7	7	7	7	7	7	8	8			6	7	7	9	7	7	7	7	6	6	0	6	6	0						
4	4	4	4	5	5	5	5	5	5	2	4	D	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
6	7	8	9	0	1	2	3	4	5	2	6	Q	Q	1	1	2	1	1	2	3	4	2	3	3	4	6	2	DQB	DQB				
+	+	+	+	+	+	+	+	+	+	+	+	1		+	+	+	+	+	-	-	-	-	-	-	-	-	-	0501					11 ce
+	+	+	+	+	+	+	+	+	+	-	-	1	-	+	+	+	+	+	-	-	+	-	+	+	-	-	-	0501	0602				4 cel
+	+	+	+	+	+	+	+	+	+	-	+	1	-	+	+	+	+	+	-	-	+	-	-	+	+	-	-	0501		0603			1 cel
+	+	+	+	+	+	+	-	w	+	-	+	1	-	+	+	+	+	+	-	-	-	-	-	-	+	-	-	0501			0604		1 cel
+	+	+	+	+	+	+	+	+	+	+	+	1		-	-	-	-	-	-	-	+	-	+	+	-	-	-	0602					22 ce
+	+	+	+	+	+	+	+	+	+	-	+	1	-	-	-	-	-	+	-	-	+	-	+	+	-	+	-	0602			0605		1 cel
+	+	+	+	+	+	+	+	+	+	-	+	1		-	-	-	-	-	-	-	+	-	+	+	+	-	-	0602	0603				2 cel
+	+	+	+	+	+	+	+	+	+	-	+	1		-	-	-	-	-	-	-	+	-	-	+	+	-	-		0603				5 cel
+	+	+	+	+	+	+	+	+	+	-	+	1		-	-	-	-	+	-	-	-	-	-	-	+	-	-			0604			4 cel
+	+	+	+	+	+	+	+	+	+	-	+	1	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-			0604	0605		1 cel
+	+	+	+	+	+	+	+	+	+	-	+	1		-	-	-	-	+	-	-	-	-	-	-	-	+	-				0605		7 cel
+	+	+	+	+	+	+	+	+	+	-	+	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0402	-				1 cel
+	+	+	+	+	+	+	+	+	+	-	-	1	4	-	-	-	-	-	-	-	-	+	-	-	-	-	-	0201	0301				1 cel

DQ2

SEROLOGY							D	N	A				
							5		7				
7	7	7	8				7		0				
5	5	5	4	D	D	D	0		0				
6	7	8	7	Q	Q	Q	5		5	DQB	DQB		
+	+	+	+	2			+		+	0201			25 cells
+	+	+	-	2	7		-		-	-	0301	04	1 cell
-	-	-	-		1	4	+		+	0201	0301		1 cell

DQ3

SEROLOGY																	D	N	A						
																	5	4	2	2	5				
7	7	8	8	8	8	8	8	8	8	8	8	8					7	5	6	6	7				
5	6	0	0	0	0	0	0	0	0	0	1	1	D	D	D		0	0	0	0	0				
9	0	1	2	3	4	5	6	7	8	9	0	1	Q	Q	Q		6	1	2	3	7	DQB	DQB		
+	+	+	+	+	+	+	+	+	+	+	+	+	7				+	+	+	-	-	0301			24 cells
+	+	+	+	+	+	+	+	+	+	+	+	+	7	8			+	+	+	+	+	0301	0302		2 cells
+	+	+	+	+	-	-	-	w	w	w	w	+		8			+	-	-	-	-		0302		3 cells
+	+	+	+	+	+	+	+	+	+	+	+	+	7		1		-	-	-	+	-		04	06	1 cell
-	+	+	+	+	-	-	-	+	+	+	+	+		8	1		-	-	w	-	-		-	02	1 cell
-	-	-	-	-	-	-	-	-	+	+	+	-			1	4	+	+	+	+	-	0301		06	1 cell
+	-	-	-	-	+	-	-	-	-	-	-	-			1	4	+	+	-	+	-	0301		02	1 cell

DQ4

SEROLOGY										DNA					
										522					
8	8	8	8	8	8					733					
1	1	1	1	5	5	D	D	D		000					
2	3	4	5	0	2	Q	Q	Q		812		DQB	DQB		
+	+	+	+	+	+	4				+	-	+	0402		26 cells
+	+	+	+	+	+	4				-	-	-			6 cell
+	+	-	w	+	+					+	-	+	0402		4 cell

DQ4

		SEROLOGY									DNA					
											522					
	!	8	8	8	8	8	8				733					
	!	1	1	1	1	5	5	D	D	D	000			D	D	D
CELL	!	2	3	4	5	0	2	Q	Q	Q	812			Q	Q	Q
32	!	8	8	8	8	8	8	4		7	1	1	1		0301	0302
41	!	8	8	8	6	8	8	4		1	1	1	1		0602	0603
44	!	8	1	8	8	1	1	4		1	1	1	1		0501	0602
52	!	8	6	6	1	1	6	4		1	1	1	1		0301	0602
57	!	8	8	8	8	1	1	4		1	1	1	1		0501	0602
72	!	8	8	8	8	8	6	4		1	1	1	1		0201	0301
	!															
26	!	8	8	1	1	1	1		2	7	8	1	8	0402		0301
36	!	8	1	1	1	8	8		1	3	8	1	9	0402		-
49	!	1	1	1	6	6	8		1	7	8	1	8	0402		0602
69	!	1	1	1	6	1	1		1	-	8	1	8	0402		0603

Part II

HLA AND DISEASE

Introduction

The number of investigations into associations between HLA and disease increased dramatically with the discovery that HLA B27 confers a relative risk for ankylosing spondylitis enormously greater than any other genetic polymorphism. More than 500 diseases have now been investigated and the strongest patterns of association are with auto-immune diseases. However, all the early work was done on Caucasian populations and my investigations were aimed at discovering if these associations with specific antigens were present in different races.

Part II contains forty four papers dealing with the relationship between HLA and several diseases in the different races. I was particularly interested in those diseases which occurred more commonly in the Black and Indian communities than in Caucasian populations because the distribution of HLA antigens varies greatly in the different races.

Chapter 2 contains eight papers dealing with the relationship of HLA to cancer. The prospective study of cancer in black patients showed an interesting association between cancer of the oesophagus and HLA B45 but a follow-up study could not confirm the original finding. These published reports led to an invitation to contribute a chapter to a book, *'Cancer of the Oesophagus'*. Other publications include a similar prospective study in Indians and the research carried out while I was Visiting Professor at the National Taiwan University in Taipei.

Diabetes mellitus has strong associations with the HLA system and the ten papers in Chapter 3 reflect the initial studies of Class I antigens and later reports covering Class II antigens as well as some interesting findings in non-insulin dependant diabetes. Another auto-immune disease, rheumatoid arthritis, was investigated as part of collaborative studies included in the International Histocompatibility Workshops and followed by detailed reports of HLA associations in Blacks and Indians.

The remaining 19 publications cover a wide variety of topics from unusual diseases such as tropical spastic paraparesis to paternity tests and even the possible influence of HLA on mate selection.

AN EVALUATION OF HUMORAL ANTIBODY RESPONSES IN PATIENTS WITH CARCINOMA OF THE CERVIX

BY

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Summary

Tumour tissue from patients with inoperable cervical carcinoma was studied to determine the significance of humoral antibody involvement. Comparative elution studies using normal and cancerous tissues revealed that various classes of immunoglobulin and complement, either singly or in combination could only be recovered from the cancerous tissues. Some cancer tissue eluates possessed antibodies which sensitized normal lymphocytes by the cytotoxicity test suggesting the host's recognition of structural modification of the tumour cell. It is possible that the various classes of immunoglobulin found in cancer tissue eluates represent antibodies to cytoplasmic constituents, cell membranes or antigen-antibody complexes. It was found that the serum from the cancer patients possessed a significantly higher incidence of "non specific" lymphocytotoxic antibodies than the controls. Our inability to associate these antibodies with specificities for normal histocompatibility antigens suggests that this type of antibody may symbolize humoral responses towards a combination of tumour-related and normal transplantation antigens. It seems apparent that their activity is of an autoimmune nature capable of altering the *in vivo* functions of the cell-mediated immune mechanism.

There is evidence to show that neoplasia in man can stimulate a host's immunological response towards tumour-associated antigens (Gold and Freedman, 1965; Hellström *et al.*, 1971). In experiments on rodents by Möller (1963) and Batchelor (1968) it was found that the formation of humoral antibodies to tumour-associated antigens blocked the rejection of these cell lines and could lead to their enhanced growth. As a result the tumour apparently remains inaccessible to the host's cell-mediated defense mechan-

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ism. Failure of the host to reject tumour cells has also been said to be due to a severely impaired cellular immune system (Keast, 1970; Alexander and Fairley, 1967). The concept that some cancers may develop as a result of a breakdown in "immunological surveillance" at a time when humoral antibody synthesis to foreign structures of the cancer cell remains active, prompted the present study which measures antibody responses in patients with cervical carcinoma.

A working hypothesis was the acceptance that (a) the recovery of various immunoglobulins from tumour cells reflects the stimulated activity of the host towards altered cell constituents and that (b) the recognition of increased antibody activity to histocompatibility antigens by the lymphocytotoxicity test can be regarded as a host's response to a hybridized antigen on the surface membranes of tumour cells. Evaluation of these serological parameters was carried out on Southern African Negro women with carcinoma of the cervix and a representative number of controls.

Materials and Methods

Patients Investigated

Two groups of women with histologically confirmed squamous cell carcinoma of the cervix were examined: (a) patients with active disease tested before treatment and (b) patients who had confirmed carcinoma of the cervix but were symptom-free five years after treatment.

Preparation of Soluble Cytoplasmic Tissue Eluates

Specimens of cervical tissue were obtained from patients with inoperable cervical carcinoma. For purposes of control normal cervical tissue was obtained from hysterectomy specimens. Pieces of tissue, approximately 20 g. in weight were stored in liquid nitrogen immediately after collection. For the preparation of cytoplasmic eluate, 5 g. (solid mass) of tissue in isotonic saline was homogenized at low speed in a tissue blender. The separation of tissue mass into microscopically recognized clusters of intact cell particles was essential for efficient removal of capillary protein by saline washings. Four saline washings were considered sufficient. Complete disruption of a 50 per cent suspension of the washed cells in saline was obtained by insonation at 25 kc per second for ten minutes in a vessel standing in iced water. On microscopy the tissue mass then appeared as remnants of broken nuclei and membrane particles. From then on the cervical tissue was treated in a similar manner to washed red cells sensitized *in vivo* by acquired haemolytic anaemia auto-antibodies. Antibody globulins, when present on the tissue, were recovered by the ether elution procedure of Vos and Kelsall (1956), modified only in that four volumes of ether was added directly to the saline suspension of the disrupted tissue. The recovered eluate was dialzyed against phosphate buffered physiological saline (0·15M, *p*H 7·1) for 24 hours at 4 °C. Particulate matter present after dialysis was removed by centrifugation and the eluate stored as a lyophilized product.

Determination of Immunoglobulin Characteristics

In view of the relatively low concentration of protein recovered from 5 g. of tissue mass (Table I), the immunoglobulins were characterized by the passive protein coupling procedure of Gold and Fudenberg (1967). A concentration of 2 mg. per ml. of protein was found most suitable for the coating of saline-washed group O red cells with 0·0375M (one per cent) chromic chloride solution diluted to 1 in 20 in a 0·15M solution of sodium chloride. The coated cells and appropriate antiserum were mixed together on agglutination plates and allowed to stand for two hours. Monospecific antisera to IgG, IgA and IgM heavy chain were used to determine the presence of different immunoglobulins. Anticomplement sera (C3 and C4) were kindly donated by Dr. L. D. Petz, Harkness Community Hospital, San Francisco.

Lymphocytotoxicity Test

For the determination of lymphocytotoxic antibodies a selected panel of 18 cell donors was used. The tests were done on Falcon microtest trays using the two-stage procedure recommended by the National Institutes of Health. Thus one μl. of serum and one μl. of cells were placed into each well under paraffin oil. After 30 minutes at room temperature 5 μl. of unabsorbed rabbit complement was added to the

TABLE I

Reactions of various specific antisera to cells coated with preparations of cytoplasmic protein obtained from cervical tissue taken from 4 normal and 8 patients with carcinoma of the cervix

Eluates from	Patient	Agglutination of coated cells by monospecific antisera*					Control serum	Total protein content of eluates in relation to mass equivalence of tissue (mg. per ml.)
		IgG	IgA	IgM	C3	C4		
Normal cervical tissues	MJ	1	0	0	0	0	0	2·3
	CM	0	0	0	0	0	0	2·5
	NS	0	0	1	0	0	0	1·8
	KM	0	0	0	0	0	0	2·7
Cancerous cervical tissue	RN	2	4	0	4	0	0	18·1
	CS	4	1	0	1	0	0	15·7
	GB	4	0	0	0	0	0	38·4
	MP	1	3	2	4	0	0	26·3
	MU	4	4	0	4	1	0	33·5
	DT	4	0	3	4	0	0	21·4
	PS	3	1	4	4	2	0	30·1
	RU	4	0	0	1	0	0	18·3

* Group O red cells were coated with tissue eluate containing 2 mg. per ml. of protein. 1, 2, 3, 4 denotes intensity of agglutination reaction. 0 denotes no agglutination.

cell-serum mixture. After a further 60 minutes at room temperature 5 μl. of freshly prepared trypan blue (0·6 per cent) in saline was placed into each well and left to stand for a further 15 minutes. After this time the excess dye was flicked off and the cells examined with an inverted microscope. Serum which killed lymphocytes with known HL-A antigen determinants, e.g. HL-A7 positive cells but not HL-A7 negative cells were identified as containing "specific" anti-HL-A. Cytotoxic antibody activity which could not be identified as "specific" when tested against the panel of 18 known HL-A cell types were classified as "non-specific". Similar methods for defining lymphocytotoxic antibodies into "specific" or "non-specific" reagents have been reported by Waters *et al.* (1971) and Kreisler *et al.* (1971).

RESULTS

To establish whether humoral antibodies were bound to tumour cells, eluates from a number of normal and cancerous cervical tissues were examined. By our method of recovering protein from cervical tissues, which is a modification of the method used to study the characteristics of autoantibodies in acquired haemolytic anaemia (Vos *et al.*, 1971). it was found that the cancerous tissue contained on a mass equivalent basis more protein than the non-cancerous tissue (Table I). Following passive coating of the protein onto red cells and evaluating their agglutination reactions for various antisera it was found that the tumour eluates often possessed a variety of immunoglobulins. These immunoglobulins were predominantly of the IgG class occurring either singly or in combination with IgM and complement component C3. No definite reactivity for anti-C4 was evident in this small series of cases. The low concentration of protein found in normal cervical tissue eluates could not be classified as immunoglobulins.

Assuming that the immunoglobulins recovered from the cancerous cervical tissues were antibodies capable of sensitizing other cell lines we then measured the presence of lymphocytotoxic antibody activity. Using a selected panel of eighteen lymphocytes which were used routinely to characterize HL-A antibody specificities it was found that four out of the eight cancerous

tissue eluates lysed lymphocytes by the cytotoxicity test (Table II). In tissue eluates CS and GB the reactions were extremely intense for all panel cells, whilst tissue eluates MU and RU only reacted with a small number of the cells. In spite of this variation the reactions could not be classified as "specific" antibodies for known HL-A determinants. Although the cancerous tissues possessed recognizable immunoglobulins (Table I) only some were found to have lymphocytotoxic antibody activity. It is hoped that further studies will determine why this difference exists.

The significance of finding cytotoxic antibodies in the cancerous tissue eluates led us to compare the incidence of these antibodies in patients with carcinoma of the cervix and in a large number of healthy women. It was important to classify antibodies capable of recognizing known combinations of HL-A types from those lacking these characteristics. Table III shows our findings in 69 Southern African Negro women with histologically confirmed squamous cell carcinoma of the cervix tested prior to treatment, 33 women examined five years after successful treatment and 1000 healthy women of the same race. There were no significant differences observed in the incidence of HL-A "specific" cytotoxic antibodies between the cancer patients and the controls (X^2 0·04, $P>0·8$). However, some inexplicable variation in the incidence of HL-A "specific" antibodies was observed between those cancer patients examined prior to treatment (21·7 per cent) and those who were symptom free five years after treatment (3·0 per cent).

In a comparative evaluation of "non specific" lymphocytotoxic antibodies the cancer patients were found to possess a significantly higher incidence of "non specific" cytotoxic antibodies than the controls (X^2 55·48, $P<0·001$). Although it may be that the increased incidence of "non specific" cytotoxic antibodies observed among the five-year-cure series was influenced by therapy, this could not be so for the patients tested prior to treatment.

We could not establish the presence of HL-A "specific" antibodies in patients with "non specific" cytotoxic antibodies by testing their serum at various dilutions. However, selective absorption studies may help to determine the nature of these unusual antibodies.

Discussion

Numerous studies have demonstrated that tumour antigens can be found in a variety of neoplasms (Prehn, 1968; Boyse *et al.*, 1968;

TABLE II

Lymphocytotoxic antibody activity in preparations of normal and cancerous cervical tissue eluate

Eluates from	Patient	Known lymphocyte panel																		Percentage kill*	HL-A specificity
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Normal cervical tissue	MJ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—
	CM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—
	NS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—
	KM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—
Cancerous cervical tissue	RN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—
	CS	+	+	+	+	(+)	+	(+)	+	(+)	+	+	(+)	(+)	+	+	+	+	+	100	Non-specific
	GB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	Non-specific
	MP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	MU	—	—	—	+	—	—	—	—	+	—	+	+	—	+	—	(+)	(+)	—	38	Non-specific
	DT	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—
	PS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—
	RU	—	—	(+)	+	+	—	—	—	(+)	+	—	—	—	—	—	—	—	—	27	Non-specific

+ Denotes strongly positive reaction, 90 to 100 per cent kill of lymphocytes.
(+) Denotes moderate positive reaction, 40 to 70 per cent kill of lymphocytes.
* Ability of eluate to react with lymphocytes of all panel cells represents 100 per cent kill.

TABLE III

The incidence of HL-A "specific" and "non-specific" lymphocytotoxic antibodies in patients with cervical carcinoma and healthy women used as controls

Lympho-cytotoxic antibody activity	Cervical cancer patients						Controls		Cancer patients versus controls
	69 patients tested prior to treatment		33 patients symptom-free five years after treatment		All cancer patients (102)		1000 multiparae		
HL-A specific	15	21·7%	1	3·0%	16	15·6%	149	14·9%	X^2 0·04, $P<0·8$
Non-specific	16	23·1%	10	30·3%	26	25·4%	54	5·4%	X^2 55·48, $P<0·001$

Gold *et al.*, 1968; Jehn *et al.*, 1970; Alexander, 1972). Under normal circumstances the presence of cell lines which differ in genetic composition from the host should induce *in vivo* an immune response resembling a homograft reaction. The inability of the host to do so may be due to a profound defect in cell-mediated immunity. Cancer studies in rodents (Möller, 1963) have shown that humoral antibodies actually block the rejection phenomen thereby enabling the tumour cell line to proliferate in the host. Evidence for linking tumour growth with the presence of a similar humoral antibody blocking mechanism was reported by Hellström *et al.* (1971). In the studies of Gold *et al.* (1968) the formation of rabbit antibodies to carcino-embryonic antigens and the actual finding of similar antibodies in patients with carcinoma suggested that tumour growth may be enhanced by an active humoral antibody responsiveness.

Using cancerous cervical tissue for the evaluation of humoral antibody involvement, we found that they contained a much greater concentration of cell-bound protein than non-cancerous tissues. These abnormally high levels of protein contained a variety of immuno-globulins, indicating the presence of some form of humoral antibody activity towards the tumour. Although no detailed studies were carried out to characterize the specificity of these antibodies, our preliminary investigations established that only some tumour tissue eluates possessed intense lymphocytotoxic activity, suggesting that the recovered immunoglobulins constitute antibodies with a variety of characteristics.

It has been shown that the presence *in vivo* of antigen-antibody determinants can result in the formation of other antibodies to these complexes (Harboe *et al.*, 1965; Abbruxxo and Christian, 1961; Kano and Milgrom, 1968). This activity is said to be influenced *in vivo* by antibodies which have been subjected to molecular transformation during their interaction with antigens. It can therefore be assumed that humoral antibodies to immune complexes may also be generated in patients with active cancer, as a consequence of released antigen-antibody determinants during the process of tumour cell necrosis. A factor to be considered in the development of secondary humoral antibody responses, particularly in proliferating carcinomas, is the overwhelming presence of released antigen-antibody complexes and their corresponding antibodies which may enhance tumour growth more effectively than the antibody which initially sensitized the surface antigen. And so the question arises whether the "blocking factors" described by Hellstrom and Hellstrom (1970) actually represent the primary antibody response to tumour-related surface antigen or the product of subsequent responses to a variety of antigen-antibody determinants.

Although the presence of "non specific" lymphocytotoxic antibodies does not provide any definite information concerning their role in tumour biology, their implication as a product of tumour immunology cannot be ignored. This is evident from the observation that "non specific" lymphocytotoxic antibodies were more often found among patients with carcinoma than in the controls ($P<0·001$). To classify them as tumour-related antibodies requires the acceptance that they possess a marked degree of cross-reactivity for normal histocompatibility determinants. That this is so is obvious from their ability to

react with lymphocytes from normal donors (Table II). It is possible that these "non specific" antibodies may have developed through a sequential process of immune responses by altered tumour antigen presentation. A similar explanation for the variability in antibody responses was recently reported in studies concerning the specificities of autoantibodies in acquired haemolytic anaemias (Vos *et al.*, 1971).

Although our findings indicate that "non specific" cytotoxic antibodies are autoimmune in nature and therefore capable of abrogating the function of the host's own cell-mediated immune system, they do not appear to have impaired the function of the non-thymus or bursa analogue dependent lymphocytes which take part in the formation of circulating immunoglobulin antibodies. If unrestricted synthesis of IgG and IgM immunoglobulins does occur, with specificities towards cancer tissue and cross-reactivity towards thymus-derived lymphocytes, it might be that the bursa analogue dependent lymphocytes lack some of the transplantation antigens which are commonly present on the thymus-derived cell lines. In studies concerning autoantibodies in patients with acquired haemolytic anaemia it has become apparent that the active synthesis of various classes of humoral antibodies towards red cells is specifically directed against the Rhesus genome (Vos *et al.*, 1971). By contrast this is seldom found in lymphoid tissue (Gurner and Coombs, 1958; Lawler and Shatwell, 1962). These observations are to some extent analogous to the concept proposed for the activity of "non specific" cytotoxic autoantibodies observed among cervical cancer patients becuause only selectivity would allow the antibody-producing cell clones to survive the effects of their own antibodies.

Figure 1 outlines the basic concepts discussed in this study.

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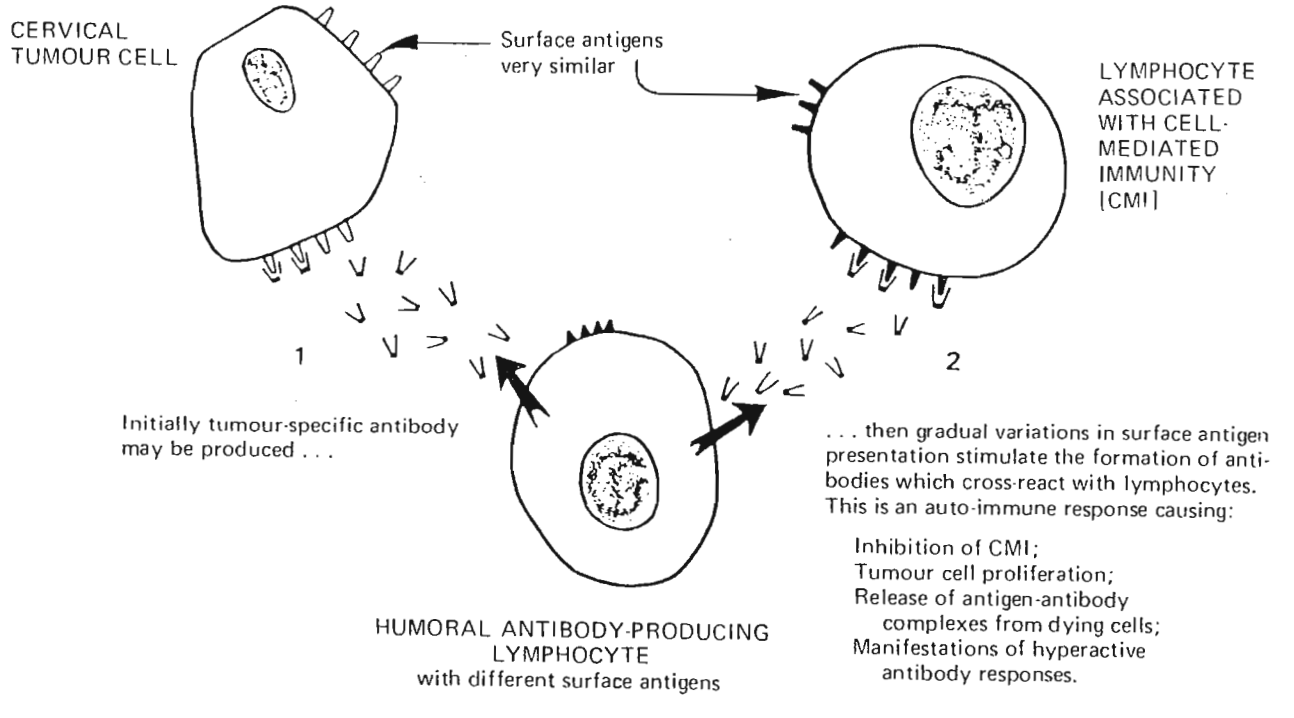

FIG. 1

Diagram of immunological events suggested by this study of patients with carcinoma of the cervix.

References

Abruzzo, J. L., and Christian, C. L. (1961): *Journal of Experimental Medicine*, **114**, 791.

Alexander, P. (1972): *Nature*, **235**, 137.

Alexander, P., and Fairly, G. H. (1967): *British Medical Bulletin*, **23**, 86.

Batchelor, J. R. (1968): *Cancer Research*, **28**, 1410.

Boyse, E. A., Old, L. J., Stockert, E., and Shigeno, N. (1968): *Cancer Research*, **28**, 1280.

Gold, P., and Freedman, S. O. (1965): *Journal of Experimental Medicine*, **121**, 439.

Gold, P., Gold, M., and Freedman, S. O. (1968): *Cancer Research*, **28**, 1331.

Gold, E. R., and Fudenberg, H. H. (1967): *Journal of Immunology*, **99**, 859.

Gurner, B. W., and Coombs, R. R. A. (1958): *Vox Sanguinis*, **3**, 13.

Harboe, M., Rau, B., and Aho, K. (1965): *Journal of Experimental Medicine*, **121**, 503.

Hellström, I., and Hellström, K. E. (1970): *International Journal of Cancer*, **5**, 195.

Hellström, I., Hellström, K. E., Sjögren, H. O., and Warner, G. A. (1971): *International Journal of Cancer*, **7**, 1.

Jehn, V. W., Nathanson, L., Schwartz, R. S., and Skinner, M. (1970): *New England Journal of Medicine*, **283**, 329.

Kano, K., and Milgrom, F. (1968): *Transplantation*, **6**, 111.

Keast, D. (1970): *Lancet*, **2**, 710.

Kreisler, M., Naito, S., and Terasaki, P. I. (1971): *Transplantation Proceedings*, **3**, 112.

Lawler, S. D., and Shatwell, H. S. (1962): *Vox Sanguinis*, **7**, 488.

Möller, G. (1963): *Journal of the National Cancer Institute*, **30**, 1177.

Prehn, R. T. (1968): *Cancer Research*, **28**, 1326.

Vos, G. H., and Kelsall, G. A. (1956): *British Journal of Haematology*, **2**, 342.

Vos, G. H., Petz, L. D., and Fudenberg, H. H. (1971): *Journal of Immunology*, **106**, 1172.

Waters, H. O., Smith, G. S., Fishkin, B., Tanaka, K. R., and Walford, R. L. (1971): *Transplantation Proceedings*, **3**, 145.

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Changeable Lymphocytotoxic Antibody Activity in Patients with Cervical Carcinoma[1]

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Abstract. In patients with cervical carcinoma examined over an extended period of time we observed lymphocytotoxic antibody activity more often in patients with terminal invasive carcinoma than in patients with preinvasive carcinoma. Antibody activity was very variable and it is postulated that this may reflect *in vivo* consumption of such antibodies as a consequence of qualitative or quantitative variations in cancerous tissue mass. In almost all instances we were unable to establish the specificity of the lymphocytotoxic antibodies with respect to known histocompatibility antigens. This suggests that their activity may be directed against a nucleus of HL-A determinants present in all human cell lines.

Introduction

In a previous study we found that extracts from cervical cancer tissues sometimes contain antibodies which sensitize normal lymphocytes by the cytotoxicity test [11]. It was suggested that this reaction represented a host's humoral antibody response to a structural modification of the tumour cell membrane. On the basis of population studies patients with cervical tumours also possessed a significantly higher incidence of so-called 'non-specific' lymphocytotoxic antibodies than a comparable control series of women of the same race ($p < 0.001$). The term 'non-specific' implies only that the structure or the origin of the corresponding antigens is not yet known. A more accurate description of these antibodies may be 'non-HL-A'. The demonstration that these cytotoxic antibodies can lyse lymphocytes of almost all normal subjects who are not suffering from the same disease also indicates that there is at this stage only an indirect association between the frequent occurrence of 'non-HL-A' antibodies and cervical tumours.

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By performing lymphocytotoxic antibody tests on stored samples of serum from the same patients collected over several weeks we were able to obtain a profile of the reaction pattern of these antibodies among patients with both invasive and preinvasive cervical tumours.

Materials and Methods

Subjects. Samples of blood were collected at weekly or fortnightly intervals from multiparous Southern African Negro women with histologically confirmed carcinoma of the cervix. Of the 14 patients studied 9 had developed invasive carcinomas with distant metastases of the bowel, bladder, vagina, vulva and kidneys and 5 were recognized to possess a preinvasive variety of carcinoma. Patients who required blood transfusions or immediate anti-tumour therapy during these follow-up studies were not included in the final analysis. Both groups of women had delivered a comparable number of livebirths, e.g. preinvasive carcinoma patients 4.7 livebirths/mother as opposed to 4.2 livebirths/mother among the invasive carcinoma patients. No reliable information could be obtained with respect to the number of abortions experienced by the two groups of women. Routine tests for treponemal infections confirmed the absence of syphilitic conditions in all the women examined.

Lymphocytotoxicity test. The presence of cytotoxic antibodies to peripheral lymphocytes was determined by testing the patient's serum against a selected panel of 23 cell donors using the modified two-stage microcytotoxicity test of BRAND *et al.* [4]. Over 40% kill of the viable lymphocytes of each donor was accepted as a positive result. The specificity of the lymphocytotoxic antibody was resolved by testing the serum against lymphocytes having many different HL-A genotypes. Cytotoxic antibodies which could not be identified as 'specific' when tested against a large panel of known HL-A cell types were classified as 'non-HL-A'.

Absorption method. To absorb serum containing lymphocytotoxic antibodies, leucocytes and platelets were obtained from 50 ml of ACD blood. The red cells were removed by dextran sedimentation and the plasma layer was centrifuged at 2,000 *g* for 20 min. The cells were washed four times in isotonic sodium chloride solution buffered to pH 7.2–7.3 with Sorensen buffer. 1 ml antiserum was added to the packed cells and absorption was carried out for 60 min at 37 °C with regular agitation of the test tube to prevent settling of the cells.

Results

To establish the serological characteristics of the lymphocytotoxic antibodies found among patients with carcinoma of the cervix uteri, follow-up studies were performed on the same patients to elucidate the complexity of the antigen involved in the formation of the so-called 'non-HL-A' lymphocytotoxic antibodies. Table I details the results of this retrospective study

Table I. Lymphocytotoxic antibody test: a follow-up study on patients with localized and disseminating carcinoma of the cervix

Patient	Age	Lymphocytotoxic antibody test (%) on day										Type of cervical carcinoma
		1	6	12	18	24	30	36	42	48	54	
M. P.	48	0[1]	0	0	0		0		13	0	0	localized
K. P.	40	0	0	0	0		0	0		0	0	localized
L. S.	38	0		0	0	10	0	0	0	15	0	localized
B. T.	36	20	0	0		0		0	0	0	0	localized
J. N.	41	0	0		0	0	0	0	0		0	localized
N. N.	70	0	0		13	13		61	87	100	100[2]	disseminating
M. D.	49	0	0	17	10	100	100	100	100			disseminating
S. M.	56	96		74		65	40	22	9	9		disseminating
M. B.	58	65		48		26		9		0		disseminating
N. R.	48	0	0		39		61		94		100	disseminating
M. L.	52	87		13		4		0		0		disseminating
H. H.	42	100	100		100		95		100	100	100	disseminating
T. M.	46	80		74		60		53		38	24	disseminating
R. B.	50	0	0		0		30		60	100	100	disseminating

[1] Absence of cytotoxic antibodies.

[2] Ability of serum to react with lymphocytes of all 23 panel cells represents 100% positive reaction (see table II, patient N. N., as an example).

Table II. Lymphocytotoxic antibody activity in patient N. N.

Follow-up samples	Known lymphocyte panel																							Percent positive reactions[2]	Specificity
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
24–9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	
30–9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	
8–10	–	–	–	8[1]	–	–	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–	8	–	13	anti-HL-A12
15–10	–	–	–	8	–	–	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–	6	–	13	anti-HL-A12
26–10	8	8	6	6	6	6	8	8	8	6	6	–	–	6	–	–	–	–	–	–	6	6	8	61	non-HL-A
30–10	8	8	8	8	8	8	8	8	8	8	8	8	–	8	8	6	6	6	–	–	6	6	8	87	non-HL-A
8–11	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	6	8	8	8	8	100	non-HL-A
14–11	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100	non-HL-A

[1] 8 denotes 90–100% kill of lymphocytes; 6 denotes 40–75% kill of lymphocytes.

[2] Ability of serum to react with lymphocytes of all 23 panel cells represents 100% positive reaction.

Table III. Lymphocytotoxic antibody follow-up study on patient S. M.

Follow-up samples	Known lymphocyte panel																							Percent positive reactions[2]	Specificity
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
20–9	8[1]	8	6	8	8	8	8	8	8	6	8	8	8	8	8	8	8	8	6	–	8	8	8	96	non-HL-A
2–10	–	8	–	8	8	8	8	8	8	8	8	–	–	8	8	8	8	8	8	–	–	8	8	74	non-HL-A
14–10	–	8	–	8	8	8	8	8	6	8	8	–	–	8	8	–	–	8	8	–	–	8	8	65	non-HL-A
21–10	–	8	–	8	6	8	–	–	–	8	8	–	–	–	–	–	–	6	8	–	–	8	–	40	non-HL-A
27–10	–	6	–	8	–	8	–	–	–	–	6	–	–	–	–	–	–	–	–	–	–	8	–	22	non-HL-A
3–11	–	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	–	9	anti-W-5
10–11	–	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	–	9	anti-W-5

[1] 8 denotes 90–100% kill of lymphocytes; 6 denotes 40–75% kill of lymphocytes.
[2] Ability of serum to react with lymphocytes of all 23 panel cells represents 100% positive reaction.

for 5 patients with localized tumours and 9 patients with disseminating tumours. The findings indicate that lymphocytotoxic antibodies were more frequently found in patients with disseminating cervical carcinoma and that such antibodies could either show a progressive increase or decline in activity as the disease progressed. The frequent absence of measurable cytotoxic antibodies in the serum of patients with localized tumours appeared as a distinguishing feature of antibody activity between the two groups.

The formation of a specific variety of HL-A antibody before the rapid development of a non-HL-A type of antibody was observed on one occasion (table II; patient N. N.). This patient initially lacked lymphocytotoxic antibody activity, then on two occasions cytotoxic antibodies for lymphocytes possessing HL-A12 determinants were detected. Subsequently the lymphocytotoxic reactions lacked specificity. Absorption experiments using cell lines lacking HL-A12 determinants failed to separate the non-HL-A cytotoxic antibody from the specific antibody. This suggests that continued *in vivo* immunization, perhaps by the growing neoplasm, has altered the initially specific nature of the antibody.

A different pattern of cytotoxic antibody behaviour was observed for patient S. M. (table III). Strong cytotoxic antibody of the so-called 'non-HL-A' variety was detected on first examination. During follow-up studies the intensity and characteristics of the cytotoxic antibody reactions gradually changed until the presence of a 'specific' variety of antibody (anti-W-5) was clearly evident. It seems improbable that this change in antibody activity was due to her sudden inability to make antibodies. A more likely explanation would be the increased availability or accessibility of the corresponding antigen through advancing tumour growth or increased blood circulation through the tumour mass. Either way one would have to assume that this loss of 'non-HL-A' antibody activity reflects *in vivo* consumption of the antibodies and not reduced synthesis. The gradual loss of 'non-HL-A' antibody activity observed in this patient also happened to take place without affecting the continuous appearence of the 'specific' variety of HL-A antibody. This again suggests that some form of *in vivo* consumption of 'non-HL-A' antibody activity is involved.

Discussion

A significant feature of the investigation was the almost complete absence of measurable lymphocytotoxic antibody activity in patients with localized cervical tumour and the remarkably variable antibody activity found in

patients with terminal disseminating cervical carcinomas. If this changeable cytotoxic antibody activity is due to *in vivo* consumption of the antibody rather than sudden variations in synthesis, then a combination of progressive tumour growth and changes in availability or accessibility of antigen sites may be the major cause of the transient appearance of the antibody. The variable pattern of cytotoxic antibody behaviour also recalls the changes of antibody reactivity commonly found among patients with autoimmune diseases [5, 8, 10]. In our previous study [11] we showed that the 'non-HL-A' type of antibody can be recovered from the patient's own cervical cancer tissue. This indicates that the activity is directed against cancerous tissues as well as normal lymphocytes. Whether the 'non-HL-A' antibodies do have autoimmune activity *in vivo* or merely act as anti-tumour membrane antibodies will be the subject of further investigation.

We suspect that the development of lymphocytotoxic antibodies among patients with cervical carcinoma occurs at a very late stage of the disease and that the initial defect is associated with a fundamental change in cervical cell growth. In this respect strong evidence has recently been presented to implicate herpes virus type 2 (HSV-2) with carcinoma of the cervix [1, 3, 9]. It has also been established that cervical tumour cells possess DNA sequences which are in part related to the HSV-2 genome [2, 6]. It was suggested that if the type 2 herpes virus has oncogenic potential for human cervical tissue that some of its function may be associated with the transformation of cells from normal to neoplastic cell lines [7]. The immunological reaction of the host against its own tumour may in this situation be very similar to a reaction of the host against allogeneic tissue graft. However, these studies suggest that intensification of humoral antibody activity may protect rather than prevent tumour cell proliferation. It is conceivable that progressive tumour growth may be facilitated by the presence of cytotoxic antibodies which are capable of blocking the effect of Tcell activity. On the other hand, no attempts have so far been made to determine whether herpes virus type 2 infection can cause a primary defect in Tcell function. Until such investigations are described no definite conclusion with respect to the 'blocking antibody' hypothesis can be reached.

References

1 Aurelian, L.; Royston, I., and Davis, H. J.: Antibody to genital herpes simplex virus: association with cervical atyphia and carcinoma *in situ*. J. nat. Cancer Inst. *45:* 455 (1970).

2 AURELIAN, L.; STRANBERG, J. D.; MELENDEZ, L. V., and JOHNSON, L. A.: Herpesvirus type 2 isolated from cervical tumour cells grown in tissue culture. Science *174:* 704 (1971).

3 AURELIAN, L.; SCHUMANN, B.; MARCUS, R. L., and DAVIS, H. J.: Antibody to HSV-2 induced tumor specific antigens in serum from patients with cervical carcinoma. Science *181:* 161 (1973).

4 BRAND, D. L.; RAY, J. G.; HARE, D. B.; KAYHOE, D. E., and MCCLELLAND, J. D.: Preliminary trials towards standardization of leucocyte typing; in TERASAKI Histocompatibility testing, p. 357 (Munksgaard, Copenhagen 1970).

5 DACIE, J. V.: The haemolytic anaemias, congenital and acquired. II. The auto-immune haemolytic anaemias; 2nd ed., vol. 2 (Grune & Stratton, New York 1962).

6 FRENKEL, N.; ROIZMAN, B.; CASSAI, E., and NAHMIAS, A.: A DNA fragment of herpes simplex 2 and its transcription in human cervical cancer tissue. Proc. nat. Acad. Sci., Wash. *69:* 3784 (1972).

7 GREEN, M.: Oncogenic viruses. Annu. Rev. Biochem. *39:* 701 (1970).

8 MILGROM, F. and WITEBSKY, E.: Autoantibodies and autoimmune diseases. J. amer. Med. Ass. *181:* 706 (1962).

9 NAHMIAS, A. J.; JOSEY, W. E.; NAIB, Z. M.; LUCE, C. F., and GUEST, B. A.: Antibodies to herpesvirus hominis types 1 and 2 in humans. Amer. J. Epidem. *91:* 547 (1970).

10 VOS, G. H.; PETZ, L. D., and FUDENBERG, H. H.: Specificity and immunoglobulin characteristics of autoantibodies in acquired hemolytic anemia. J. Immunol. *106:* 1172 (1971).

11 VOS, G. H.; HAMMOND, M. G.; VOS, D.; GROBBELAAR, B. G.: AUSLANDER, H. P., and MARESCOTTI, G.: An evaluation of humoral antibody responses in patients with carcinoma of the cervix. J. Obstet. Gynaec. brit. Cwlth. *79:* 1040 (1972).

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HLA and Cancer in South African Negroes

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Five hundred patients with cancer were tested for 32 HLA antigens and the antigen frequencies compared with those of 500 control subjects matched for race, sex and age. Although the overall frequencies showed no significant differences, detailed analysis with regard to site of cancer, age and the number of antigens detected at each locus revealed significant differences. Phenotype tables and haplotype frequencies have been included.

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There are significant differences in the frequencies of HLA antigens in various races. The Negroes of South Africa have higher frequencies of A28, A29, Aw30, Bw42, Bw 17 and TT than Caucasians and lower frequencies of A1, A11, B5, B27 and Bw40 (Hammond et al. 1975). The overall cancer incidence in South African Negroes is similar to that in Caucasians but there are differences in the incidence rates for different sites of cancer. A survey of cancer in Durban Negroes (Schonland & Bradshaw 1968) showed that cancer of the esophagus is the commonest male malignancy and it has the highest reported incidence in the world. Cancer of the liver and lung are the next most frequent in males. In females, cancer of the cervix has the highest incidence and is nearly four times more frequent than in Caucasians.

Materials and Methods

Blood samples were taken from 500 confirmed cancer cases over a period of 18 months. Confirmation was obtained by cytology, histology or hematology. Blood samples were also taken from 500 control subjects, not suffering from malignancies, matched for race, age and sex and 180 antisera were used to define 13 antigens at the A locus, 17 at the B locus and two at the C locus. Their specificity was confirmed during the Sixth Histocompatability Workshop by testing them in parallel with the workshop sera against the cells of 100 unrelated Negroes of the Zulu tribe (Hammond et al. 1975). The N.I.H. standard microcytotoxicity test was used throughout.

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Results

Table 1 gives the antigen frequencies in controls, cancer cases and in subgroups according to site of cancer. Table 2 shows the percentage frequency of individuals with only one detectable antigen at the first and second locus in relation to age and site of cancer. Tables 3–6 give the phenotype distributions at each locus in the cancer patients and in the control group. Gene frequencies were estimated from Table 1 using the formula $G = 1 - \sqrt{1 - f}$ where f is the antigen frequency, and the χ^2 between observed and expected fre-

Table 1
Percentage frequency of HLA antigens

	Control 500	Cancer 500	Cervix 143	Oesophagus 101	Breast 61	Lung and larynx 41	Liver 35
HLA-A1	5.0	8.4	9.8	6.9	9.8	2.4	17.1**
HLA-A2	20.6	22.0	16.1	28.7	19.7	24.4	17.1
HLA-A28	21.2	20.4	23.1	17.8	27.9	22.0	14.3
HLA-A3	14.2	13.8	18.2	12.9	18.0	4.9	5.7
HLA-A11	0.2	0.2	0	0	0	0	0
HLA-Aw23	17.2	18.4	23.1	17.8	19.7	9.8	17.1
HLA-Aw24	4.8	7.0	5.6	8.9	4.9	7.3	14.3
HLA-Aw25	15.6	14.2	9.1	18.8	13.1	29.3	11.4
HLA-Aw26	9.0	9.4	8.4	11.9	6.6	4.9	8.6
HLA-A29	17.0	18.0	18.9	16.8	18.0	14.6	20.0
HLA-Aw30	39.4	33.6	37.1	36.6	26.2	24.4	31.4
HLA-Aw31	11.4	12.8	9.8	8.9	16.4	19.5	17.1
HLA-Aw32	1.8	2.4	0.7	1.0	3.3	2.4	2.9
1 antigen	22.6	19.0	20.3	12.9*	16.4	29.3	22.9
HLA-B5	1.2	2.0	3.5	0	1.6	0	0
HLA-Bw35	6.2	5.6	4.9	4.0	4.9	4.9	11.4
HLA-B18	3.8	4.6	4.2	5.9	4.9	0	8.6
HLA-Bw15	4.2	3.2	4.2	4.0	1.6	0	2.9
HLA-Bw16	3.2	2.0	0.7	2.0	1.6	4.9	5.7
HLA-Bw21	0.6	1.4	2.8	2.0	1.6	0	0
HLA-B7	17.8	20.0	20.3	19.8	26.2	17.1	20.0
HLA-Bw22	0	0	0	0	0	0	0
HLA-Bw42	25.0	25.2	30.1	24.8	19.7	12.2	25.7
HLA-B27	0.6	0.2	0	0	0	0	0
HLA-B8	15.8	15.4	16.8	13.9	21.3	4.9	17.1
HLA-B14	5.2	5.0	7.0	4.0	3.3	7.3	2.9
HLA-B12	15.8	13.2	11.2	15.8	8.2	17.1	14.3
HLA-TT	7.2	8.0	6.3	15.8**	4.9	7.3	5.7
HLA-B13	4.8	3.4	2.1	4.0	4.9	2.4	2.9
HLA-Bw17	41.2	41.0	36.4	42.6	45.9	48.8	34.3
HLA-Bw40	1.0	1.2	0	0	0	2.4	2.9
1 antigen	42.8	40.4	44.1	36.6	37.7	51.2	34.3
HLA-C2	13.0	12.4	9.1	15.8	6.6	24.4	20.0
HLA-C3	6.0	9.2	8.4	7.9	11.5	7.3	8.6

* uncorrected $p < 0.05$

** uncorrected $p < 0.005$

Table 2

Percentage frequency of individuals with only one detectable antigen at the first and second locus in relation to age and site of cancer

	Controls		All cancers		Cervix		Oesophagus		Breast		Lung & larynx		Liver	
Age	< 40	> 60	< 40	> 60	< 40	> 60	< 40	> 60	< 40	> 60	< 40	> 60	< 40	> 60
Number	161	89	140	98	52	15	15	24	16	14	10	15	9	9
1 antigen at A locus	23	21	22	15	23	13	7	8	13	36	40	7*	33	22
1 antigen at B locus	47	43	38	37	37	40	20	29	25	43	80	33**	56	33
1 antigen at both A and B loci	13	11	9	6	8	0	0	0	6	14	40	7*	22	11

* $p < 0.05$
** $p < 0.02$

Table 3
A locus phenotypes of 500 cancer patients

HLA-A	1	2	3	w23	w24	w25	w26	11	28	w29	w30	w31	w32	Blank
1	2	3	6	3	1	3	5	0	3	6	8	1	1	
2		15	13	8	4	8	6	1	14	11	18	9	0	
3			8	6	0	3	4	0	9	5	12	2	1	
w23				12	1	8	4	0	10	7	21	10	2	
w24					1	3	2	0	2	7	9	4	1	
w25						9	0	0	8	11	11	7	0	
w26							1	0	6	7	9	1	1	
11								0	0	0	0	0	0	
28									7	12	21	8	2	
w29										6	16	2	0	
w30											27	14	2	
w31												6	1	
w32													1	
Blank														1

$\chi^2_{78} = 70.03$
$0.7 < p < 0.8$

quencies was calculated. Table 7 shows the most common haplotype frequencies for the controls and cancer patients.

Discussion

There are no significant differences in the HLA antigen frequencies when comparing all the cancer cases with the controls. When considering various sites of cancer, however, there are differences worthy of comment. HLA–A1 has a significantly higher frequency in cancer of the liver than in the controls and the antigen TT is significantly increased in cancer of the esophagus. The probabilities are both less than 0.005, but when corrected for the number of antigens tested they are no longer significant.

Table 4
A locus phenotypes of 500 control subjects

HLA-A	1	2	3	w23	w24	w25	w26	11	28	w29	w30	w31	w32	Blank
1	1	1	2	2	0	1	2	0	2	3	8	3	0	
2		10	7	9	3	3	4	1	10	16	31	6	2	
3			8	8	3	9	3	0	7	7	13	14	0	
w23				8	0	15	4	0	10	5	20	5	0	
w24					4	2	2	0	1	1	6	2	0	
w25						11	0	0	5	7	20	5	0	
w26							4	0	7	6	9	3	1	
11								0	0	0	0	0	0	
28									19	10	29	5	1	
w29										8	15	5	2	
w30											32	13	1	
w31												6	0	
w32													2	
Blank														0

$\chi^2_{78} = 74.23$
$0.5 < p < 0.6$

Table 5
B locus phenotypes of 500 cancer patients

HLA-B	5	7	8	12	13	14	17	27	w5	w10	w15	w16	w18	w21	w22	w42	TT	Blank
5	2	0	1	2	0	0	5	0	0	0	0	0	0	0	0	0	0	
7		21	8	5	2	2	27	1	3	1	2	2	1	1	0	22	2	
8			14	3	3	0	25	0	3	3	0	0	0	1	0	12	4	
12				20	2	3	19	0	1	0	1	0	0	0	0	10	0	
13					4	1	2	0	0	0	0	0	0	0	0	3	0	
14						6	4	0	0	0	0	0	1	0	0	6	2	
17							81	0	4	0	2	2	6	2	0	15	11	
27								0	0	0	0	0	0	0	0	0	0	
w5									7	0	1	1	1	0	0	4	3	
w10										2	0	0	0	0	0	0	0	
w15											1	0	1	0	0	7	1	
w16												1	0	0	0	4	0	
w18													7	0	0	5	1	
w21														2	0	0	1	
w22															0	0	0	
w42																31	7	
TT																	8	
Blank																		18

$\chi^2_{136} = 144.16$
$0.3 < p < 0.4$

Table 6
B locus phenotypes of 500 control subjects

HLA-B	5	7	8	12	13	14	17	27	w5	w10	w15	w16	w18	w21	w22	w42	TT	Blank
5	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	
7		24	4	10	3	0	14	1	6	1	2	4	2	0	0	16	2	
8			26	9	4	2	17	0	1	0	1	1	0	0	0	11	2	
12				15	2	4	25	0	2	0	3	1	2	0	0	5	0	
13					4	0	6	0	2	1	0	0	1	0	0	1	0	
14						4	9	0	1	0	0	0	3	0	0	0	3	
17							71	1	5	1	4	2	2	0	0	39	9	
27								1	0	0	0	0	0	0	0	0	0	
w5									4	0	0	0	0	1	0	7	2	
w10										1	0	0	0	0	0	1	0	
w15											7	0	1	0	0	2	1	
w16												2	1	1	0	4	0	
w18													6	0	0	0	1	
w21														1	0	0	0	
w22															0	0	0	
w42																35	3	
TT																	12	
Blank																		9

$\chi^2_{136} = 174.4$
$0.025 > p > 0.01$

The frequency of individuals with only one detectable antigen at the first or second locus ranges from 12.9% at the first locus for patients with cancer of the esophagus to 51.2% at the second locus for patients with cancer of the lung or larynx. The frequency of 12.9% is significantly different from that in the controls ($p < 0.05$) and this might mean that individuals who are heterozygous at the first locus are more susceptible to cancer of the esophagus. Moreover, this frequency is significantly different from the frequency of 29.3% in patients with cancer of the lung or larynx ($p < 0.02$). At the second locus the same trend is apparent but the differences are not significant. Gerkins et al. (1974) and Macurova et al. (1975) presented data indicating increased heterozygosity in aged persons. Table 2 shows that there are no significant differences in the frequency of individuals with or without cancer in the age groups less than 40 or greater than 60. However, the frequencies in patients with cancer of the lung or larynx show significant differences between those younger than 40 and those older than 60. At the A locus the difference is significant at the 0.05% level while at the B locus $p < 0.02$. This might mean that young people who are homozygous at the A or B loci or at both are more susceptible to cancer of the lung or larynx. Thus we may say that among Negroes with cancer, those who are homozygous at the A or B locus or at both may be more liable to cancer of the lung or larynx while those who are heterozygous may be more likely to get cancer of the esophagus. Unfortunately the numbers of individuals in these subgroups are small and the statistical inferences should be treated with great reserve.

Table 7 shows the most common haplotype frequencies for the controls and cancer patients calculated from the phenotypes by the method of Mattiuz et al. (1970). The most common haplotype is Aw30, Bw42 which might be called a negroid haplotype in the same way that A1, B8 is a Caucasoid haplotype. Several other haplotypes show significant linkage disequilibrium, notably A1, B7; A2, TT; A24, B7; Aw25, B12; A29, B12; A29, B13.

Table 7
Haplotype frequencies in cancer patients and controls (All values × 10^3)

Haplotype		Controls	Cancer
Aw 30	Bw 42	75*	67*
A 28	Bw 17	37	38
Aw 30	Bw 17	34	32
A 2	Bw 17	31	39
Aw 25	Bw 17	30	11
A 3	Bw 17	28	34*
Aw 30	B 8	25	19
Aw 25	B 12	22*	17*
A 29	B 12	19*	23*
Aw 26	Bw 17	18	19
A 1	B 7	17*	24*
A 3	B 8	14	22*
A 2	Bw TT	13*	17*
A 29	B 13	13*	13*
Aw 24	B 7	12*	22*
Aw 31	Bw 35	9	11*

* Absolute value of Δ greater than twice the S.E.

References

Gerkins, V. R., Ting, A., Menck, H. T., Casagrande, J. T., Terasaki, P. I., Pike, M. C. & Henderson, B. E. (1974) HLA heterozygosity as a genetic marker of long-term survival. *J. Nat. Cancer* 52, 6, 1909.

Hammond, M. G., Appadoo, B. & Brain, P. (1975) HLA antigens in Bantu and Indians. *Histocompatibility Testing 1975*, Munksgaard, Copenhagen.

Macurova, H., Ivanyi, P., Sajdlova, H. & Trojan, J. (1975) HLA antigens in aged persons. *Tissue Antigens* 6, 269.

Mattiuz, P. L., Ihde, D., Piazza, A., Ceppellini, R. & Bodmer, W. F. (1970) New approaches to

the population genetic and segregation analysis of the HLA system. *Histocompatibility Testing 1970* p. 193. Munksgaard, Copenhagen.

Schonland, M. & Bradshaw, E. (1968) Cancer in the Natal African and Indian 1964–66. *Int. J. Cancer* **3**, 304.

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HLA and Cancer in South African Indians

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Two-hundred-and-forty-nine Indian cancer patients were tested for 39 HLA antigens and the antigen frequencies were compared with those of 603 control subjects. Comparisons were also made between cancer patients and controls for each ethnic group and for each site of cancer. There was an increase in the frequency of the HLA antigens A11 and Bw52 in patients with malignancies. Heterozygosity at the B locus was significantly increased in patients with cancer of the breast. The *Aw24, B17* haplotype was also associated with breast cancer.

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A survey of cancer in the Negro and Indian populations of Durban (Schonland & Bradshaw 1968) showed that the overall cancer incidence in females of both races and in Negro males is as high as in most Western countries, but Indian males have a low overall cancer incidence which is not readily explained. We have previously reported our findings with regard to HLA and cancer in South African Negroes (Hammond et al. 1977a). The Indian population can be divided into four major ethnic groups and we have shown that there are differences in the frequencies of the HLA antigens in these groups. (Hammond et al. 1974).

Material and Methods

The Indian Population

The Indians of Natal are the descendents of immigrants who arrived about a century ago to work on the sugar plantations. They are composed of Dravidians from Southern India and Aryans from Northern India. In South Africa, the Dravidians can be divided into Tamil and Telegu speakers while the Aryans can more conveniently be divided by religion into Hindus from the north-east (mostly Hindi speaking) and Muslims from the north-west. Intermarriage between these four groups is rare and there has been almost no admixture with other races. (Mistry 1965).

The Indian population of greater Durban is over 430,000 and the proportion of each ethnic group is roughly as follows:

Race	*Religion*	*Language*	*Percentage*
Aryan	(a) Hindu	1. Hindi	26
		2. Gujerati	3
	(b) Muslim	1. Urdu	9
		2. Gujerati	3

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Dravidian			
	(a) Hindu	1. Tamil	38
		2. Telegu	12
Others			9

All Indians who were classified as "other" have been omitted from this survey.

Blood samples were taken from 249 confirmed cancer cases over a period of 2 years. Confirmation of carcinoma was obtained by cytology or histology and confirmation of leukemia by hematology. Controls consisted of 603 unrelated donors or staff members, 140 of whom have been typed with workshop sera and another 250 who were typed concurrrently with the cancer patients. Two-hundred-and-ten antisera were used to define 14 antigens at the A locus, 22 at the B locus and three at the C locus. Their specificity was confirmed during the Sixth and Seventh Histocompatibility Workshops. The subdivision of Bw40 in Indians into Bw40.1, Bw40.2, and of B5 into four components was reported at the Seventh Workshop (Hammond et al. 1975, 1977b). In this report, B5 cells that were not Bw51 or Bw52 were classed as Bw5 IND. The N.I.H. standard microcytotoxicity test was used throughout.

Results

The control group, 250 unrelated donors typed concurrently with the cancer patients were matched for age, sex and racial subgroup. A comparison of antigen frequencies between this control group and another group of 353 unrelated donors showed no significant differences and consequently the data were combined to provide a larger control population.

The numbers of patients and controls in each of the four racial subgroups are shown in the column headings of Table 1. The Tamil and Telegu results are combined in the Dravidians, and Hindi and Muslim are combined to form the Aryan category. The total is shown in the column "Total Indians".

The significance of the difference between the frequency of each antigen in the control group and in the cancer patients for each of the four racial groups was calculated using a computer program for 2 x 2 chi-squares. If the difference was significant the calculations were repeated using Yates' correction or, if any number in the 2 x 2 table was less than 10, Fisher's exact method was used. The same procedure was followed in examining the total population and the Dravidian and Aryan sub-groups. The frequencies that were still significantly different are marked with asterisks in Table 1.

We then looked in more detail at those antigens which showed significantly different frequencies. In Table 2 the frequencies of these antigens in patients with cancer of the breast and patients with cancer of the cervix are compared with the control group. Fisher's exact method was used to calculate probabilities.

Gene frequencies were estimated from Table 1 using the formula $g = 1 - \sqrt{1 - f}$ where f is the antigen frequency, and the haplotype frequencies were estimated from the phenotype data by the method of Mattiuz et al. (1970).

Discussion

Significant differences between the antigen frequencies in patients and controls are marked with asterisks in Table 1. However, when corrected for the number of antigens and the four race groups (39 x 4) only one shows borderline significance. The very high frequency of A11 in Tamil cancer patients has a chi-square value of 14.35 and

Table 1
HLA antigen frequencies in per cent

	TAMIL		TELEGU		HINDI		MUSLIM		DRAVIDIAN		ARYAN		TOTAL INDIANS	
	Controls 288	Cancer 118	Controls 122	Cancer 40	Controls 133	Cancer 66	Controls 60	Cancer 25	Controls 410	Cancer 158	Controls 193	Cancer 91	Controls 603	Cancer 249
A1	30.6	26.3	35.2	47.5	16.5	24.2	23.3	24.0	32.0	31.6	18.7	24.2	27.7	28.9
A2	30.6	41.5	41.0	22.5	21.1	27.3	35.0	44.0	33.7	36.7	25.4	31.9	31.0	34.9
A3	15.3	14.4	12.3	5.0	17.3	6.1	8.3	8.0	14.4	12.0	14.5	6.6	14.4	10.0
A11	25.0	44.1**	25.4	20.0	37.6	36.4	15.0	24.0	25.1	38.0*	30.6	33.0	26.9	36.1*
Aw23	0.7	0.8	2.5	0	0	1.5	0	0	1.2	0.6	0	1.1	0.8	0.8
Aw24	29.2	24.6	18.9	42.5*	30.8	27.3	33.3	28.0	26.1	29.1	31.6	27.5	27.9	28.5
A25	1.7	2.5	1.6	2.5	1.5	1.5	3.3	4.0	1.7	2.5	2.1	2.2	1.8	2.4
A26	8.0	4.2	4.9	7.5	3.8	3.0	11.7	4.0	7.1	5.1	6.2	3.3	6.8	4.4
A28	13.9	8.5	9.0	12.5	20.3	15.2	15.0	16.0	12.4	9.5	18.7	15.4	14.4	11.6
A29	0.7	1.7	0.8	0	0.8	1.5	0	0	0.7	1.3	0.5	1.1	0.7	1.2
Aw30	3.1	4.2	4.9	7.5	3.0	7.6	6.7	12.0	3.7	5.1	4.1	8.8	3.8	6.4
Aw31	2.4	2.5	2.5	0	6.0	4.5	5.0	4.0	2.4	1.9	5.7	4.4	3.5	2.8
Aw32	2.1	0.8	3.3	2.5	3.0	3.0	3.3	4.0	2.4	1.3	3.1	3.3	2.7	2.0
Aw33	7.3	3.4	5.7	0	6.0	10.6	5.0	8.0	6.8	2.5	5.7	9.9	6.5	5.2
1 antigen	29.5	20.3	32.0	30.0	32.3	28.8	35.0	20.0	30.2	22.8	33.2	26.4	31.2	24.1
B5	37.8	31.3	36.0	52.5	37.6	27.2	33.3	36.0	37.3	36.7	36.3	29.7	37.0	34.2
B7	15.6	11.9	13.9	5.0	6.8	10.6	10.0	8.0	15.1	10.1	7.8	9.9	12.8	10.0
B8	5.9	5.1	6.6	10.0	3.8	1.5	8.3	0	6.1	6.3	5.2	1.1	5.8	4.4
B13	6.9	5.1	9.0	5.0	6.0	4.5	3.3	4.0	7.6	5.1	5.2	4.4	6.8	4.8
B14	0	0	0	0	0	1.5	3.3	0	0	0	1.0	1.1	0.3	0.4
B15	9.7	13.6	12.3	10.0	13.5	13.6	11.7	24.0	10.5	12.7	13.0	16.5	11.3	14.1
B16	2.1	7.6	2.5	2.5	2.3	1.5	1.7	0	2.2	6.3	2.1	1.1	2.2	4.4
B17	22.9	30.5	23.0	32.5	16.5	21.2	16.7	16.0	22.9	31.0	16.6	19.8	20.9	26.9
B18	1.4	2.5	2.5	0	5.3	6.1	5.0	4.0	1.7	1.9	5.2	5.5	2.8	3.2
Bw21	1.7	0	1.6	0	2.3	4.5	1.7	4.0	1.7	0	2.1	4.4	1.8	1.6
Bw22	2.8	6.8	2.5	10.0	1.5	3.0	1.7	12.0	2.7	7.6	1.6	5.5	2.3	6.8*
B27	1.7	0.8	0	0	6.8	0	3.3	4.0	1.2	0.6	5.7	1.1	2.5	0.8
Bw35	18.8	13.6	25.4	7.5	18.8	18.2	21.7	32.0	20.7	12.0	19.7	22.0	20.4	15.7
B37	4.9	5.9	4.1	2.5	2.3	7.6	0	0	4.6	5.1	1.6	5.5	3.7	5.2

Table 1 (Continued)

	TAMIL		TELEGU		HINDI		MUSLIM		DRAVIDIAN		ARYAN		TOTAL INDIANS	
	Controls 288	Cancer 118	Controls 122	Cancer 40	Controls 133	Cancer 66	Controls 60	Cancer 25	Controls 410	Cancer 158	Controls 193	Cancer 91	Controls 603	Cancer 249
Bw40.1	17.0	14.4	24.6	10.0	12.8	21.2	16.7	12.0	19.3	13.3	14.0	18.7	17.6	15.3
Bw40.2	13.5	18.6	9.0	20.0	15.8	21.2	0	4.0	12.2	19.0	10.9	16.5	11.8	18.1
Bw42	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bw44	9.4	5.9	4.1	15.0	21.1	19.7	18.3	20.0	7.8	8.2	20.2	19.8	11.8	12.4
Bw45	0	0	0	0	0	0	0	4.0	0	0	0	1.1	0	0.4
Bw51	20.8	16.1	26.2	25.0	18.8	4.5*	13.3	8.0	22.5	18.4	17.1	5.5*	20.8	13.7
Bw52	10.5	12.7	5.7	25.0*	15.0	10.6	15.0	20.0	9.0	15.8	15.0	13.2	11.0	14.9
Bw5 IND	4.5	0.8	1.6	0	1.5	7.6	5.0	8.0	3.7	0.6	2.6	7.7	3.3	3.2
Bw53	2.1	1.7	2.5	2.5	2.3	4.5	0	0	2.2	1.9	1.6	3.3	2.0	2.4
1 antigen	27.8	25.4	23.0	17.5	27.1	16.7	43.3	16.0	26.3	23.4	32.1	16.5*	28.4	20.9
Cw2	1.4	4.2	0.8	0	3.8	1.5	0	0	1.2	3.2	2.6	1.1	1.7	2.4
Cw3	6.6	14.4	9.8	7.5	10.5	9.1	6.7	8.0	7.6	12.7	9.3	8.8	8.1	11.2
Cw4	8.0	2.5	9.0	0	4.5	1.5	0	8.0	8.3	1.9*	3.1	3.3	6.6	2.4

* uncorrected $P < 0.01$.

** uncorrected $P < 0.0005$.

Table 2
HLA antigen frequencies in per cent

	TAMIL			TELEGU		
	Control	Breast	Cervix	Control	Breast	Cervix
	288	53	25	122	14	11
A11	25.0	43.4*	52.0*	25.4	21.4	27.3
Aw24	29.2	32.1	20.0	18.9	57.1*	18.2
Bw22	2.8	7.5	12.0	2.5	7.1	18.2
Bw51	20.8	9.4	28.0	26.2	14.3	45.5
Bw52	10.5	13.2	8.0	5.7	42.9**	18.2
1 antigen	27.8	13.2	32.0	23.0	0	27.3

	HINDI			MUSLIM			DRAVIDIAN		
	Control	Breast	Cervix	Control	Breast	Cervix	Control	Breast	Cervix
	133	30	16	60	11	3	410	67	36
A11	37.6	40.0	37.5	15.0	27.3	33.3	25.1	38.8	44.4
Aw24	30.8	23.3	37.5	33.3	54.5	0	26.1	37.3	19.4
Bw22	1.5	6.7	0	1.7	0	0	2.7	7.5	13.9*
Bw51	18.8	6.7	0	13.3	18.2	0	22.5	10.4	33.3
Bw52	15.0	6.7	18.8	15.0	18.2	0	9.0	19.4	11.1
1 antigen	27.1	10.0	18.8	43.3	18.2	33.3	26.3	10.4*	30.6

	ARYAN			INDIAN		
	Control	Breast	Cervix	Control	Breast	Cervix
	193	41	19	603	108	55
A11	30.6	36.6	36.8	26.9	38.0	41.8
Aw24	31.6	31.7	31.6	27.9	35.2	23.6
Bw22	1.6	4.9	0	2.3	6.5	9.1*
Bw51	17.1	9.8	0	20.8	10.2	21.8
Bw52	15.0	9.8	15.8	11.0	15.7	12.7
1 antigen	32.1	12.2	21.1	28.4	11.1***	29.3

* Uncorrected $P < 0.01$. ** Uncorrected P (exact) = 0.00046.
*** Uncorrected P (exact) = 0.00005.

a P value after correction of 0.078. Table 2 shows that the frequency of A11 is increased in both cancer of the breast and cancer of the cervix in Tamils. The increase of Aw24 in Telegu patients with breast cancer is not significant after correction.

The inverse relationship between Bw51 and Bw52 in cancer of the breast and cancer of the cervix is most noticeable in the Telegu although the same trend is seen in the Tamil. Bw51 has an increased frequency in cancer of the cervix, while Bw52 is increased in cancer of the breast. The increase is greatest in Telegus with breast cancer. This relationship is not found in the Aryan Indians. Bw22 shows an increase

Table 3

Haplotype frequencies with significant linkage disequilibrium. (All values × 10^3)

	TAMIL		TELEGU		HINDI		MUSLIM		DRAVIDIAN		ARYAN		INDIAN	
	Control	Cancer	Control	Cancer	Control	Cancer	Control	Cancer	Control	Cancer	Control	Cancer	Control	Cancer
	288	118	122	40	133	66	60	25	410	158	193	91	603	249
1, 17	62**	67*	96**	106	27	40	28	36	72**	76*	27	39	57**	62*
1, 37	20*	0	16	0	7	12	0	0	19*	0	5	9	15**	2
2, 40.1	37*	41	41	0	5	28	45	35	39*	28	17	29	32*	28
24, 7	30	16	21	9	0	27	9	17	28*	14	9	25	18	18
24, 17	0	17	0	12	0	0	0	10	0*	16	0	0	0*	8
24, 52	20	0	6	8	28	37	25	57	16	0	27	42	20*	13
33, 44	13*	8	4	0	26*	29	7	18	10*	6	20*	26	13**	14*

* Absolute value of $\Delta > 2$ S.E.

** Absolute value of $\Delta > 3$ S.E.

in all cancer patients and this increase is seen in both types of cancer, except in Muslims.

The frequency of individuals with only one detectable antigen at the B locus is reduced in cancer of the breast in all four ethnic groups and when considering the Indians as a whole the exact $P = 0.00005$. This should be multiplied by eight (four ethnic groups x 2 loci) to give a $P = 0.0004$, but even if we were to consider the individuals with only one detectable antigen as possessing some rare, as yet serologically undectable antigen, and apply a correction of $39 \times 4 \times 2$ we arrive at a $P = 0.016$. However, this low frequency means that more of these patients are heterozygous at the B locus in contrast to patients with cancer of the cervix who have frequencies similar to the control population.

Gerkins et al. (1974) investigated the number of antigens present at each locus in old and young people with and without cancer. The results showed a trend towards homozygosity in cancer patients but the results included all types of cancer. In an earlier study of HLA and cancer in South African Negroes (Hammond et al. 1977) we did not find any significant differences in the number of antigens detected at the A or B loci in cancer of the breast but heterozygosity at the A locus was increased in cancer of the esophagus.

Table 3 shows the haplotype frequencies with significant linkage disequilibrium. The *A1, B17* haplotype is the most common and typical of Asian Indians, with the highest frequency occurring in the Telegu speaking Dravidians from Southern India. The contrast in frequency of the *A1, B37* haplotype and the *Aw24, B17* haplotype is noteworthy. Cancer patients do not show the significant linkage disequilibrium that is evident in the control population. The delta value is positive for the *A1, B37* haplotype but it is negative for the *A24, B17* haplotype. The presence of the *Aw24, B17* haplotype may indicate susceptibility to cancer of the breast because the frequency of this haplotype was .024 in all Indians with cancer of the breast and .044 in Dravidians with cancer of the breast, whereas there were no patients with cancer of the cervix who had the antigens Aw24 and B17 together.

References

Gerkins, V. R., Ting, A., Menck, H. T., Casagrande, J. T., Terasaki, P. I., Pike, M. C. & Henderson, B. E. (1974) HLA Heterozygosity as a genetic marker of long-term survival. *J. Nat. Cancer Ins. 52;* 6, 1909.

Hammond, M. G., Appadoo, B. & Brain, P. (1974) Subdivision of HLA5 and comparative studies of the HLA polymorphism in South African Indians. *Tissue Antigens* 4, 42.

Hammond, M. G., Appadoo, B. & Brain, P. (1975) HLA antigens in Bantu and Indians. *Histocompatibility Testing 1975.* Munksgaard, Copenhagen.

Hammond, M. G., Appadoo, B. & Brain, P. (1977a) HLA and cancer in South African Negroes. *Tissue Antigens* 9, 1.

Hammond, M. G., Appadoo, B. & Brain, P. (1977b) HLA antigens in South African Negroes and Indians. *Histocompatibility Testing 1977,* Munksgaard, Copenhagen.

Mattiuz, P. L., Ihde, D., Piazza, A., Ceppellini, R. & Bodmer, W. F. (1970) New approaches to the population. Genetic and segregation analysis of the HLA system. *Histocompatibility Testing 1970,* Munksgaard, Copenhagen.

Mistry, S. D. (1965) Ethnic groups of Indians in South Africa. *S. Afr. Med. J.* 39, 691.

Schonland, M. & Bradshaw, E. (1968) Cancer in the Natal African and Indian. *Int. J. Cancer* 3, 304.

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Tissue Antigens (1980), 16, 254–255 SHORT COMMUNICATION

HLA and Cancer of the Esophagus in South African Negroes

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In a previous investigation (Hammond et al. 1977) we found an increased frequency of HLA–Bw45 in Negroes with cancer of the esophagus (uncorrected $P < 0.005$). This was not significant after correcting for the number of antigens tested but nevertheless we felt that a follow-up study was necessary because of the very high incidence of this cancer in the Negro population and the relatively high frequency of HLA–Bw45 in Negroes compared to other races.

A further 153 patients with confirmed cancer of the esophagus were HLA typed using almost the same set of 180 sera as in the original investigation. Table 1 shows the antigen frequencies of the two groups of patients, the total number of patients and the controls.

The frequency of HLA–Bw45 in the second group of patients was not significantly different from the frequency in controls, which means that our original observation was probably due to chance. In the first group there was also a significant decrease in the frequency of patients with only one detectable antigen at the A locus but this was not confirmed in the second group.

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References

Hammond, M. G., Appadoo, B. & Brain, P. (1977) HLA and cancer in South African Negroes. *Tissue Antigens* 9, 1–7.

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Table 1
Percentage frequency of HLA antigens in Negroes with cancer of the esophagus

HLA	Group I[a] 101	Group II 153	Total 254	Controls 756
A1	6.9	7.2	7.1	5.8
A2	28.7	17.6	22.1	20.1
A3	12.9	11.1	11.8	13.5
A11	0	0	0	0.1
Aw23	17.8	17.6	17.7	19.2
Aw24	8.9	6.5	7.5	3.3
A 25	18.8	15.7	16.9	13.9
A 26	11.9	12.4	12.2	7.5
A 28	17.8	18.3	18.1	20.0
A 29	16.8	16.3	16.5	16.7
Aw30	36.6	37.3	37.0	39.6
Aw31	8.9	10.5	9.8	12.6
Aw32	1.0	4.6	3.2	1.6
1 Antigen	12.9	24.8	20.1	26.2
B 5	0	1.3	0.8	2.7
B 7	19.8	24.8	22.8	16.0
B 8	13.9	19.6	17.3	13.9
B 13	4.0	2.0	2.8	4.8
B 14	4.0	4.6	4.3	6.1
B 15	4.0	0.7	2.0	5.8
B 16	2.0	2.6	2.4	2.4
B 17	42.6	32.7	36.6	38.1
B 18	5.9	8.5	7.5	3.8
Bw21	2.0	2.6	2.4	0.5
Bw22	0	0	0	0
B 27	0	0	0	0.3
Bw35	4.0	5.2	4.7	7.3
B 40	0	1.3	0.8	1.6
Bw42	24.8	25.5	25.2	27.7
Bw44	15.8	17.6	16.9	16.0
Bw45	15.8	7.2	10.6	6.4
1 Antigen	36.6	47.1	42.9	46.2

[a] Hammond et al. (1977).

Cancer of the Esophagus

Volume I

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HLA AND CANCER OF THE ESOPHAGUS

M. G. Hammond

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I. THE HLA SYSTEM

The major histocompatibility system of man is called HLA. It refers to a genetic region on the short arm of chromosome 6[1] that plays a dominant role in the survival of grafts. The letters HL stand for Human Leukocyte and the A originally stood for the A locus, but in 1975 HLA was made the official designation for the whole region.[2]

At first only two loci were recognized, A and B, and these two have been studied the most. Nearly all the work on transplantation, disease associations, and population studies has been done on antigens of the A and B loci. The C locus was first proposed in 1970,[3] but because of difficulties in defining the antigens, only eight have so far been recognized.[4] The D locus was included in 1975[2] and more recently, the DR (D-related) locus has been defined using B lymphocytes.[5]

Each well-defined antigen is identified with a letter for the locus and a number. Historically, the numbers used for the A and B loci do not overlap but the C, D, and DR antigens are numbered starting with one. Antigens identified during the International Histocompatibility Workshops are prefixed with a W, and when complete agreement is reached, the W is dropped. Table 1 lists the currently identifiable antigens at each locus.

A haplotype is the combination of closely linked HLA genes on the same chromosome transmitted from parent to child. Two haplotypes, one from each parent, embody all the HLA genes in any individual. Thus, there is a maximum of two A and two B loci antigens present. In those individuals where only one antigen is identified at a locus, there is a strong probability of homozygosity.[6]

Not all the genes have been defined but the combined frequency of unidentified genes is only about 2% at the A locus and about 4% at the B locus in European populations, while in other populations the frequency of unidentified genes is larger.[7]

The frequencies of individual antigens vary widely in different population groups and Table 2 compares the antigen frequencies in Caucasians, Negroes, and Asian Indians. It is noteworthy that some antigens are restricted to certain populations.

Linkage disequilibrium is the phenomenon of two genes occurring on the same haplotype significantly more frequently than would be expected by chance. Thus, the A1 and B8 genes are present on the same chromosome in the Caucasian population about four times more frequently than would be expected from random matings. Table 3 shows that linkage disequilibrium between two genes can be characteristic of some populations.

II. HLA AND DISEASE

The histocompatibility locus (H-2) of mice has been shown to be involved in susceptibility to cancer[8] and the discovery that specific immune response genes, Ir, are located in the H-2 complex of mice[9] led to numerous studies in man.

Kourilsky et al.[10] and Amiel et al.[11] were the first to study the HLA antigens of patients with malignant diseases, but in general only weak associations have been found between HLA antigens and cancer. The combined relative risk of the antigen, HLA A1, in 25 independent investigations of Hodgkin's disease was highly significant but the risk of 1.38 was not nearly as great as that found for some HLA antigens and nonmalignant diseases such as ankylosing spondylitis,[12] where the combined relative risk was 87.4 in Caucasians.

III. HLA AND CANCER OF THE ESOPHAGUS

The overall cancer incidence in South African Negroes is similar to that in Cauca-

Table 1
WHO-RECOGNIZED HLA SPECIFICITIES (BROAD SPECIFICITIES ARE SHOWN IN BRACKETS)

HLA-A	HLA-B	HLA-D
A1	B5	Dw1
A2	B7	Dw2
A3	B8	Dw3
A9	B12	Dw4
A10	B13	Dw5
A11	B14	Dw6
Aw23 [9]	B15	Dw7
Aw24 [9]	Bw16	Dw8
A25 [10]	B17	Dw9
A26 [10]	B18	Dw10
A28	Bw21	Dw11
A29	Bw22	Dw12
Aw30	B27	
Aw31	Bw35	
Aw32	B37	
Aw33	Bw38 [16]	HLA-DR
Aw34	Bw39 [16]	
Aw36	B40	DR1
Aw43	Bw41	DR2
	Bw42	DR3
	Bw44 [12]	DR4
	Bw45 [12]	DR5
HLA-C	Bw46	DRw6
	Bw47	DR7
Cw1	Bw48	DRw8
Cw2	Bw49 [21]	DRw9
Cw3	Bw50 [21]	DRw10
Cw4	Bw51 [5]	
Cw5	Bw52 [5]	
Cw6	Bw53	
Cw7	Bw54 [22]	
Cw8	Bw55 [22]	
	Bw56 [22]	
	Bw57 [17]	
HLA-B	Bw58 [17]	
	Bw59	
Bw4	Bw60 [40]	
Bw6	Bw61 [40]	
	Bw62 [15]	
	Bw63 [15]	

sians, but there are differences in the incidence rates for different sites of cancer. A survey of cancer in Durban Negroes by Schonland and Bradshaw[13] showed that cancer of the esophagus is the most common male malignancy and that it has, in this population, one of the highest reported incidences in the world. They reported an age-adjusted incidence rate of 26.1 per 100,000 for Negro males and 8.3 for Negro females.

Our first investigation determined the HLA antigens of 500 confirmed cancer cases

Table 2
PERCENTAGE FREQUENCY OF HLA ANTIGENS IN THREE RACIAL GROUPS

	Caucasian 1100	Negro 1000	Asian Indians 706
A1	29.7	6.5	27.2
A2	45.3	21.3	32.0
A3	29.6	13.3	14.4
A11	12.4	0.1	27.1
Aw23	1.9	18.4	0.6
Aw24	16.8	3.9	27.5
A25	3.7	15.3	2.0
A26	4.9	8.5	6.2
A28	8.7	20.3	14.3
A29	5.5	16.3	1.0
Aw30	4.5	37.6	4.0
Aw31	5.2	10.9	3.4
Aw32	2.3	1.8	2.4
Aw33	0.8*	0.7*	7.8*
One antigen	28.7	25.1	30.2
B7	26.2	18.2	12.5
B8	22.0	14.1	6.2
B13	4.6	4.8	6.5
B14	7.2	6.2	0.3
B15	12.0	5.2	10.8
Bw16	3.2	2.3	2.4
B17	7.5	38.5	21.5
B18	2.5	4.0	3.3
Bw21	1.0	0.8	2.0
Bw22	4.6	0	2.7
B27	6.3	0.3	2.3
Bw35	12.5	6.5	21.5
B37	0.7	0	4.2
Bw41	0.3*	1.0*	0*
Bw42	0	25.5	0
Bw44	28.9	16.4	11.8
Bw45	0.9	7.6	0.1
Bw46	0*	0*	0*
Bw51	9.5*	1.8*	21.2*
Bw52	1.4	0	10.1
B5 IND	0	0	3.1
Bw53	0.3	2.2	1.7
Bw60	13.2	1.2	16.9
Bw61	0.2	0	12.2
One antigen	35.2	43.4	26.8
Cw1	8.0*	5.0*	0*
Cw2	6.0	1.2	14.1
Cw3	14.6	8.8	7.7
Cw4	14.3*	15.0*	14.0*
Cw5	12.0*	2.0*	4.0*
	*N = 300	*N = 146	*N = 150

over an 18 month period.[14] The most common malignancy was cancer of the cervix (143 cases), followed by cancer of the esophagus with 101 cases. Table 4 compares the frequency of HLA antigens in these patients with the frequency in 500 control subjects who were typed concurrently. The frequency of the antigen HLA Bw45 is significantly

Table 3
CHARACTERISTIC HAPLOTYPE FREQUENCIES ($\times 10^3$) IN THREE DIFFERENT RACES

	Caucasian N = 1000	Negro N = 1000	Indian N = 706
A1, B7	10	19**	9
A1, B8	90**	0	8
A1, B17	21**	7	58**
A1, B37	2	0	16**
A2, Bw44	67*	12	4
A2, Bw45	1	17**	1
A3, B7	65**	1	12
A3, B8	12	17**	1
Aw24, B7	13	14**	17
Aw24, Bw52	2	0	19*
A25, Bw44	4	19*	1
A26, B17	2	24**	3
A29, B13	1	14**	0
A29, Bw44	16*	23**	0
Aw30, B13	5*	3	8*
Aw30, Bw42	0	78**	0
Aw31, Bw35	1	12**	1
Aw33, Bw44	0	1	14**

* $\Delta > 2SE$ ** $\Delta > 3SE$

greater in patients with cancer of the esophagus ($p < 0.005$), but in studies of this kind a correction must be applied by multiplying the probability by the number of comparisons. In this case the frequencies of 32 antigens were compared and the resulting probability was no longer significant. The frequency of patients with only one detectable antigen at the A locus was 12.9%. This is significantly different from the frequency in the controls at the 5% level and this might mean that individuals who are heterozygous at the A locus are more susceptible to cancer of the esophagus. Data has been presented[15,16] indicating increased heterozygosity in aged persons, but Table 5 shows that both young (< 40 years) and old (> 60 years) groups of patients have a decreased frequency of homozygosity compared to controls.

These findings prompted a follow-up investigation and an additional 141 confirmed cases in Durban were tested. Epidemiological studies have shown that the Transkei and Ciskei (part of the hinterland of the port of East London) are regions with a very high incidence of esophageal cancer.[17,18] Dr. E. F. Rose of the National Research Institute for Nutritional Diseases has sent 67 blood samples to us for HLA typing. The total of 309 was divided into two groups for analysis: 93 Xhosas, Fingoes, and others who originated from the high-incidence areas of Transkei and Ciskei, and 216 Zulus. Analysis of the 1000 random controls revealed that 55 were Xhosa but there were no significant differences between the frequencies of the HLA antigens of Xhosa and Zulu. This is not surprising because all sub-Saharan Negroes are broadly alike in genetic constitution.[19]

Table 6 delineates the antigen frequencies in both groups of patients and in all the patients, while in Table 7 selected frequencies are shown in more detail. The antigen, A10, consists of A25 and A26. We see that the increased frequency of A10 in the total group of patients is caused by the increased frequency of A26 and also that this increase is due to the significant increase in Xhosas. Correcting the probability by mul-

Table 4
PERCENTAGE FREQUENCY OF HLA ANTIGENS IN 101 PATIENTS WITH CANCER OF THE ESOPHAGUS COMPARED WITH CONTROLS

	Controls 500	Cancer of the esophagus 101
A1	5.0	6.9
A2	20.6	28.7
A3	14.2	12.9
A11	0.2	0
Aw23	17.2	17.8
Aw24	4.8	8.9
A25	15.6	18.8
A26	9.0	11.9
A28	21.2	17.8
A29	17.0	16.8
Aw30	39.4	36.6
Aw31	11.4	8.9
Aw32	1.8	1.0
Aw33	N.T.	N.T.
One antigen	22.6	12.9
B7	17.8	19.8
B8	15.8	13.9
B13	4.8	4.0
B14	5.2	4.0
B15	4.2	4.0
B16	3.2	2.0
B17	41.2	42.6
B18	3.8	5.9
Bw21	0.6	2.0
B27	0.6	0
Bw35	6.2	4.0
Bw42	25.0	24.8
Bw44	15.8	15.8
Bw45	7.2	15.8
Bw51	1.2	0
Bw53	N.T.	N.T.
Bw60	1.0	0
One antigen	42.8	36.6
Cw2	13.0	15.8
Cw3	6.0	7.9

tiplying by the number of antigens tested yielded a $p < 0.016$ and a relative risk of 2.8. The frequency of A26 in the small number of Xhosa controls was only 9.1% so that the increase in Xhosa patients does not seem to be because of an increased frequency of the A26 antigen in the Xhosa population.

The initial findings of an increase in Bw45 and increased heterozygosity was not confirmed in the larger sample but the increased frequency of Cw2 reaches borderline significance (after correction, $p < 0.033$) in the total group of patients with a relative risk of 1.7.

Haplotype frequencies were estimated from the population data by the method of Mattiuz et al.,[20] and some of these are shown in Table 8. There are no significant

Table 5
PERCENTAGE FREQUENCY OF INDIVIDUALS WITH ONLY ONE DETECTABLE ANTIGEN AT THE FIRST AND SECOND LOCUS IN RELATION TO AGE

		Controls		Cancer of esophagus	
	Age	< 40	> 60	< 40	> 60
	Number	161	89	15	24
] antigen at A locus		23	21	7	8
] antigen at B locus		47	43	20	29
] antigen at both A and B loci		13	11	0	0

Table 6
PERCENTAGE FREQUENCY OF HLA ANTIGENS IN XHOSA AND ZULU PATIENTS WITH CANCER OF THE ESOPHAGUS

	Xhosa (93)	Zulu (216)	Total (309)
A1	5.4	6.5	6.1
A2	20.4	21.8	21.4
A3	11.8	11.1	11.3
Aw23	17.2	18.5	18.1
Aw24	5.4	6.9	6.5
A25	12.9	18.1	16.5
A26	20.4	13.0	15.2
A28	18.3	17.6	17.8
A29	12.9	16.7	15.5
Aw30	41.9	37.5	38.8
Aw31	8.6	9.3	9.1
Aw32	5.4	2.8	3.6
One antigen	19.4	20.3	20.1
B7	24.7	22.7	23.3
B8	14.0	18.1	16.8
B13	0	3.2	2.3
B14	8.6	4.2	5.5
B15	6.5	1.4	2.9
B16	4.3	2.8	3.2
B17	37.6	34.3	35.3
B18	8.6	6.5	7.1
Bw21	1.1	2.8	2.3
B27	2.2	0	0.6
Bw35	8.6	4.6	5.8
B40	2.2	0.5	1.0
Bw42	23.7	25.5	24.9
Bw44	7.5	18.1	14.9
Bw45	8.6	10.6	10.0
Bw51	3.2	0.5	1.3
One antigen	38.7	44.5	43.7
Cw2	21.5	22.2	22.0
Cw3	8.6	12.0	11.0

Table 7
SELECTED ANTIGEN FREQUENCIES IN PATIENTS WITH CANCER OF THE ESOPHAGUS

	Controls (1000)	Cancer of the esophagus		
		Xhosa (93)	Zulu (216)	Total (309)
A25	15.3	12.9	18.1	16.5
A26	8.5	20.4***	13.0	15.2**
A10	23.8	33.3	31.1	31.7*
One antigen at A locus	25.8	19.4	20.3	20.1
Bw45	7.6	8.6	10.6	10.0
Cw2	14.1	21.5	22.2*	22.0**

Uncorrected p* < 0.005
** < 0.001
*** < 0.0005

Table 8
HAPLOTYPE FREQUENCES ($\times 10^3$) IN PATIENTS WITH CANCER OF THE ESOPHAGUS

	Xhosa (93)	Zulu (216)	Total (309)
A1, B7	21	28*	26**
A2, Bw45	26	26*	26**
A3, B8	20	15	17
Aw24, B7	21	25*	24*
A25, Bw44	3	26	19
A26, B17	44	17	25
A29, B13	0	14*	10*
A29, Bw44	27	26	27*
Aw30, Bw42	83*	66*	71**
Aw31, Bw35	4	9	7

* $\Delta > 2SE$ ** $\Delta > 3SE$

differences between patients and controls. In another study of HLA antigens and cancer in the Indian population of Durban[20] we found only 18 cases of esophageal cancer out of a total of 250 patients (Table 9). The small numbers necessitated the use of Fisher's exact method[21] for calculating probabilities and only the increased frequency of Aw32 was significant with $p = 0.01$. However, when corrected for the number of antigens tested, this was no longer significant. The HLA and Disease Registry[12] provided data on the HLA antigen frequencies in Caucasians with cancer of the esophagus.[22] The increased frequency of HLA B7 carried a relative risk of 2.4 but after correction this was not significant. Also shown in Table 9 are the frequencies found by Hashemi et al.[23] in Iranians living in the Caspian littoral, an area noted for the high incidence of esophageal cancer.[24] A preliminary report (quoted by Simons and Amiel[25]) of a significant increase of B40 in 71 patients was not confirmed in this larger series. Their detailed analysis of the four ethnic groups studied showed an increase of B18 in Persians with cancer of the esophagus but they suggest that this may be a chance event.

Table 9
PERCENTAGE FREQUENCY OF HLA ANTIGEN IN CAUCASIANS, ASIAN INDIANS, AND IRANIAN PATIENTS WITH CANCER OF THE ESOPHAGUS

HLA		Caucasian[22] (N = 47)	Asian Indian[20] (N = 18)	Iranians[22] (N = 151)
A1		27.7	5.6	22.5
A2		51.1	33.3	22.5
A3		36.2	16.7	27.8
A11		4.3	22.2	12.6
Aw23	12.8		5.6	29.1
Aw24			16.7	
Aw25	10.6		0	12.6
Aw26			0	
A28		12.8	16.7	9.3
A29	33.8	4.3	0	
Aw30		4.3	16.7	
Aw31		N.T.	11.1	
Aw32		12.8	16.7	
Aw33		N.T.	16.7	
B7		44.7	11.1	7.9
B8		8.5	0	5.3
B13		8.5	5.6	2.6
B14		12.8	0	7.9
B15		8.5	11.1	3.3
B16		N.T.	11.1	6.6
B17		6.4	22.2	6.6
B18		8.5	0	12.6
Bw21		0	5.6	6.6
Bw22		4.3	5.6	11.9
B27		6.4	0	2.0
Bw35		12.8	16.7	33.8
B37		N.T.	0	2.6
Bw44	40.4		16.7	10.6
Bw45			5.6	
Bw51	8.5		0	35.1
Bw52			27.8	
B5IND		N.T.	0	N.T.
Bw53		N.T.	5.6	N.T.
Bw60	8.5		16.7	11.3
Bw61			11.1	

This needs to be confirmed. Thus it would appear that if there is a "susceptibility" gene within the HLA region, then it must be associated with different HLA antigens in different populations.

REFERENCES

1. Francke, U. and Pellegrino, M. A., Assignment of the major histocompatibility complex to a region of the short arm of human chromosome 6, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 1147, 1977.
2. WHO-IUIS Terminology Committee, Nomenclature for factors of the HLA system, in *Histocompatibility Testing 1975*, Kissmeyer-Nielsen, F., Ed., Munksgaard, Copenhagen, 1975, 5.
3. Sandberg, L., Thorsby, E., Kissmeyer-Nielsen, F., and Lindholm, A., Evidence of a third sub-locus within the HLA chromosomal region, in *Histocompatibility Testing 1970*, Terasaki, P. I., Ed., Munksgaard, Copenhagen, 1970, 165.
4. WHO-IUIS Terminology Committee, Nomenclature for factors of the HLA system, in *Histocompatibility Testing 1980*, Terasaki, P. I., Ed., Munksgaard, Copenhagen, 1980.
5. WHO-IUIS Terminology Committee, Nomenclature for factors of the HLA system, in *Histocompatibility Testing 1977*, Bodmer, W. F., Ed., Munksgaard, Copenhagen, 1977.
6. Kissmeyer-Nielsen, F. and Thorsby, E., Human transplantation antigens, *Transplant. Rev.*, 4, 155, 1970.
7. Pickbourne, P., Piazza, A., and Bodmer, W. F., Population analysis, in *Histocompatibility Testing 1977*, Bodmer, W. F., Ed., Munksgaard, Copenhagen, 1977, 259.
8. Lilly, F., The influence of H-2 type on gross virus leukemogenesis, *Transpl. Proc.*, 3, 1239, 1971.
9. Benacerraf, B. and Katz, D. H., The nature and function of histocompatibility linked immune response genes, in *Immunogenetics and Immunodeficiency*, Benacerraf, B., Ed., Medical and Technical Publishing, London, 1975, 117.
10. Kourilsky, F. M., Dausset, J., Feingold, N., Dupuy, J. M., and Bernard, J., Leucocyte groups and acute leukaemia, *J. Natl. Cancer Inst.*, 41, 81, 1968.
11. Amiel, J. L., Study of the leucocyte phenotypes in Hodgkin's disease, in *Histocompatibility Testing 1967*, Munksgaard, Copenhagen, 1967, 79.
12. Ryder, L. P., Andersen, E., and Svejgaard, A., *HLA and Disease Registry Third Report*, Munksgaard, Copenhargen, 1979.
13. Schonland, M. and Bradshaw, E., Cancer in the Natal African and Indian 1964-66, *Int. J. Cancer*, 3, 304, 1968.
14. Hammond, M. G., Appadoo, B., and Brain, P., HLA and Cancer in South African Negroes, *Tissue Antigens*, 9, 1, 1977.
15. Gerkins, V. R., Ting, A., Menck, H. T., Casagrande, J. T., Terasaki, P. I., Pike, M. C., and Henderson, B. E., HLA heterozygosity as a genetic marker of long-term survival, *J. Natl. Cancer Inst.*, 52, 6, 1909, 1974.
16. Macurova, H., Ivanyi, P., Sajdlova, H., and Trojan, J., HLA antigens in aged persons, *Tissue Antigens*, 6, 269, 1975.
17. Warwick, G. P. and Harington, J. S., Some aspects of the epidemiology and etiology of esophageal cancer with particular emphasis on the Transkei, South Africa, *Adv. Cancer Res.*, 17, 81, 1973.
18. Robertson, M. A., Harington, J. S., and Bradshaw, E., The cancer pattern in African gold miners, *Br. J. Cancer*, 25, 395, 1971.
19. Tobias, P. V., The biological invalidity of the term Bantu, *S. Afr. J. Sci.*, 67, 517, 1971.
20. Hammond, M. G., Appadoo, B., and Brain, P., HLA and Cancer in South African Indians, *Tissue Antigens*, 14, 296, 1979.
21. Fisher, R. A., *Statistical Methods for Research Workers*, 10th ed., Oliver & Boyd, Edinburgh, 1946.
22. Terasaki, P. I., Perdue, S. T., and Mickey, M. R., *Genetics for Human Cancer*, Raven Press, New York, 1977, 321.
23. Hashemi, S., Dowlatshahi, K., Day, N. E., Kmet, J., Takasugi, M., Mohagheghpour, N., and Modabber, F. Z., Esophageal cancer studies in the Caspian Littoral of Iran: introductive assessment of the HLA profile in patients and controls, *Tissue Antigens*, 14, 422, 1979.
24. Kmet, J. and Mahboubi, E., Esophageal cancer in the Caspian Littoral of Iran: initial studies, *Science*, 175, 846, 1972.
25. Simons, M. J. and Amiel, J. L., HLA and malignant diseases, in *HLA and Disease*, Dausset, J. and Svejgaard, A., Eds., Munksgaard, Copenhagen, 1977, 212.

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PRELIMINARY RESULTS OF HLA CLASS I AND CLASS II ANTIGENS IN CHINESE WITH NASOPHARYNGEAL CARCINOMA◊

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INTRODUCTION

Simons *et al.* (1) were the first to report an association between nasopharyngeal carcinoma (NPC) and HLA antigens in Chinese patients. Expanded studies on 153 patients by Simons *et al.* (2) showed a borderline increase in HA A2 and Bw46. Simons *et al.*.(3) reviewed the data collected during the Second Asia-Oceania Histocompatibility Workshop with respect to NPC in Chinese. The close linkage disequilibrium between Bw46 and DRw9 in controls was not seen in NPC patients but there was a high frequency of blanks. The HLA profile showed differences between newly diagnosed patients and long-term survivors.

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These interesting findings led to the inclusion of NPC as one of the diseases studied in the Third Asia-Oceania Histocompatibilty Workshop and the report by Chan *et al.* (4) confirmed the previous findings in southern Chinese and also reported that HLA B17(58) had a lower frequency in long term survivors. In contrast northern Chinese showed none of these associations but instead showed an increased frequency of HLA B35.

A comprehensive review by Simons (5) points out that the original findings have been amply confirmed in several reports of both overseas Chinese and in mainland Chinese but only in southern Chinese patients

MATERIALS AND METHODS

SUBJECTS

Patients and controls were of Chinese descent and resident in Taipei. The patient group consisted of 74 unrelated confirmed cases of nasopharyngeal carcinoma who were attending the ENT clinic at the National Taiwan University Hospital.. The control group consisted of 200 unrelated random staff, blood donors and parents of patients awaiting transplantation. Class II antigens were determined in 97 controls.

HLA TYPING

Lymphocytes were isolated on a density gradient and T and B cells were separated by means of nylon wool columns (6) in the first phase of the investigation. Many of the patients, however, had very low lymphocyte counts and we changed to using immunomagnetic beads (Dynal) for separating B cells (7). The yield of B cells was significantly improved but we were still unable to perform satisfactory HLA Class II typing on all the patients.

A complete set of Tenth International Histocompatibility Workshop antisera was used in a two-stage microlympho-cytoxicity test (8) to determine the HLA antigens in the patients. Antigen assignment was based on the Antigen Society reports in the proceedings of the Tenth Workshop (9). Commercial typing trays designed for use in Oriental populations (One Lambda) were used in parallel and appeared satisfactory for nearly all specificities. Some difficulty in antigen assignment was found for A30/A31 and Bw57/Bw62/Bw75 when they occurred together. The antigen frequencies of the control population was based on typing with these commercial trays.

ANTIBODY TESTS:

Antibodies against EBV early antigens (anti-EA) and viral capsid antigens (anti-VCA) in IgG and IgA were tested by the indirect immunofluorescent techniques (10).

RESULTS AND DISCUSSION

The frequency of HLA A2 was increased in the patients (62.2% vs 47.0%, p 0.05) and although the corrected p-value was not significant, this confirms earlier reports. The frequency of B46 was increased but this was not significant. There were no singificant differences at the C locus. HLA DR9 was increased (27.3% vs 21.7%) but not significantly so and there were no differences at the DQ locus. These figures are in agreement with previous reports of increased frequencies of A2, B46 and DR9. The difference was greatest with A2 and least with DR9, giving the impression that the A locus has the most influence. The joint occurrence of combinations of these antigens in patients and controls showed increasing relative risks up to a value of 2.3 for the combination of A2, B46, DR9.

A very interesting finding was the decreased frequency of the All antigen in patients (33.8% vs 60.0%, p 0.0005). This may indicate a protective effect that is in linkage disequilibrium with this A locus antigen although Svejgaard et al (11) has explained the difficulties in establishing a negative correlation. Table 1 shows the frequency of selected HLA antigens in all patients and in three broad categories. Patients in group A are descendants of families who have lived in Taiwan for many generations. Group B are from the central provinces on the mainland and group C are Cantonese. It is difficult to establish statistical significance with small numbers as happens when subdividing into small groups but some interesting trends have emerged. The A2, B46 and DR9 antigens do not have increased frequencies in patients from the central provinces. This agrees with the report of Chan et al. (4) HLA All was decreased in all groups. B57 was increased in patients with origins on the mainland but not in those from Taiwan. The joint occurrence of A2, B46, DR9 was more frequent in patients from Taiwan. Dividing the patients according to the extent of the carcinoma showed that B46 and DQwl were greatly increased in patients where the cancer had a limited spread whereas DR9 was not. HLA B57 was increased in all stages (Table 2).

Not all the patients were tested for the presence of antiEA and anti-VCA in IgA an IgG. As shown in Table 3, DR9 was decreased in those patients with antibodies. The difference in frequency for B46 was less marked and was not observed for A2. HLA All was not present in patients with anti-EA and markedly decreased in patients with anti-VCA in IgG. Thus, it seems that Chinese with All who make antibodies are less likely to develope NPC. An interesting finding was the increase in B13 and DRw6 in patients with antibodies and a decrease in patients with low titre of antibodies. There was also an inverse relationship between DQwl and DQw3 in patients with and without antibodies which may be caused by linkage disequlibrium with DRw6 and DR9.

Table 1. Selected antigen frequencies in NPC patients

	RANDOM	ALL	A	B	C
N=	200	74	54	9	11
A2	47	62	67	33	64
All	60	34	35	33	27
B35	5	9	7	0	27
B46	22	31	39	11	9
B57	2	19	7	33	27
Cwll	18	16	20	0	9
DR9	22	27	33	13	11
DQwl	38	53	53	63	44
D#w3	75	62	67	25	67
A2 B46 DR9	10	21	25	13	11

A=Taiwan B=Central mainland C=Cantonese

Table 2 Selected antigen freqencies in NPC patients

	RANDOM	I	II	III	IV
N=	2OO	6	30	16	l2
A2	47	67	50	75	58
All	60	50	43	19	42
B35	5	0	7	19	17
B46	22	50	23	44	8
B57	2	17	20	19	17
Cwll	18	17	10	25	8
DR9	22	0	28	36	10
DQwl	38	80	55	57	30
DQw3	75	20	62	50	70
A2 B46 DR9	10	0	21	29	0

I, II, III, lV Stages of NPC

Table 3 Antigen frequencies in NPC patients with antibodies

	RANDOM	ALL	IgA EA	IgG EA	IgA VCA	IgG VCA
N=	2OO	74	7	7	18	26
A2	47	62	71	86	56	69
All	60	34	0	0	22	15
B13	5	9	29	29	28	19
B46	22	31	14	29	22	27
DRw6	11	12	43	29	22	17
DR9	22	27	0	14	6	9
DQwl	38	53	86	71	56	61
DQw3	75	62	29	43	39	48
A2 B46 DR9	10	21	0	15	0	4

The calculations to estimate haplotype frequencies only showed significant linkage disequilibrium in the total group because of the small numbers involved when considering subgroups. Significant linkage disequalibrium was present for A2, B46 andB46, DR9 in both control groups, and patients, but for A33, B57 and B57.Dr3, it was only present in the patients, and in fact, the frequency of these latter pairs was extremely low in the random controls.

REFERENCES

1. M.J. Simons, G.B. Wee *et al.*, Lancet I: 142-143 (1975). 2. MJ Simons, GB Wee *et al.*, Natl Cancer Inst Monogr 47:147-151 (1977).
3. M.J. Simons, S.H. Chan *et al.*, Proceedings of the Second Asia and Oceania Histocompatibility Workshop Conference. Immuno-publishing, Toorak, Australia p369 (1981).
4. S.H. Chan,C.T. Chew *et al.*, Aizawa M(ed). HLA in Asia-Oceania. Hokkaido, University Press, Hokkaido Japan p396 (1986).
5. M.J. Simons. Hawkins BR (eds.) Human leucocyte antigens in Chinese. Hong Kong University Press, Hong Kong (1987).
6. J.A. Danilov, G. Ayoub *et al.*, Terasaki, P.I. (ed) Histocompatibility Testing 1980. Los Angeles, UCLA Tissue Typing Laboratory, p287 (1980).
7. F. Vartdal, G. Gaudernack *et al.*, Tissue Antigens 28: 301 (1986).
8. K.K. Mittal, M.R. Mickey *et al.*, Transplantation 6: 913 (1968).
9. B. Dupont (ed) Immunobiology of HLA, vol 1, Histocompatibility Testing 1987. Springer-Verlag, New York (1989).
10. A. Kawamura, M. Takada *et al.*, Gann 61, 55-71 (1970)
11. A. Svejgaard, P. Platz *et al.*, Transplant Rev. 22:3 (1975)

Table 1: *CENTRES INVOLVED IN THE 11TH WORKSHOP HODGKIN'S DISEASE STUDY*

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11th International Histocompatibility Workshop Hodgkin's Disease Study.

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Introduction

Amiel (1), in his introduction to the first study of the human histocompatibility (HLA) antigens and disease, postulated that as susceptibility to the Gross virus, which gives rise to spontaneous leukaemias in mice, associates with the mouse histocompatibility (H2) antigens (2), the same mechanism might operate in human susceptibility to viral disease. As Hodgkin's Disease (HD) was suspected to be of viral origin, a suggestion that has been in, out and is now back in fashion (3), this was the disease chosen for this first study. Careful analysis of the serological results indicated that one antigen, then called 4C, related to what are now called B35, B5 and B18, was significantly raised in patients with HD.

Since then a number of other studies have confirmed the association with these and other Class I HLA antigens (4-10). If data from several studies are pooled an increase in susceptibility to HD is seen in association with HLA-A1, B5, B8 and B18 (10). Nevertheless the relative risks were small and variable in the random patient studies and so the most convincing evidence for the role of HLA in susceptibility to HD came from studies of multi case HD families with more than one affected member, in which an excess of HLA identity between pairs of affected sibs was observed (11).

Following the early HLA Class I studies, Class II, specifically HLA-DR alleles, were investigated in HD but no significant associations were seen (10,12,13). As improved methods of typing for HLA-DP were developed, such as restriction fragment length polymorphism (RFLP) (14,15), it was decided to investigate the possibility that alleles at this locus might reveal a Hodgkin's Disease susceptibility gene. In 1989 Bodmer *et al* (16) described the results of a study using RFLP to type a small number of patients and controls. In this study a significant decrease in a fragment associated with DPw2 was seen in patients compared to controls along with a non-significant increase in a fragment associated with DPw3, 5 and 6.

Following the pilot study mentioned above, an international collaboration was set up as part of the 11th International Histocompatibility Workshop to study the association of HLA-DPB with Hodgkin's Disease using a panel of sequence specific oligonucleotide (SSO) probes in conjunction with the techniques of enzymic amplification of DNA and dot blotting. Twenty officially named DPB alleles were defined by DNA sequencing techniques (17,18).

This study was carried out at two levels, firstly to look for an association between HLA-DPB and overall susceptibility to Hodgkin's Disease and secondly to look for associations of particular alleles with the clinical course of the disease. There is evidence for impaired immunity at presentation in Hodgkin's Disease, and this may correlate adversely with survival (19). The role of HLA in immune function is well established and this could make HLA alleles possible factors in determining survival in patients with Hodgkin's Disease. Some of the early published studies analysed HLA results according to survival, HLA-A1 and B8 being found in increased frequency in long term survivors. The HLA types in long term survivors can be compared with those of patients who died shortly after the onset of disease to explore the role of HLA in influencing disease progression. For example in an early study investigating HLA in Hodgkin's Disease, Falk and Osoba (4) found an increase in antigens A1, B5, formerly A5, and B8, formerly A8, in patients with Hodgkin's Disease as a whole, B8 being particularly prevalent in patients who had survived more than 5 years. In addition, the frequency of HLA-A3 was increased in patients with recent onset, suggesting that this could be associated with poor prognosis. Osoba and Falk studied prospectively 79 previously untreated patients who were diagnosed between 1972 and 1973 at The Princess Margaret Hospital, Toronto. The HLA phenotype Aw19 was found to be a highly significant prognostic factor, on univariate as well as multivariate analyses and was independent of stage, age, histology or sex (20). The significance of HLA-Aw19 was also confirmed by comparing the frequency of this antigen between patients in good and bad survival groups. Another recent study reported a significant increase in HLA-B5 in patients who relapsed (21). This is of interest as a preliminary analysis of the patients reported by Osoba et al (20) had also associated this antigen with poor survival, being present relatively frequently in patients who had died within three years of diagnosis (22).

Thus examination of a possible correlation of the frequency distribution of HLA-DPB alleles with clinical parameters is an important component of the study.

The HLA-DPB typing was carried out at the DNA level using a panel of 25 sequence specific nucleotides (SSOs) as described in the Hodgkin's Disease abstract (Tonks *et al* volume II). A total of 741 patients with Hodgkin's Disease and 686 controls from 17 centres in 12 countries were included in the typing analysis. Table 1 shows a list of the collaborating clinical centres and laboratories involved. The populations included Caucasoid, Black and Oriental patients with ethnically matched controls.

Methods

The clinical centres were asked to provide their collaborating tissue typing laboratory with an EDTA blood sample, 5-10 mls of serum and clinical details for each patient. They were also asked to provide histological slides for central analysis. The laboratories extracted DNA from the blood samples provided and HLA-DPB typed both the patient and controls according to the workshop protocol (DNA Methodology report, this volume). Eight homozygous typing cells were sent to each laboratory for typing as controls. The completed typing data were then sent to the disease study organisers along with copies of the autoradiographs and the clinical pro-formas for analysis. The HLA-DPB alleles were assigned using a computer program designed by A. Wasik and J. G. Bodmer (ICRF). Relative risks and χ^2 were calculated on the patients and controls to whom alleles had been assigned (23). Data from each centre was analysed against their matched controls and then data from ethnic groups compared (table 2). Using the scores for each SSO, χ^2 and RR for different sequence motifs were also calculated.

The information obtained from the clinical questionnaires (table 3) was used to construct a clinical database. All completed pro-formas were checked at St. Bartholomew's Hospital for any obvious inconsistency. Clinical information for analysis was available on 551 patients from 12 centres.

Survival analyses could not be performed on the entire set of data as all patients typed were alive relatively recently, at least at the time of venesection. Historical data from patients treated and followed-up at St. Bartholomew's Hospital was used to illustrate the differences between the population studied for this workshop and an 'unselected' population.

Proportions of patients achieving complete remission (CR) in different prognostic groups were compared using the χ^2 test with Yates's correction (24). Duration of remission curves were plotted using standard life table methods (25) and compared using the log rank method (26). The significance of prognostic factors in determining the achievement of CR was evaluated by logistic

regression analysis, whereas duration of CR differences were determined using a stepwise linear regression method based on Cox's proportional hazards model (27).

Results

a. Overall analysis

Analysis of the data comparing the frequencies of HLA-DPB alleles in patients and controls was carried out for each patient group separately (table 2) . In addition, sets of data from closely related populations were combined if the heterogeneity between the them was shown to be small. On this basis the nine sets of data from centres in Britain, France, Germany, Italy, Hungary and the U.S.A were combined into a relatively homogeneous Caucasoid group and data from Taiwan and Japan were combined into an Oriental group.

Increased risk with DPB1*0301 in Caucasoids

The combined Caucasoid population, made up of the European and American patients (544) and controls (464), showed the allele DPB1*0301 to have an increased risk (RR 1.95, P<1%) for Hodgkin's Disease. This confirmed the observation of an increase in the RFLP fragment associated with DPw3 seen in the pilot study (16). Two individual data sets in which this allele was seen to be significantly increased were from France (RR 6.19, P<1%) and Germany (RR 2.69, P<5%), though all the other data showed a trend towards an increase in this allele in patients compared to controls (Table 2). No other allele showed a disturbed frequency in the combined Caucasoid data.

Decreased risk with DPB1*0401 in Orientals

The DPB1*0401 allele was seen to be significantly decreased (RR 0.148, p<1%) in patients compared to controls in the Oriental population, consisting of the data from Japan and Taiwan. The same observation was made in several other individual data sets, particularly from Japan, and the U. K (Marsden).

Individual patient groups

No significant associations were seen in the South African data as a whole, however in an independent analysis of 21 Cape Coloureds Jacobs et al (workshop communication) calculated the RR to be 3.8 for DPB1*0301, in agreement with the prior hypothesis.

Two data sets from Israel were analysed. A trend towards an increase in the DPB1*0401 was seen. However, there were technical difficulties in the assignment of DPB alleles in one of the sets (from Haifa) and since the controls were used for both groups, these results are not conclusive.

The previously reported decrease in DPB1*0201, while seen in the German data was not confirmed overall.

Hypervariable region analysis

Since HLA-DPB alleles have the interesting property of being composed of a relatively small number of variable stretches of sequence, or motifs, which are shuffled to form the different alleles, a given motif may be found in several different alleles. Therefore if the susceptibility site were encoded by a particular sequence motif, a higher relative risk would be expected with this motif than with any of the alleles containing it. Analysis of these sequence motifs, based on probe frequencies, while confirming the results of the allele analysis, did not reveal any more significant correlation between susceptibility to HD and a single motif than that seen in the allele analysis. SSOs DPB3502 (FV) and DPB5504 (DED) both specific for DPB1*0301 showed a significant increase in some groups; for example DPB5504 was significantly increased in the French and German data, while DPB3502, was significantly increased in the Hungarian data. In the combined Caucasoid group a number of motifs associated with the DPB1*0301 allele were significantly increased; DPB0902 (VYQL), DPB3502 (FV), DPB5504 (DED), DPB6502 (QKDL), DPB6903 (LLEEK), DPB7602 (M) and DPB8503 (DEAV). SSOs specific for the DPB1*0401 allele showed the same trend as the allele in the Oriental populations; the probe DPB3501 (FA) was significantly reduced in the combined Oriental data and also in the Japanese and the U. K. (Marsden) data.

Linkage disequilibrium

Since the level of recombination between the DP and DR loci is now estimated to be about 1% (28) the question arises whether an association of HD with alleles at other HLA loci would have been seen in this study. In the 11th Workshop DP DNA report (this vol) significant linkage disequilibria were seen between DPB1*0301 and both DRB1*0301 and DRB1*1302 in random Caucasoids and between DPB1*0401 and DRB1*1302 in Japanese. This indicates that DRB1*1302 is associated with the DPB1*0301 allele, increased in HD in Caucasoids, but with DPB1*0401 in Oriental patients, where this allele is reduced in HD. Data on HLA loci other than DP were available only on a small number of patients in this study but in the French data which showed a significant relative risk for DPB1*0301, there was no significant linkage disequilibrium with DPB1*0301 in the patients although linkage disequilibria were seen in the French controls with both DR3 and B18.

In the overall analysis of linkage disequilibria (LD) between DP alleles and alleles at other loci, data from random individuals from other populations in the 11th Workshop were studied. These were: France (192 individuals), Italy (359), Germany (65), UK (44), U.S.A (123) and Japan (314). For the HLA-A locus no alleles were found in significant LD with DPB1*0301 in Caucasoids or with DPB1*0401 in Japanese. For HLA-B only very weak LD were seen for DPB1*0301 with B7 and B50 in Italy and B27 in France. A weak non significant LD was seen in Japanese between B44 and DPB*0401.

b Clinical analysis

To discover whether any HLA-DPB allele was associated with clinical state or progression of the disease, subsets of the patients were compared.

Characteristics of the overall patient population are shown in table 4.

HLA-DPB and remission rate

The overall complete remission rate was high (79.5%). If patients in equivocal complete remission (29) are included, the remission rate is 89%. The proportion of patients who do not respond to initial therapy is very small (1.5%), considerably lower than in other published series. The duration of remission is long, being significantly better in comparison with all previously untreated patients treated at St Bartholomew's Hospital over a 24 year period (figure 1a). This, coupled with the fact that all these patients are alive, demonstrates the significant, albeit inadvertent, selection of patients in this study. The distribution of patients is heavily skewed towards a predominance of survivors, in a good prognostic group.

The duration of remission in patients with different HLA-DPB alleles was compared. On univariate analysis HLA-DPB1*0901 was associated with with shorter overall duration of remission (figure 1b). In the Japanese population, where this allele is most prevalent, the remission duration of the eight patients with DPB1*0901 was significantly less (Figure 1c). There was no significant correlation between HLA-DPB1*0301 (Figure 1d) or other HLA-DPB alleles and remission duration.

HLA-DP and other clinical features

The distribution of HLA-DPB alleles in patients who had a positive family history of Hodgkin's Disease was identical to the overall distribution.

There was a statistically significant increase in the frequencies of HLA-DPB1*0202 and DPB1*0501 in patients with Lymphocyte Deplete Hodgkin's Disease; however, this histological subtype is the least common one and as numbers are small, this finding should be treated with caution.

There was no correlation between HLA-DPB type and stage, extent of disease, outcome to initial therapy, presentation blood count, serum albumin or erythrocyte sedimentation rate.

Discussion.

The most interesting and significant findings to emerge from this study were

a] an increased frequency of DPB1*0301 in Caucasoid patients compared with controls.

b] a decreased frequency of DPB1*0401 in Oriental patients compared with controls.

c] shorter remission duration associated with HLA-DPB1*0901.

While it is impractible to carry out a prospective study on patients to ascertain the relationship of HLA type to disease progression, an alternative approach is available; to type a cohort of patients retrospectively, using stored material. This was performed for a group of patients with poor prognosis from St. Bartholomew's Hospital. The results (unpublished data) showed a highly significant increase in the frequency of HLA-DPB1*0901 in this group, compared with patients in follow up (largely composed of 'survivors') or normal controls. The poor remission duration associated with HLA-DPB1*0901 in these patients provides independent verification of this Workshop result. This finding also suggests that DPB1*0901 or a gene in strong linkage disequilibrium with it predicts a poor outcome.

In conclusion it appears from this study that the HLA association with Hodgkin's Disease, up until now most securely indicated by HLA concordance in affected sibs, is to be found closer to the HLA-DPB locus than to other loci previously studied. This is confirmed by the analysis of linkage disequilibria of DPB1*0301 and *0401 in random donors from the populations studies which show that there are no HLA-A, B or DR alleles consistently in strong LD with these DPB alleles and which might be considered candidates for the primary association with Hodgkin's Disease.

Though the relative risks seen with DPB alleles are highly significant, the increased risk overall is still relatively modest. It is therefore possible that future studies which will be directed towards confirming these results should also examine genes near to HLA-DP which have been shown to be polymorphic (Nomenclature report). The interesting suggestion that DPB1*0901 is associated with

poorer remission duration will be further investigated for both its immunological and clinical implications.

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Table 2: Relative Risk analysis of DPB1*0301 and DPB1*0401 alleles

Country	Group	No. Patients	No. Controls	DPB1*0301 allele				DPB1*0401 allele			
				RR	95% C. L.	χ^2	Sig.	RR	95% C. L.	χ^2	Sig.
United Kingdom	London (Bart's)	38	65	1.537	0.59, 4.02	0.78		0.804	0.34, 1.93	0.24	
	Sutton (Marsden)	32	[65]	2.524	0.98, 6.49	3.73		**0.265**	0.11, 0.63	**8.97**	**
	Southampton	55	85	1.359	0.62, 3.00	0.58		0.784	0.38, 1.62	0.43	
	Manchester	39	40	1.556	0.51, 4.73	0.61		0.595	0.22, 1.61	1.06	
France	Nantes	69	49	**6.191**	2.08, 18.46	**9.36**	**	0.478	0.21, 1.07	2.57	
Germany	Hamburg	53	59	**2.699**	1.10, 6.63	**4.72**	*	1.943	0.89, 4.26	2.78	
Italy	Milan	31	19	2.804	0.73, 10.77	2.30		0.778	0.24, 2.48	0.18	
Hungary	Budapest	39	40	1.386	0.53, 3.62	0.45		0.519	0.20, 1.36	1.81	
USA	Stanford	188	107	1.614	0.92, 2.83	2.81		**1.789**	1.09, 2.93	**5.36**	*
[1]**Combined Caucasoid populations**		**544**	**464**	**1.955**	1.46, 2.62	**20.36**	***	0.903	0.70, 1.16	0.63	
Japan	Chiba	48	43	2.000	0.49, 8.24	0.93		**0.149**	0.04, 0.60	**7.25**	**
Taiwan	Taipei	17	20	4.072	0.43, 39.01	1.52		0.146	0.02, 1.13	3.49	
[2]**Combined Oriental populations**		**65**	**63**	2.440	0.74, 8.02	2.16		**0.148**	0.05, 0.47	**10.55**	**
South Africa	Cape Town, Durban	30	99	1.418	0.46, 4.40	0.37		0.488	0.20, 1.19	2.49	
Israel	Haifa	44	32	4.667	0.77, 28.43	2.83		**3.000**	1.17, 7.68	**5.32**	*
Israel	Tel Hashomer	32	[32]	4.801	0.75, 30.89	2.77		**2.963**	1.08, 8.15	**4.50**	*

] Duplicate controls * significant at the 5% level ** significant at the 1% level ***significant at the 0.1% level

[1] Heterogeneity χ^2 for DPB1*0301 = 6.42 for 9 degrees of freedom

[2] Heterogeneity χ^2 for DPB1*0401 = 0.18 for 2 degrees of freedom

Table 3: Clinical Information

Age

Ethnic Origin

Family History (Hodgkin's, Non-Hodgkin's or other malignancy)

History of Infectious Mononucleosis

Histology

Date of Diagnosis

Stage, clinical/pathological

Number of sites of disease

Therapy details

Outcome

Recurrence details

Pre-treatment:

- Erythrocyte Sedimentation Rate
- Serum Albumin
- Full blood count with differential

Blood group

Table 4: Characteristics of the overall patient population

TOTAL	551
HISTOLOGY	
Nodular Sclerosis	281
Lymphocyte Predominant	62
Mixed Cellularity	136
Lymphocyte Deplete	21
Unspecified	21
STAGE	
I	88
II	203
III	152
IV	73
?	35
B SYMPTOMS	212
OUTCOME	
Complete Remission	400
Complete Remission (u)	46
Partial Remission	47
Fail	8
Early Death	2
Not specified	48
RECURRENCE	78
INFECTIOUS MONONUCLEOSIS	
YES	36
NO	392

Figure legends:

Fig. 1a

Remission duration of patients in HLA study compared with all patients treated at St. Bartholomew's Hospital, London.

Fig. 1b

Remission duration of patients with the DPB1*0901 allele compared with the rest.

Fig 1c

Remission duration of Japanese patients with and without the DPB1*0901 allele.

Fig 1d

Remission duration of patients with the DPB1*0301 allele compared with the rest.

References

(1) Amiel, J. In *Histocompatibility Testing 1967*; (Ed. J. Dausset , J. Colombani)) p. 79 , Munksgaard, Copenhagen, (1967).

(2) Lilly, F., Boyse, E. A. and Old, L. J. *Lancet* **ii**, 1207, 1964.

(3) Weiss, L. M., Movahed, L. A., Warnke, R. A. and Sklar, J. *N. Engl. J. Med.* **320**, 502, 1989.

(4) Falk, J. and Osoba, D. *Lancet* **ii**, 1118, 1971.

(5) Thorsby, E., Bratlie, A. and Engeset, A. In *Histocompatibility Testing 1972*.; (Ed., P. I. Terasaki), Munksgaard, Copenhagen, (1973).

(6) Kissmeyer-Nielsen, F., Kjerbye, K. E. and Lamm, L. U. *Transplant Rev* **22**, 168, 1975.

(7) Kissmeyer-Nielsen, F., Lamm, L. U., Kjerbye, K. E., Bjotn Jensen, K., Nordentoft, A. M., Thorling, K. and Hastrup, J. In *Histocompatibility Testing 1972*; (Ed., J. Dausset), Munksgaard, Copenhagen, (1973).

(8) Bodmer, W. F. *Natl. Cancer Inst. Monogr.* **36**, 127, 1973.

(9) Bjorkholm, M., Holm, G., Johansson, B., Mellstedt, H. and Moller, E. *Tissue Antigens* **6**, 247, 1975.

(10) Hors, J. and Dausset, J. *Immunol Rev* **70**, 167, 1983.

(11) Dausset, J., Colombani, J. and Hors, J. *Cancer Surv* **1**, 119, 1982.

(12) Hansen, J. A., Joung, C. W., Whitsett, C., Case, D. C., Jersild, C., Good, R. A. and Dupont, B. In *HLA and Malignancy*; (Ed., G. P. Murphy), p. 217 A. R. Liss, New York, , (1977).

(13) Welsh, K. I., Amlot, P. and Batchelor, J. R. *Tissue Antigens* **17**, 91, 1981.

(14) Bodmer, J., Bodmer, W., Heyes, J., So, A., Tonks, S., Trowsdale, J. and Young, J. *Proc Natl Acad Sci U S A* **84**, 4596, 1987.

(15) Hyldig, N. J., Morling, N., Odum, N., Ryder, L. P., Platz, P., Jakobsen, B. and Svejgaard, A. *Proc Natl Acad Sci* **84**, 1644, 1987.

(16) Bodmer, J. G., Tonks, S., Oza, A. M., Lister, T. A. and Bodmer, W. F. *Lancet* **1**, 1455, 1989.

(17) Koster, d., Hs, Kenter, M. J., D'Amaro, J., Luiten, R. M., Schroeijers, W. E., Giphart, M. J. and Termijtelen, A. *Immunogenetics* **34**, 12, 1991.

(18) Bugawan, T. L., Angelini, G., Larrick, J., Auricchio, S., Ferrara, G. B. and Erlich, H. A. *Nature* **339**, 470, 1989.

(19) Rijswijk, v. R., de Meijer, A., Sybesma, J. P. and Kater, L. *Cancer* **57**, 1489, 1986.

(20) Osoba, D., Falk, J. A., Sousan, P., Ciampi, A. and Till, J. E. *Cancer* **46**, 1825, 1980.

(21) Prazak, J. and Hermanska, Z. *Eur J Haematol* **43**, 50, 1989.

(22) Falk, J. and Osoba, D. *Prog Clin Biol Res* **16**, 205, 1977.

(23) Svejgaard, A., Jersild, C., Nielsen, L. S. and Bodmer, W. F. *Tissue Antigens* **4**, 95, 1974.

(24) Armitage, P. *Statistical Methods in Medical Research* Halstead: (1971).

(25) Kaplan, E. S. and Meier, P. *American Statistical Association Journal* **53**, 457, 1958.

(26) Peto, R., Pike, M. C., Armitage, P., Breslow, N. E., Cox, D. R., Howard, S. V., Mantel, N., McPherson, K., Peto, J. and Smith, P. G. *Br J Cancer* **35**, 1, 1977.

(27) Cox, D. R. *J. Roy. Statistical Society* **34**, 187, 1972.

(28) Begovich, A. B., McClure, G. R., Helmuth, R. C., Fildes, N., Bugawan, T. L., Erlich, H. A. and Klitz, W. J. *Immunology* **in press**, 1992.

(29) Lister, T. A., Crowther, D., Sutcliffe, S. B. *et al. J. Clin. Oncol.* **7**, 1630, 1989.

Fig. 1a REMISSION DURATION – 'DP' PATIENTS vs SBH 'CONTROLS'

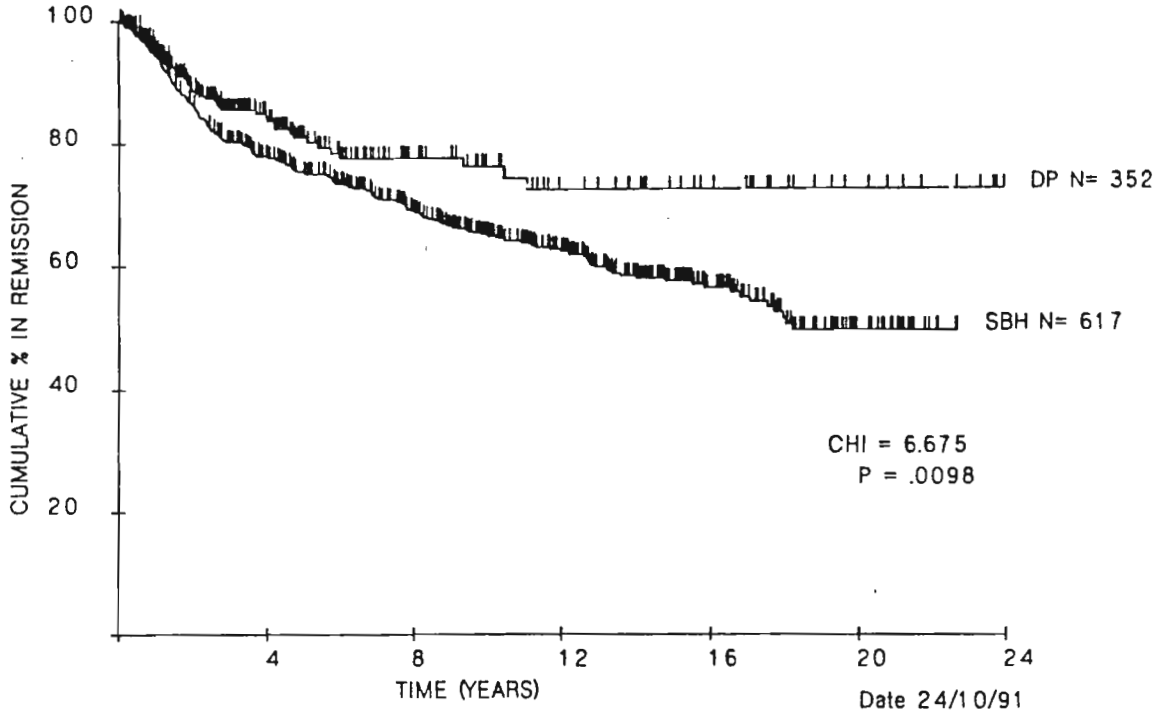


Fig. 1b REMISSION DURATION – DP 0901 vs REST

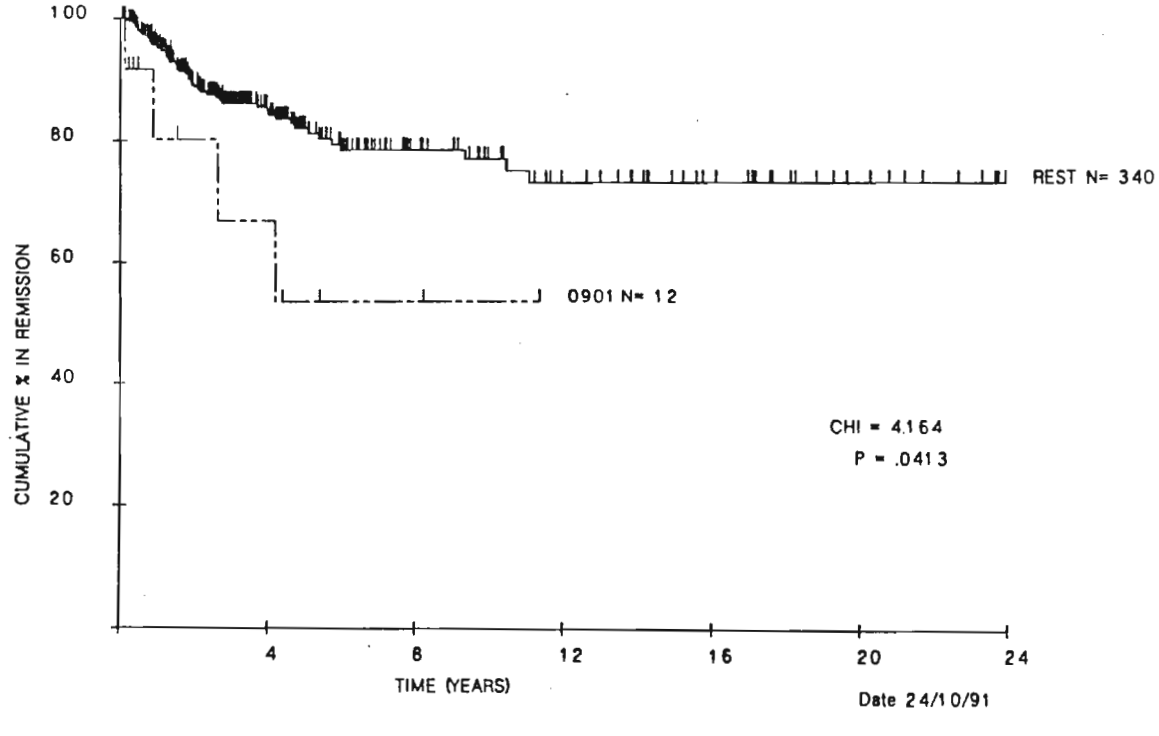


Fig 1c

REMISSION DURATION (MIK PTS ONLY) - 0901 vs REST

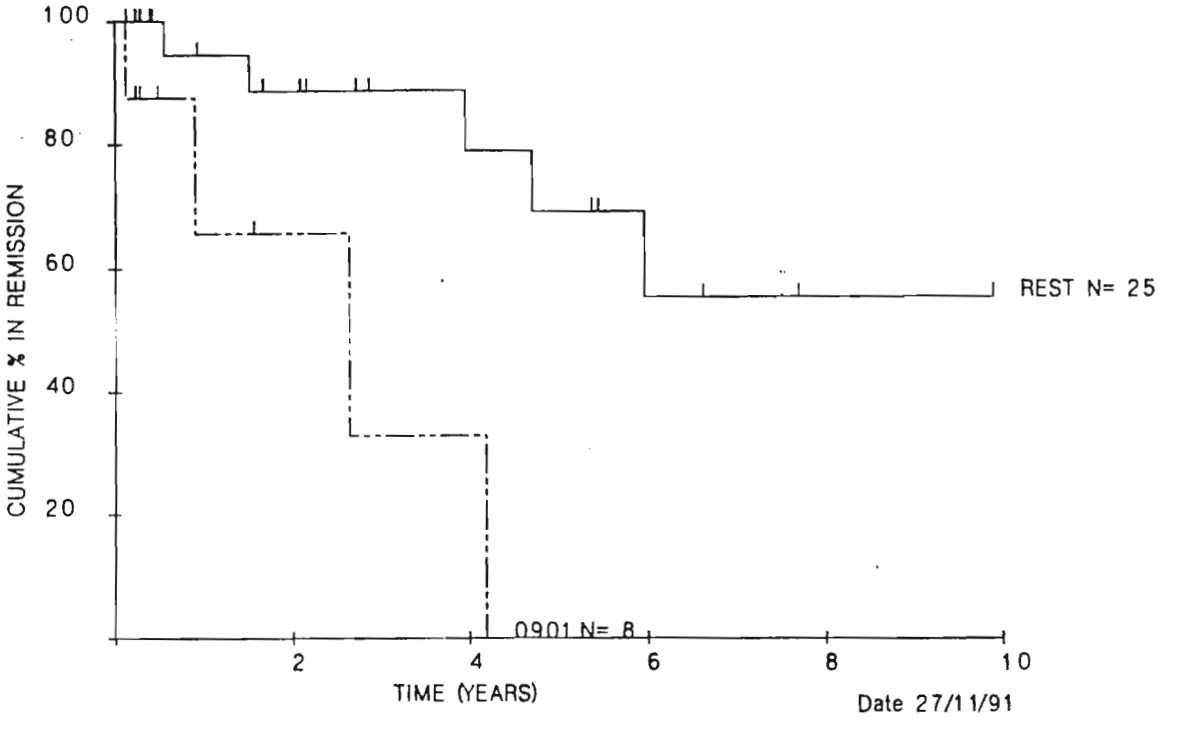
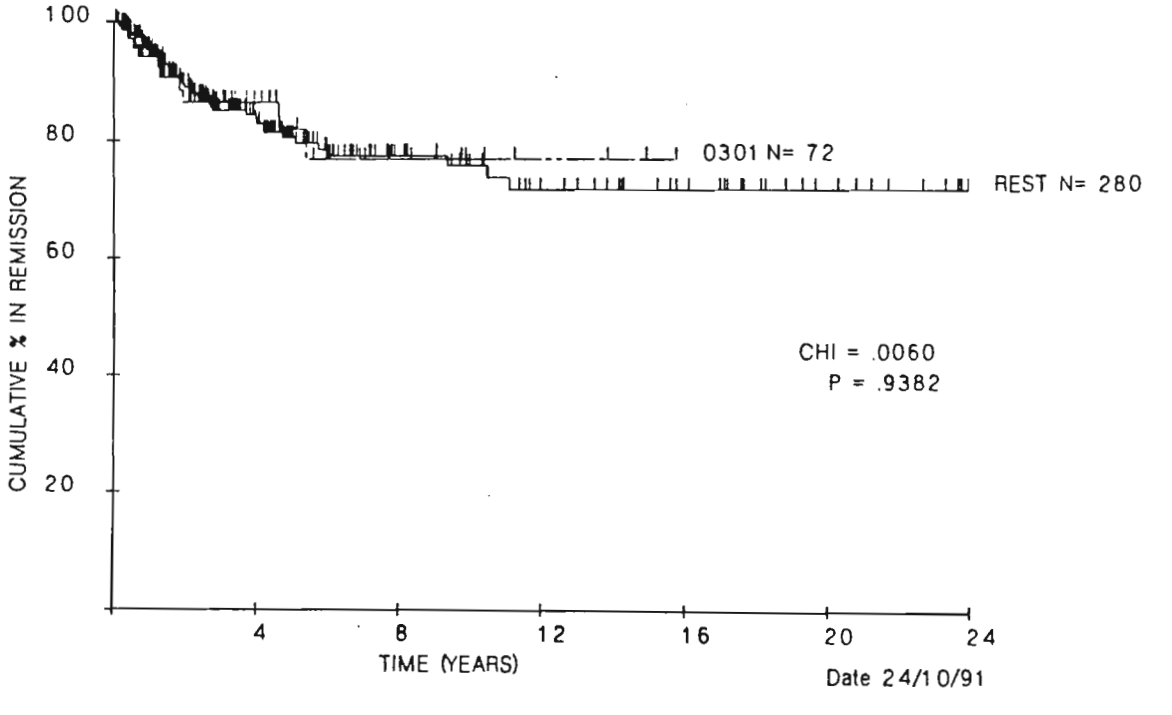


Fig 1d.

REMISSION DURATION - DP 0301 vs REST



Tissue Antigens (1980), 15, 244–248

HLA and Insulin Dependent Diabetes in South African Indians

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The HLA antigens of 44 Asian Indians with juvenile-onset, insulin-dependent diabetes were determined. The frequency of HLA–B8 was increased but that of HLA–B15 was not. There was a significant increase in the frequency of some of the subdivisions of B5.

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Studies of the HLA system in diabetes mellitus have broadened our insight into the role of genetic mechanisms in its development and have highlighted the significant genetic heterogeneity of the disorder (West 1978, Fajans et al. 1978, Cahill 1979). Most of these studies have been carried out on white Caucasian populations and have shown that, whereas insulin-dependent diabetès (IDDM) has certain clear associations with the HLA antigens B8, B15 and B18 (Nerup et al. 1977), non-insulin dependent diabetes does not have such direct relationships.

Studies of the HLA system in non-Caucasian populations have revealed an association between HLA–B8 and IDDM in Black Americans (Cahill 1979), but no association between either B8 or B15 and diabetes in Japanese. In contrast, in the latter group, diabetes has been associated with Bw54, a variant of Bw22 (Kawa et al. 1977, 1978), and with B12 (Nakao et al. 1977). In addition, both groups of workers also reported a decreased incidence of B5 in their diabetics. Kawa et al. (1979) reported that the decreased incidence of B5 was due to the significant decrease in the frequency of Bw52.

Within the European Caucasian population itself there is considerable variation in the distribution of HLA antigens (Cahill 1979). There have been few (if any) studies of the HLA relationship to IDDM in non-European Caucasian populations.

Material and Methods

The diabetic subjects were seen either at the King Edward VIII Hospital, Durban, which is the main teaching hospital of the University of Natal, or at one of the satellite hospitals in the group. The IDDM patients were characterized by onset under 35 years and a dependence on insulin for control of symptoms and for the prevention of basal ketosis (West 1978). The antigen frequencies were compared with those found in a healthy control population, many of whom were typed for Inter-

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national Workshops (Hammond et al. 1975, 1977).

The Indian subjects studied represent two ethnic subgroups – the Aryans and the Dravidians – whose predecessors came to South Africa more than 100 years ago from north and south India, respectively (Mistry 1965).

A total of 180 antisera were used in a two-stage microlymphocytotoxicity test to determine the HLA antigens of 44 Indians with IDDM. Lymphocytes were isolated on a Ficoll-Hypaque density gradient.

Results

Table 1 shows the antigen frequencies in Aryans, Dravidians and in all the Indian patients compared with the controls. HLA–B5 has been subdivided into Bw51, Bw52 and B5 IND. This last category probably includes the specificity Bu = 8W59. The distribution of antigens at the A and B loci conform to Hardy-Weinberg equilibrium. Estimates of haplotype frequencies are not very reliable for small numbers but no linkage disequilibrium was evident. Some haplotypes are shown in Table 2.

Probabilities were calculated by χ^2 or, if any of the numbers in the 2 × 2 table were less than 4, by Fisher's exact method. Probabilities have been corrected by multiplication by the number of antigens tested.

There were no significant differences at the A locus. At the B locus, HLA–B8 was increased in the total Indian sample (13.6% vs 5.9% N.S.) this being due to the high frequency in Dravidians (21.7% vs 6.4%), but was not significant after correction for the number of antigens tested. The frequency of B15 was unaltered. The frequency of B13 was also increased in the total Indian sample but this was due to the higher incidence in Aryans (19.1% vs 4.8%). This difference was not significant.

None of the Indians with IDDM was found to have B40.1 but the corrected probability was not significant. B40.2 was increased, but the frequency of B40 overall was approximately the same in the total Indian sample. This inverse relationship in the subdivisions of B40 was also seen in the subdivision of B5. Bw52 was significantly increased in Dravidians with IDDM (corrected $P < 0.04$) and B5 IND was significantly increased when considering all Indians (corrected $P < 0.02$), while Bw51 was decreased. The same trend was also present in Aryan Indians. The splitting of B5 and B40 into subdivisions still poses problems which may be solved in the future by better sera and International Workshops. These results should therefore be treated with reserve. The overall increase of B5 (43.2% vs 34.2%) contrasts with the decrease of B5 observed in European Caucasians (Nerup et al. 1977) and in Japanese (Kawa et al. 1979, Nakao et al. 1977).

Discussion

Studies on White Caucasian populations have shown that both HLA–B8 and B15 are associated with an increased risk of IDDM. Rotter & Rimoin (1978) have hypothesized that there are two distinct forms of IDDM, one associated with B8 and characterized by autoimmunity, microangiopathy and a stronger association with the D locus antigen Dw3. It has also been noted that B7 and Dw2 have lower than normal frequencies in B8 positive diabetics. The B15 type is characterized by antibody response to exogenous insulin and a stronger association with the C locus antigen, Cw3, but it now appears that these

Table 1
Percentage frequency of HLA antigens in Indians with JOD

	ARYAN		DRAVIDIAN		TOTAL INDIAN	
	Control	JOD	Control	JOD	Control	JOD
HLA	N 208	21	424	23	632	44
A1	18.8	23.8	32.2	26.1	27.9	25.0
A2	25.5	23.8	34.2	34.8	31.3	29.6
A3	14.9	9.5	14.4	13.0	14.6	11.4
A11	31.3	28.6	25.7	30.4	27.5	29.6
Aw23	0	0	0.9	4.4	0.6	2.3
Aw24	30.8	33.3	25.2	30.4	27.1	31.8
A25	2.4	9.5	1.7	0	1.9	4.6
A26	5.8	4.8	6.6	0	6.3	2.3
A28	18.3	23.8	12.5	0	14.4	11.4
A29	1.0	0	0.7	0	0.8	0
Aw30	3.9	14.3	4.0	4.4	4.0	9.1
Aw31	5.3	0	2.6	0	3.5	0
Aw32	2.9	0	2.4	0	2.5	0
Aw33	7.2	14.3	7.6	13.0	7.4	13.6
One antigen	32.2	14.3	29.3	43.5	30.2	29.6
B7	7.2	14.3	15.1	8.7	12.5	11.4
B8	4.8	4.8	6.4	21.7*	5.9	13.6
B13	4.8	19.1	7.8	4.4	6.8	11.4
B14	1.0	0	0	0	0.3	0
B15	12.5	9.5	10.1	13.0	10.9	11.4
B16	2.4	0	2.1	0	2.2	0
B17	17.8	19.1	22.9	8.7	21.2	13.6
B18	5.8	0	1.7	4.4	3.0	2.3
Bw21	1.9	0	1.7	8.7	1.7	4.6
Bw22	1.9	0	2.8	0	2.5	0
B27	5.3	0	1.2	4.4	2.5	2.3
Bw35	20.2	4.8	20.8	8.7	20.6	6.8
B37	2.4	14.3	5.0	0	4.1	6.8
B40.1	10.6	0	14.9	0	13.4	0
B40.2	13.9	38.1*	16.5	17.4	15.7	27.3
Bw42	0	0	0	0	0	0
Bw44	19.7	14.3	8.3	13.0	12.0	13.6
Bw45	0.5	0	0	0	0.2	0
B5	33.7	28.6	34.4	56.5	34.2	43.2
Bw51	23.6	4.8	21.9	8.7	22.5	6.8
Bw52	7.7	9.5	8.7	30.4**	8.4	20.5*
Bw53	1.4	4.8	2.1	0	1.9	2.3
B5 IND	2.4	14.3	3.8	17.4*	3.3	15.9**
One antigen	32.2	28.6	26.4	30.4	28.3	29.6

*Uncorrected $P < 0.01$.
**Corrected $P < 0.04$.

Table 2
Selected haplotype frequencies (× 10^3) in Indians with JOD

Haplotype	ARYAN		DRAVIDIAN		TOTAL INDIAN	
	Control	JOD	Control	JOD	Control	JOD
	208	21	424	23	632	44
1, 17	27	44	70**	19	56**	30
1, 37	7	47	19*	0	15**	22
24, 7	0	14	27*	45	17	31
24, 52	19	0	16	54	17*	21
30, 13	7	47	7	0	7*	22
33, 44	18	21	12*	20	14*	20

*Δ > 2SE.
**Δ > 3SE.

are secondary to the increase in Dw4 (Christy et al. 1979). When both B8 and B15 are present their effect is additive.

In the present study, B8 and not B15 was associated with IDDM. This association was confined to the Dravidians and the relative risk (RR) was 2.5 which is the same as that found in European Caucasians. It is known that the northern populations of India were subjected to successive waves of infiltration of Mongoloid races from the northeast. These waves did not penetrate to the south of India, which is occupied by Dravidian races who originated in western Asia and settled in India in prehistoric times.

The Aryans with IDDM show similarities with Japanese, i.e. there is no increase in the frequency of B8, B15 or B5, while the Dravidian patients show an increase in B8, as found in European Caucasians and an increase in Bw52 which is the opposite of the finding in Japanese.

References

Cahill, G. F. (1979) Human evolution and insulin-dependent (IDD) and non-insulin dependent diabetes (NIDD). *Metabolism* **28**, 389.

Christy, M., Green, A., Christau, B., Kromann, H., Nerup, J., Platz, P., Thomsen, M., Ryder, L. P. & Svejgaard, A. (1979) Studies of the HLA system and insulin-dependent diabetes mellitus. *Diabetes Care* **2**, 209.

Fajans, S. S., Cloutier, M. C. & Crowther, R. L. (1978) Clinical and etiological heterogeneity of idopathic diabetes mellitus. *Diabetes* **27**, 1112.

Hammond, M. G., Appadoo, B. & Brain, P. (1975) HLA antigens in Bantu and Indians. *Histocompatibility Testing 1975*, p. 173. Munksgaard, Copenhagen.

Hammond, M. G., Appadoo, B. & Brain, P. (1977) HLA antigens in South African Negroes and Indians. *Tissue Antigens* **10**, 230.

Kawa, A., Nakamura, S., Kono, Y., Maeda, Y. & Kanehisa, T. (1979) A negative association of HLA Bw52 with Graves' disease and insulin-dependent diabetes mellitus with juvenile-onset among Japanese population. *Experientia* **35**, 547.

Kawa, A., Nakazawa, M., Kono, Y., Sakaguchi, S., Nakamura, S. & Kanehisa, T. (1978) HLA Bw54 and B5 in Japanese diabetics with juvenile-onset and insulin dependency. *Experientia* **34**, 669.

Kawa, A., Nakazawa, M., Sakaguchi, S. Nakamura, S., Kono, Y., Hazeki, H. & Kanehisa, T. (1977) HLA system in Japanese patients with diabetes mellitus. *Diabetes* **26**, 591.

Mistry, J. D. (1965) Ethnic groups of Indians in South Africa. *S. Afr. Med. J.* **39**, 691.

Nakao, Y., Fukunishi, T., Koide, M., Akasawa, K., Ikeda, M., Yahata, M. & Imura, H. (1977) HLA antigens in Japanese patients with diabetes mellitus. *Diabetes* **26**, 736.

Nerup, J., Cathelineau, Cr., Seignalet, J. & Thomsen, M. (1977) HLA and endocrine

diseases. In *HLA and Disease*, eds. Dausset, J. & Svejgaard, A., p. 149. Munksgaard, Copenhagen.

Rotter, J. I. & Rimoin, D. L. (1978) Heterogeneity in diabetes mellitus – update. *Diabetes* 27, 599.

West, K. J. (1978) *Epidemiology of diabetes and its vascular lesions.* Elsevier, New York.

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Diabetologia

HLA and Insulin-dependent Diabetes in South African Negroes

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Summary. The HLA antigens of 57 South African negroes with juvenile-onset, insulin-dependent diabetes were determined. The frequency of B8 was increased (29.8% vs 13.9%) as was the frequency of B14 (17.5% vs 6.1%). The frequency of patients with either one of these cross-reactive antigens was significantly increased after correction for the number of antigens tested (45.6% vs 19.2%, P (corrected) <0.005).

Key words: HLA, insulin-dependent diabetes, negroes.

Numerous studies of the HLA system in diabetes have shown clear associations between insulin-dependent diabetes mellitus (IDDM) and certain HLA antigens. Increased frequencies of HLA B8, B15, B18, Cw3, Dw3, Dw4, DRw3 and DRw4 have been found in white Caucasian populations with this disease [1]. An association between HLA B8 and IDDM has also been found in South Africa Indians [2] and in American Blacks [3, 4], but not in Japanese with IDDM [5, 6].

Materials and Methods

The diabetic patients attended the Diabetic Clinic of the King Edward VIII Hospital, Durban which is the main teaching hospital of the University of Natal Medical School. The patients were characterised by an acute onset of illness below 35 years old, and a dependence on insulin for control of symptoms and prevention of ketosis [7]. They were typed over a two year period for the HLA-A-B-C antigens and the frequencies were compared with those found in a healthy control population, many of whom were typed for International Workshops [8, 9]. The Negro population of Durban consists mainly of Zulus and the patients and controls studied by us were of pure descent. A total of 180 antisera were used in a two-stage microlymphocytotoxicity test [10] to determine the HLA antigens of 57 Negroes with IDDM. Lymphocytes were isolated on a Ficoll-Hypaque density gradient [11]. Frequency differences were tested for significance with a X^2 test (without Yates' correction) and the resulting probabilities corrected by multiplication by the number of antigens tested.

Results

Table 1 shows the antigen frequencies in the IDDM patients compared with the controls. The distribution of alleles at the A and B loci conform to Hardy-Weinberg equilibrium.

There were no significant differences between diabetics and control subjects at the A and C loci. The frequency of HLA B8 was increased in the diabetics (29.8%) compared to the controls (13.9%) but this was not significant after correction for the number of antigens tested. The frequency of HLA-B14, on the other hand, was significantly increased even after correction (17.5% vs 6.1%, P (corrected) <0.04). As HLA-B8 and B14 form part of a cross-reacting group, the number of patients and controls with either of these antigens were compared. The difference in the frequencies (45.6%) was highly significant (P_c <0.004). The relative risk (3.5) was about the same as for B14 alone (3.3) but greater than the relative risk for B8 alone (2.6).

There was a slightly stronger negative association between Bw42 and IDDM. The relative risk was 0.25 and the uncorrected p <0.005. This was no longer significant after correction for the number of antigens tested.

Discussion

Nerup et al. [12] discuss the possibility that there are two genes conferring increased risk of IDDM: one associated with B8 and the other with B15 or B18. In

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Table 1. Percentage frequency of HLA antigens in Negroes with insulin-dependent diabetes mellitus (IDDM)

HLA	Control n = 756	IDDM n = 57		Control n = 756	IDDM n = 57
A1	5.8	7.0	B7	16.0	22.8
A2	20.1	14.0	B8	13.9	29.8[a]
A3	13.5	10.5	B13	4.8	3.5
A11	0.1	0	B14	6.1	17.5[b]
Aw23	19.2	26.3	B15	5.8	1.8
Aw24	3.3	3.5	B16	2.4	3.5
A25	13.9	5.3	B17	38.1	21.1
A26	7.5	7.0	B18	3.8	5.3
A28	20.0	24.6	Bw21	0.5	1.8
A29	16.7	12.3	Bw22	0	0
Aw30	39.6	36.8	B27	0.3	0
Aw31	12.6	10.5	Bw35	7.3	3.5
Aw32	1.4	8.8	B37	0	0
Aw33[d]	2.7	3.5	Bw60	1.6	3.5
Only one antigen detected	23.6	29.8	Bw61	0	0
			Bw41[d]	2.1	3.5
Cw1[d]	0	1.8	Bw42	27.7	8.8[a]
Cw2[d]	18.5	21.1	Bw44	16.0	14.0
Cw3[d]	9.6	17.5	Bw45	6.4	10.5
Cw4[d]	15.8	17.5	Bw51	2.7	0
Cw5[d]	4.1	3.5	Bw52	0	0
			Bw53	3.4	3.5
B8+B14	19.2	45.6[c]	Only one antigen detected	41.1	45.6

[a] $P < 0.005$
[b] $P < 0.001$
[c] $P < 0.0001$
[d] N = 146 (Number of controls)

all the Caucasian populations studied to date the association with B8 has been a constant finding. The relationship with B15 and/or B18 has been confined to certain population groups. The association of these antigens appears to be secondary to the increased frequency of DRw3/Dw3 and DRw4/Dw4 [1, 13].

This study has demonstrated an increased frequency of B8 and of B14 in South African Negroes with IDDM. Since these antigens form a cross-reacting group it is probable that the same susceptibility gene is associated with either of these antigens in Negroes. Alternatively, it may be postulated that another susceptibility gene, associated with B15 or B18 in Caucasians, is linked to B14 in Negroes. This latter explanation seems less likely.

The Bw42 antigen has been detected only in Black populations. It is, however, one of the cross-reacting antigens associated with B7 which may be linked to some protective mechanism against IDDM [12]. The protective effect is not associated with HLA B7 in Negroes, indeed, the frequency of the antigen is greater in the IDDM group than in the controls. The relatively low frequency of Bw42 in Negroes with IDDM may have a bearing on the low prevalence of IDDM in Negroes. Alternatively, it may be a reflection of the increased frequency of B8 and B14. The difficulties of establishing the significance of a negative association between HLA and disease have been discussed by Svejgaard et al. [13].

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References

1. Ryder LP, Andersen E, Svejgaard A (1979) HLA and disease registry, third Rep. Munksgaard, Copenhagen
2. Hammond MG, Asmal AC (1979) HLA and insulin-dependent diabetes in South African Indians. Tissue Antigens
3. Cahill GF (1979) Human evolution and insulin dependent (IDD) and non-insulin dependent diabetes (NIDD). Metabolism 28: 389–393
4. Duquesnoy RJ, MacDonald MJ, Mullins P, Hackbarth SA, Trasman HS, Levitsky LL (1979) Increased frequency of HLA-Dw3 in North American Black patients with juvenile onset diabetes. Tissue Antigens 13: 369–372
5. Kawa A, Nakazawa M, Sakaguchi S, Nakamura S, Komo Y, Hazeki H, Kanehisa T (1977) HLA system in Japanese patients with diabetes mellitus. Diabetes 26: 591–595
6. Nakao Y, Fukunishi T, Koide M, Akasawa K, Ikeda M, Yahata M, Imura H (1977) HLA antigens in Japanese patients with diabetes mellitus. Diabetes 26: 736–739
7. West KJ (1978) Epidemiology of diabetes and its vascular lesions. Elesevier, New York
8. Hammond MG, Appadoo G, Brain P (1975) HLA antigens in Bantu and Indians. In: Kissmeyer-Nielsen F (ed) Histocompatibility testing 1975. Munksgaard, Copenhagen, p 173–178
9. Hammond MG, Appadoo B, Brain P (1977) HLA in Non-Caucasian populations. In: Bodmer WF (ed) Histocompatibility testing 1977. Munksgaard, Copenhagen, p 407–408
10. Terasaki PI, McClelland JD (1964) Microdroplet assay of human serum cytotoxins. Nature 204: 998–1000
11. Boyum A (1968) Separation of leucocytes from blood and bone marrow. Scand J Clin Lab Invest 21: 97
12. Nerup J, Cathelineau Cr, Seignalet J, Thomsen M (1977) HLA and endocrine disease. In: Dausset J, Svejgaard A (eds) HLA and disease. Munksgaard, Copenhagen, p 149–167
13. Christy M, Green A, Christau B, Kromann H, Nerup J, Platz P, Thomsen M, Ryder LP, Svejgaard A (1979) Studies of the HLA system and insulin-dependent diabetes mellitus. Diabetes Care 2: 209–214
14. Svejgaard A, Jersild C, Staub Nielsen L, Bodmer WF (1974) HLA antigens and disease. Statistical and genetical considerations. Tissue Antigens 4: 95–105

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Joint Report

INSULIN-DEPENDENT DIABETES MELLITUS

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Introduction

Since the discovery of Singal and Blajchman (1) of an association between insulin dependent diabetes mellitus (IDDM) and HLA-B15 and later between both B15 and B8 and IDDM (2), a large number of studies has been carried out on HLA and IDDM (3,4 for references). These studies have shown that there are even stronger associations between IDDM and HLA-DW3 and DW4 than with B8 and B15, respectively (5,6), and that HLA-DR3 and DR4 seem to be increased to the same extent as DW3 and DW4 (7). HLA typing of affected sibpairs has shown a considerable increase of HLA identical sibpairs and a strong decrease of sibpairs not sharing HLA haplotypes (8).

Various genetic models have been advanced to explain these observations: (a) a recessive model with incomplete penetrance (9,10) (b) a dominant model with incomplete penetrance (11), and (c) more complicated models involving two HLA-linked susceptibility genes associated with HLA-DW3 and 4, respectively (12), and perhaps even an HLA-DW2-associated gene conferring resistance (13,14). When the IDDM part of the 8th Workshop was planned it was uncertain which of these models fitted the findings best although evidence against both the dominant and the recessive models was available (3).

Plans for the IDDM study

The plans for the IDDM part of the 8th Workshop were prepared by a committee consisting of A. Green and M. Hauge, University of Odense; M. Christy and J. Nerup, Steno Memorial Hospital, Copenhagen; and the authors of this report. The major goal of the IDDM study was to provide more data which would enable the distinction between various genetic models of IDDM. In addition, we had the following scopes: (a) to study the HLA associations in various ethnic groups, (b) to investigate whether the HLA association(s) differ between familial and nonfamilial cases, and (c) to search for heterogeneity within IDDM in relation to the HLA determinants associated with IDDM.

To approach these goals, we recommended that investigators participating in the study (a) select a homogeneous population living in a defined geographical area; (b) include all patients who had diagnosed IDDM during a certain period in that area; (c) determine which of these patients had at least one affected sib; (d) randomly select 10 to 20 affected sibpairs and type them and their parents; and (e) randomly select and type 30 to 40 patients having no first degree relatives affected with IDDM but having one or more unaffected sibs aged 20 or more at the time. Obviously, this procedure was not possible for all investigators. In particular, complete determination was difficult although desirable because earlier studies (15) indicated that the ascertainment method might influence the results. The call for studying affected sibpairs was abandoned for investigators studying particularly interesting ethnic groups, e.g., African and American Blacks, Japanese, and Basques. The reason for not typing healthy sibs or the parents of nonfamilial cases was to save serum and time and seemed justified because the information inherent in such relatives is limited.

The following diagnostic criteria for IDDM were recommended: the disease should have onset before age 40, and should be idiopathic, ketosis-prone, and the patients should be nonobese and insulin-dependent (i.e., not just insulin treated).

The clinical information required on all diabetics typed appears from the punch card format shown in Table 1 (this card was first called 'DM' but later '44' by the Los Angeles analyzers).

The family history requested for each propositus is exemplified in Table 2.

Participants and data obtained

Table 3 gives the names and institutions of the investigators of the 24 groups participating in this study and Table 4 summarizes the number of propositi used in this analysis. In some cases, more data were received but not analyzed because the information received was ambiguous. It appears that 636 nonfamilial, 158 familial, and 85 'unknown' propositi were included in the study. Because parents, affected sibs, and sometimes unaffected sibs were also typed for the familial cases, the total number of typings performed exceeds 1200. These typings include both HLA-A,B,C, and DR typings, although the latter were not always successful. In addition, we sought and obtained information from the participants about the HLA-A,B,C, and DR types of about 1600 controls, most of whom were typed with the 8th Workshop sera.

Results

Familial versus nonfamilial IDDM. Only a limited number of groups had studied and given information about sufficient numbers of both nonfamilial cases (group B patients) selected according to the strict criterion of at least one healthy sib aged 20 or more and patients with affected first degree relatives (group A patients). It appears from Table 5 that there are no significant differences between these two groups concerning the frequencies of the DR3 and DR4 antigens or the DR3,4 phenotype although there is a tendency to a higher frequency for each of these

antigens and in particular for the DR3,4 phenotype among the familial propositi. However, when the phenotypes involving only DR3 and/or DR4 were pooled, there was a significant difference between familial and nonfamilial cases. Nevertheless, because all the differences in Table 5 are quite small and because most data sets did not allow distinction between familial and nonfamilial cases, it was considered justified (and necessary) to pool familial and nonfamilial cases in most of the remaining analyses.

HLA-B versus DR associations. These associations were studied in a number of Caucasian populations. Table 6 summarizes the results of these analyses. It appears that DR3 is significantly increased both in B8 positive and in B8 negative patients compared to B8 positive and B8 negative

Table 1. Patient coding form.

01 02	D M	Card Number	
03 04 05		Typing Lab.	Use 8. work-shop code
06 07 08		Individual ID-number	
09 10		Pedigree number	
11		Ascertainment: Proband = 1, other = 0	
12		IDDM in first degree relatives = 1, isolated case = 0	Family history
13		Other types of DM in first degree relatives = 1, no = 0	
14		IDDM in other relatives, yes = 1, no = 0	
15 16		Age at onset (years)	
17 18		Month of onset (01 = January, ..., 12 = December)	
19 20 21		Present weight in kilograms	
22 23 24		Present height in centimeters	
25		Episodes of ketoacidosis No = 0, Yes = 1	
26 27		Duration of insulin treatment (years)	
28 29 30 31		Present insulin requirement (IU. pr. kg. day, two decimal places)	
32 33 34		Type of insulin (fill out name(s):)	
35		Species of insulin (Pork = P, Beef = B, mixed = M, other = X)	
36 37 38		Present fasting blood glucose (mg. pr. 100 ml)	
39 40 41		Present postprandial blood glucose (mg. pr. 100 ml)	
42 43 44		Present daily glucose excretion in urine (g pr. 24 hours)	
45		Ketonuria presently	No = 0, Yes = 1
46		Retinopathy presently	
47		Abnormal tendon reflexes presently	
48		Decreased vibration sense presently	
49		Proteinuria presently	
50 51 52		Present serum-creatinine (m-mol pr. litre)	
53 54 55 56		Fasting C-peptide level (pico-mol pr. litre, two decimal places)	
57		ICA never detected = 0, present = 1, earlier = 2, not investigated = blank	
58 59		ICA first investigated (years after diagnosis)	
60 61		ICA persisted (years after diagnosis, if still present = 99)	
62 63		Other endocrine disorders,	00 = no 01 = Graves' disease 02 = Hypothyroidism 03 = Addison's disease 04 = Pernicious anemia 05 = Hypergonadotropic hypogonadism 06 = Idiopathic hypoparathyroidism 07 = other example: 13 = both Graves' and Addison's disease

Give comments, name(s) of other chronic diseases and name, full address and phone number of the person who filled out this form:
(use reverse side)

controls. In contrast, B8 is not increased either in DR3 positive or in DR3 negative patients compared to the corresponding control groups. Accordingly, it can be concluded that the deviation seen for B8 in IDDM is entirely secondary to the increase of DR3. In analogy, the increases of B15 and B18 appear to be secondary to increases of DR4 and DR3, respectively, and the decrease of B7 is secondary to that of DR2 (the significant decrease of B7 in DR2 positive patients may be considered a chance deviation). The significant heterogeneity seen in some of these comparisons is probably due to the fact that the associations between DR antigens and IDDM and between HLA-B antigens and DR antigens vary considerably among the populations studied.

Because of these observations and because the time available for the analyses was rather limited, we decided to concentrate the remaining studies on the DR antigens alone.

Unfortunately, sufficient control materials allowing us to analyze the DR3 versus the BfF_1 frequencies were not available.

DR antigen frequencies (Table 7). For the patients, we have shown both the Los Angeles and the local DR antigen assignment, but because local DR antigen assignments were used for the controls, we have also used local assignments for the patients in the calculations of relative risks. The following picture emerges: DR1 shows no significant deviations in any population; DR2 is decreased in all populations studied; DR3 is increased in almost all populations except perhaps the Japanese. The highest relative risk is seen for the Basques but it is also high for Yugoslavians and non-Ashkenazi Jews; DR4 is uniformly increased in all populations including the Japanese. The relative risk for DR4 positives is generally higher than for DR3 positives; DR5 was decreased in most populations; DRW6 was not analyzed because this antigen was poorly defined by the disease serum set; DR7 was decreased in most populations; and DRW8 showed no significant deviations in Caucasians but was significantly increased in Japanese.

In brief, DR4 was increased in all IDDM patients studied throughout the world and DR3 was increased in all

Table 2

IDDM - Family sheet.

TYPING LAB CODE: PEDIGREE No. IF TYPED IN EIGTH WORKSHOP:

	ID-no. if typed in 8. workshop	Sex F=female M=male	Ethnic group Use 8. workshop code	Year of birth	Non-diabetic=N If DM, Y. of diagn. IDDM / Other types of DM	Alive=A If deceased Y. of death	HLA-phenotype if typed outside 8. workshop	Comments
Proband								
Father		M						
Mother		F						
1. sibling 2. sibling 3. sibling etc.								
1. child 2. child etc.								

This scheme should be filled out for each proband typed as part of the workshop (isolated as well as familial cases).

Typing Lab code = positions 3-5 of Card 01; Pedigree no. = positions 35-36 of Card 01.

If information on more than three generations is available, please fill out more sheets.

Note: Information on all non-diabetic relatives is also required (sex, year of birth, death, etc.), irrespectively whether they are alive now or not.

Please give name, full address and phone number of the person who filled out this sheet:

Table 4.

LAB-code	Bw code	Ethnic Group	No. of propositl In: sporadic	familial	affected sibships	unknown	Normal controls
BER	21	German	59	15	14		53
BOD	19	English				17	44
BRA	24	Ashk.jew	33	4	3		46
"	25	non-A.jew	25	10	4		35
BRG	02	Amer.Black	25	7	} 11		36
"	11	Amer.Cauc.		9			
BRT	17	Czeck.	9	4	1		74
BSH	14	Australian	23	13	12		57
CEP	30	[illegible]	23	11	10		57
DEM	"34"	[illegible]	49	2	2		44
GUT					2		
HAM	01	Afr.Black	31			9	54
"	13	As.Ind.	16	1		4	41
HAN					1		
JUJ	06	Japanese	54			2	104
KAS	31	Yugosl.	32	10	8		63
KRE	29	Spanish	29	12	10		52
MOL	26	Swedish	20	2	1		83
MYR	15	Austrian			1	53	150
RAF					17		133
RUB	02	Amer.Black	5				{ 26 Spanish
"	"34"		20	1			{ 25 non-"
"	00	unknown	5				
SAI	06	Japanese	29				
SUF					1		
SVE	26	Danish	44	20	20		174
TII	32	Finnish	17	13	6		49
TSU	06	Japanese	64				116
VIL	29	Spanish	24	24	10		75
		Total	636	158	134	85	1.591

Table 5. Familial versus non-familial IDDM.

Investigator	No. of cases Fam.	Non-F.	DRw3 Per cent pos. Fam.	Non-F.	Odds Ratio	DRw4 Per cent pos. Fam.	Non-F.	Odds Ratio	DRw3, 4 Per cent pos. Fam.	Non-F.	Odds Ratio	DRw3 and/or 4 alone Per cent pos. Fam.	Non-F.	Odds Ratio
BER	15	59	40	51	.66	73	47	2.82	13	15	.98	73	51	2.47
BSH	13	5	69	80	.70	85	80	1.53	62	60	1.10	92	60	5.95
CEP	11	15	64	60	1.14	73	73	.95	36	40	.88	64	60	1.14
KAS	10	15	90	67	3.32	70	60	1.47	70	27	5.48	80	33	6.49
SVE	20	44	60	59	1.03	75	80	.75	45	41	1.18	75	80	.75
TII	12	5	50	20	3.00	92	100	.70	50	20	3.00	58	40	1.91
Combined	81	143			1.10			1.33			1.46			1.90
χ^2 sign.					.11			.82			1.58			4.48*
χ^2 heterog.					3.50			2.97			4.19			6.32

Familial cases involved one or more affected first degree relatives (usually sibs) in addition to the propositus. Non-familial cases had no affected first degree relatives and at least one healthy sib at the age of 20 or more.

Odds ratio were calculated for the frequencies of the phenotypes indicated in familial versus non-familial cases.

χ^2 sign. = chi square (1 d.f.) for the deviation of the odds ratio from unity.

χ^2 heterog. = chi square for heterogeneity between the individual odds ratios.

*: $p < .05$

The comparisons under "DRw3" and "DRw4" involve all DRw3-positive and DRw4-positive patients against DRw3-negative and DRw4-negative patients, respectively. The "DRw3,4" comparison compares the DRw3,4-phenotype against all other phenotypes. In the "DRw3 and/or 4 alone" comparison, patients having only DRw3 and/or 4 (but no other detectable DR antigens) were tested against the remaining patients.

Table 6. HLA-B versus DR associations (Caucasians).

Antigens		Lab.	Odds Ratio				
B	DR		B-pos.	B-neg.	DR-pos.	DR-neg.	
8	3	BER	3.00	2.77	1.17	1.08	
		BSH	2.24	3.16	1.59	2.24	
		DEM	1.89	17.02*	.60	5.36	
		KAS	3.26	27.05*	.19	1.59	
		MYR	1.21	1.87	1.30	2.00	
		SVE	3.85	5.58*	.56	.81	
		TIL	.55	4.09	.42	3.18	
		MOL	3.00	1.51	1.22	.61	
		VIL	16.33	4.58	1.50	.42	
		Combined	2.21	4.93	.76	1.44	
			5.53	70.38	1.47	1.31	χ^2 sign.
			5.63	19.03	8.67	4.43	χ^2 het.
			8	8	8	8	d.f.
15	4	BER	4.22	5.46*	.75	.97	
		BSH	19.00	3.35	4.05	.71	
		DEM	25.00	5.91*	1.06	.25	
		KAS	17.00	12.47*	.41	.30	
		MYR	3.21	5.16*	.70	1.13	
		SVE	45.00*	4.70*	2.81	.29	
		TIL	21.00	12.76*	1.05	.64	
		MOL	4.37	16.69*	.63	2.42	
		VIL	7.00	5.97*	.53	.45	
		Combined	7.79	6.07	1.16	.77	
			35.73	125.78	.471	.761	χ^2 sign.
			7.41	7.18	12.14	4.64	χ^2 het.
			8	8	8	8	d.f.
18	3	BER	1.17	3.83*	1.07	3.48	
		BSH	65.00*	2.76	4.57	.19	
		DEM	10.43	8.01*	2.25	1.73	
		KAS	2.14	13.26*	.27	1.67	
		TIL	3.00	1.92	1.00	.64	
		MOL	2.33	1.78	3.53	2.70	
		VIL	15.92*	3.52	1.58	.35	
		Combined	4.79	3.83	1.48	1.28	
			15.07	47.51	1.78	.60	χ^2 sign.
			9.80	11.27	7.35	9.76	χ^2 het.
			6	6	6	6	d.f.
7	2	BER	.10*	.09*	1.80	1.54	
		BSH	.27	.36	.65	.86	
		DEM	.14	.07	.69	.33	
		KAS	.02*	.76	.24	11.57*	
		MYR	.04*	.28	.26	2.07	
		SVE	.09*	.06*	.50	1.07	
		TIL	.15	.27	.29	.54	
		MOL	.22	1.12	.31	1.58	
		VIL	.44	3.57	.18	1.47	
		Combined	.13	.37	.46	1.28	
			37.37	19.12	4.42	1.54	χ^2 sign.
			6.44	30.09	4.25	14.64	χ^2 het.
			8	8	8	8	d.f.

Explanation: Odds ratios in column "B-pos." give the risk of developing IDDM for individuals having both the B and DR antigen in question as compared to controls having both antigens. For example, for "BER", the odds ratio of 3.00 for "B-pos." is the risk of IDDM for Dw3-positives among B8-pos. patients compared to B8-pos. controls, i.e. Dw3 is increased even in B8-pos. patients. In analogy, DR3 is also increased in B8-neg. patients (odds ratio = 2.77), while B8 is not increased in DR3-pos. or DR3-neg. patients (odds ratios = 1.17 and 1.08, respectively).

Table 7. DR antigen frequencies.

Antigen	Lab.	Patients, L.A. %	N	Patients, local %	N	Controls, loc. %	N	rel. risk
DR 1	BER			14.9	74	24.5	53	.5
	BOD	47.1	17	47.1	17	29.5	44	2.1
	BRT	0	13	7.7	13	4.1	74	2.5
	BSH	11.8	34	11.1	36	26.3	57	.4
	KAS	0	18	14.3	42	20.6	63	.7
	KRE			13.5	37	26.9	52	.4
	MOL			9.1	22	15.7	83	.6
	MYR			18.9	53	18.7	150	1.0
	SVE	9.4	64	9.4	64	20.7	174	.4
	TII			6.7	30	26.5	49	.2
	VIL	13.6	44	14.6	48	9.8	137	1.7
	Combined				436		936	0.72
						p. significance = .04		
						p. heterog.		> .05
DR 2	BER			6.8	74	39.6	53	.1
	BOD	0	17	0	17	27.3	44	.1
	BRT	0	13	0	13	25.7	74	.1
	BSH	5.9	34	5.6	36	22.8	57	.2
	KAS	27.8	18	19.0	42	34.9	63	.5
	KRE			2.7	37	30.8	52	.1
	MOL	-		9.1	22	26.5	83	.3
	MYR			5.7	53	28.7	150	.2
	SVE	4.7	64	4.7	64	30.5	174	.1
	TII			3.3	30	26.5	49	.1
	VIL	2.3	44	14.6	48	12.0	137	1.3
	Combined				436		936	.25
						p. significance		$< 10^{-10}$
						p. heterog.		= .01
DR 3	BER			48.6	74	15.1	53	5.1
	BOD	47.1	17	47.1	17	29.5	44	2.1
	BRT	30.8	13	30.8	13	25.7	74	1.3
	BSH	70.6	34	66.7	36	35.1	57	3.6
	KAS	77.8	18	76.2	42	22.2	63	10.6
	KRE			59.5	37	17.3	52	6.6
	MOL			27.3	22	32.5	83	.8
	MYR			37.7	53	22.0	150	2.1
	SVE	59.4	64	59.4	64	28.2	174	3.7
	TII			33.3	30	20.4	49	1.9
	VIL	47.7	44	52.1	48	18.9	137	4.6
	Combined				436		936	3.30
						p. significance		$< 10^{-10}$
						p. heterog.		= 0.008
DR 4	BER			55.4	74	7.5	53	13.6
	BOD	58.8	17	58.8	17	25.0	44	4.1
	BRT	53.8	13	69.2	13	17.6	74	9.6
	BSH	70.6	34	66.7	36	31.6	57	4.2
	KAS	50.0	18	66.7	42	15.9	63	10.0
	KRE			51.4	37	21.2	52	3.8
	MOL			59.1	22	25.3	83	4.1
	MYR			58.5	53	23.3	150	4.6
	SVE	78.1	64	76.6	64	32.2	174	6.7
	TII			83.3	30	26.5	49	12.5
	VIL	38.6	44	54.2	48	12.0	137	18.7
	Combined				436		936	6.36
						p. significance		$< 10^{-10}$
						p. heterog.		> .05
DR 5	BER			8.1	74	1.9	53	3.3
	BOD	0	17	0	17	22.7	44	.1
	BRT	7.7	13	69.2	13	24.3	74	.7
	BSH	14.7	34	0	36	26.3	57	0
	KAS	16.7	18	7.1	42	42.9	63	.1
	KRE			5.4	37	15.4	52	.4
	MOL			0	22	10.8	83	.2
	MYR			11.3	53	25.3	150	.4
	SVE	4.7	64	4.7	64	6.9	174	.7
	TII							
	VIL	4.5	44	8.3	48	14.9	137	.6
	Combined				406		887	.39
						p. significance		$= 7 \times 10^{-6}$
						p. heterog.		= .02

Table 7 continued.

Antigen	Lab.	Patients, L.A. %	N	Patients, local %	N	Controls, loc. %	N	rel. risk
DR 7	BER			12.2	74	5.7	53	2.1
	BOD	5.9	17	5.9	17	25.0	44	.3
	BRT	7.7	13	15.4	13	36.5	74	.4
	BSH	2.9	34	2.8	36	17.5	57	.2
	KAS	0	18	2.4	42	20.6	63	.1
	KRE			10.8	37	34.6	52	.3
	MOL			4.5	22	10.8	83	.5
	MYR			20.8	53	26.0	150	.8
	SVE	4.7	64	4.7	64	24.1	174	.2
	TII			3.3	30	10.2	49	.4
	VIL	18.2	44	18.8	48	17.1	137	1.2
	Combined				436		936	.51
						p. significance		$= 10^{-4}$
						p. heterog.		= .02
DR 8	BER							
	BOD	0	17	0	17	2.3	44	.8
	BRT	30.8	13					
	BSH	2.9	34	2.8	36	5.3	57	.7
	KAS	5.5	18	4.8	42	4.8	63	1.2
	KRE			4.5	22	3.6	83	1.6
	MOL							
	MYR							
	SVE	9.4	64	9.4	64	7.5	174	1.3
	TII			3.3	30	2.0	49	1.6
	VIL	2.3	44	0	48			
	Combined				259		470	1.19
						p. significance		> .05
						p. heterog.		> .05

Table 7 continued. Special groups.

Antigen	Lab.	Patients, L.A. %	N	Patients, local %	N	Controls, local %	N	rel. risk
DR 1	DEM			18.4	49	31.8	44	.5
	RUB/34			25.0	20	19.2	26	1.4
	BRA/24	11.8	17	10.8	37	6.5	46	1.7
	BRA/25	6.7	30	2.9	35	14.3	35	.2
	HAM/13	0	11	0	16	2.4	41	.8
	BRG/02	6.7	30	3.1	32	16.7	36	.2
	HAM/01		14	9.7	31	3.7	54	2.6
	JUJ	13.0	54	12.7	55	9.6	104	1.4
	TSU	13.1	61	10.9	64	9.5	116	1.2
	SAI§			3.4	29	10.7	792§	.4
	Combined Jap. §				145			1.08
						p. significance >		.05
						p. heterogen. >		.05
DR 2	DEM			0	49	22.7	44	0 ***
	RUB/34			0	20	19.2	26	.1
	BRA/24	5.9	17	5.4	37	17.4	46	.3
	BRA/25	0	30	0	35	25.7	35	0 **
	HAM/13	18.2	11	12.5	16	29.3	41	.4
	BRG/02	0	30	3.1	32	30.6	36	.1 **
	HAM/01		14	6.5	31	13.0	54	.5
	JUJ	11.1	54	10.9	55	35.6	104	.2 **
	TSU	11.5	61	10.9	64	33.6	116	.3 **
	SAI§			13.8	29	35.6	792	.3 *
	Combined Jap. §				145			.27
						p. significance =		6×10^{-7}
						p. heterogen. >		.05

Table 7 continued. Special groups.

Antigen	Lab.	Patients, L.A. %	N	Patients, local %	N	Controls, local %	N	rel. risk
DR 3	DEM			87.8	49	34.1	44	12.7 ***
	RUB/34			30.0	20	19.2	26	1.8
	BRA/24	41.2	17	40.5	37	13.0	46	4.3 **
	BRA/25	66.7	30	65.7	35	14.3	35	10.4 ***
	HAM/13	27.3	10	25.0	16	9.8	41	3.0
	BRG/02	43.3	30	37.5	32	13.9	36	3.5 *
	HAM/01		14	41.9	31	33.3	54	1.4
	JUJ	3.7	54	3.6	55	0	104	9.8
	TSU	0	61	0	64	1.7	116	.4
	SAI§			6.9	29	1.9	792	4.6
	Combined Jap. §				145			3.25
						p. significance =		.02
						p. heterogen. >		.05
DR 4	DEM			34.7	49	6.8	44	6.4 **
	RUB/34			80.0	20	34.6	26	6.8 **
	BRA/24	88.2	17	78.4	37	37.0	46	5.9 ***
	BRA/25	83.3	30	85.7	35	20.0	35	21.1 ***
	HAM/13	54.5	11	56.3	16	4.9	41	20.0 ***
	BRG/02	40.0	30	40.6	32	5.6	36	9.6 **
	HAM/01		14	35.5	31	7.4	54	6.3 **
	JUJ	53.7	54	58.2	55	46.2	104	1.6
	TSU	78.7	61	82.8	64	47.4	116	5.2 ***
	SAI§			86.2	29	40.7	792	8.3 ***
	Combined Jap. §				145			3.63
						p. significance =		1.5×10^{-8}
						p. heterogen. =		.003

Antigen	Lab.	Patients, L.A. %	N	Patients, local %	N	Controls, local %	N	rel. risk
DR 5	DEM			0	49	13.6	44	.1 *
	RUB/34			30.0	20	19.2	26	1.8
	BRA/24	0	17	8.1	37	47.8	46	.1 ***
	BRA/25	3.3	30	11.4	35	40.0	35	.2 *
	HAM/13	9.1	11	12.5	16	19.5	41	.7
	BRG/02	6.7	30	3.1	32	11.1	36	.3
	HAM/01		14	12.9	31	20.4	54	.6
	JUJ	0	54	0	55	3.8	104	.2
	TSU	8.2	61	7.8	64	4.3	116	1.9
	SAI§			0	29	3.4	792	.5
	Combined Jap. §				145			.97
						p. significance >		.05
						p. heterogen. >		.05
DR 7	DEM			14.3	49	14.3	44	.1 ***
	RUB/34			15.0	20	19.2	26	.9
	BRA/24	17.6	17	21.6	37	21.7	46	1.0
	BRA/25	6.7	30	14.3	35	17.1	35	.8
	HAM/13	9.1	11	12.5	16	19.5	41	.7
	BRG/02	23.3	30	12.5	32	11.1	36	1.1
	HAM/01		14	22.6	31	14.8	54	1.7
	JUJ	1.9	54	0	55	1.0	104	.6
	TSU	0	61	0	64	2.6	116	.3
	SAI§			6.9	29	.6	792	13.0 *
	Combined Jap. §				145			2.82
						p. significance >		.05
						p. heterogen. =		.006

Table 7 continued. Special groups.

Antigen	Lab.	Patients, L.A. %	N	Patients, local %	N	Controls, local %	N	rel. risk
DR 8	DEM			2.0	49	0	44	2.8
	RUB/34							
	BRA/24	5.9	17	2.7	37	4.3	46	.7
	BRA/25	0	30	2.9	35	2.9	35	1.0
	HAM/13	9.1	11	0	16	2.4	41	.8
	BRG/02	3.3	30					
	HAM/01		14	6.5	31	1.9	54	3.0
	JUJ	24.1	54	18.2	55	13.5	104	1.4
	TSU	27.9	61	28.8	59	8.5	116	4.3 **
	SAI§			44.8	29	16.8	792	4.0 **
	Combined Jap. §				145			3.0
						p. significance =		3.1×10^{-6}
						p. heterogen. >		.05

L.A. = Los Angeles, "local" = local antigen assignment.
The special ethnic groups were as follows: DEM=Basques; RUB/34='Spanish', Puerto Rican; BRA/24=Ashkenazi Jews; BRA/25=Non-Ashkenazi Jews; HAM/13=Asian Indians ('Tamil'); BRG/02=American Blacks; HAM/01=African Blacks (Zulu). JUJ, TSU, and SAJ = Japanese.
Combined estimates of relative risks have been calculated for the Caucasian groups not listed as special, and for the three Japanese groups. One, two, and three asterisks indicate significance at the 5, 1, and .1 per cent level, respectively. These p-values and those for the combined estimates are uncorrected.
§ No local controls were received from SAJ's laboratory and so we have used Los Angeles controls for these data which is not strictly correct because these controls include those from JUJ and TSU but the error(s) are unlikely to be large.

Table 8. Relative risks for some DR phenotypes.

	DR3 vs (0+X) R.R.	χ^2	3, 4 vs (0+X) R.R.	χ^2	4 vs (0+X) R.R.	χ^2	3, X vs (0+X) R.R.	χ^2	4,X vs (0+X) R.R.	χ^2
BER	9.6	14.0	24.5	14.5	10.3	14.0	4.7	6.5	10.3	14.0
BOD	10.2	4.8	18.4	8.5	18.4	4.7	1.3	.1	3.2	2.5
BRA-A	10.2	3.9	42.8	17.7	23.8	15.2	7.3	4.1	9.3	9.1
-Non-A	114.3	12.6	190.6	25.2	42.5	13.8	5.4	2.3	20.0	9.9
BRT	2.7	.6	14.5	8.9	18.6	11.3	2.2	.7	11.8	7.9
BSH	11.4	8.6	43.0	20.7	16.0	9.9	.6	.3	.6	.2
CEP	10.4	4.3	137.9	21.3	56.8	12.8	12.9	7.8	10.0	6.0
KAS	47.4	14.1	1027.0	30.5	43.9	10.5	20.0	12.8	20.8	12.3
KRE	14.7	12.1	99.7	17.1	7.2	4.0	2.0	.9	2.7	2.5
MOL	8.3	4.7	24.6	13.0	28.3	14.5	1.1	.8	13.2	7.4
MYR	7.6	12.0	13.1	18.4	9.2	17.7	1.6	.6	4.1	8.8
SVE	9.2	10.5	80.1	45.4	17.0	22.2	4.4	4.7	5.2	6.1
TII	.0	.1	15.0	14.6	9.2	9.4	1.4	.1	12.8	12.0
VIL	25.2	12.8	14.3	19.7	20.6	10.8	5.3	6.9	6.9	10.1
Combined risk	10.53		33.06		15.05		3.32		6.32	
χ^2_1	102.71		253.48		164.38		31.20		92.54	
χ^2_k	84.7		21.91		6.55		16.71		16.32	
d.f.	13		13		13		13		13	
-95	6.68		21.49		10.39		2.18		4.34	
+95	16.59		50.86		24.18		5.07		9.20	
RUB Span.	15.0	5.2	21.0	7.0	9.0	4.5	3.9	1.4	17.2	9.8
DEM	123.7	25.1	328.6	23.4	10.6	4.1	9.1	9.5	10.6	5.4
HAM-As.Ind.	1.8	.2	38.2	9.4	20.0	10.7	2.3	.8	5.5	2.5
HAM-Afr.Bl.	1.2	.1	11.8	7.3	43.4	10.9	3.3	3.2	1.7	.3
BRG-Am.Bl.	14.5	7.9	43.4	10.1	81.9	14.8	2.3	1.1	2.9	1.1
	DR0 vs (0+X)		4, 8 vs (0+X)		4 vs (0+X)		8, X vs (0+X)		4, X vs (0+X)	
JUJ	1.8	.6	25.1	14.9	3.5	8.8	1.8	.6	1.7	1.2
TSU	9.5	7.1	13.0	18.9	5.9	14.5	6.8	4.6	4.2	9.2
Jap. Combined risk	3.84		16.2		4.43		3.18		2.0	
χ^2_1	5.57		33.4		22.5		3.92		0.73	
χ^2_k	2.11		.41		.69		1.26		1.65	
-95	1.26		6.30		2.39		1.01		1.41	
+95	11.73		41.63		8.18		9.97		5.49	

χ^2_1 = chi square for significance (1 d.f.) of relative risk differing from unity. χ^2_k = chi square for heterogeneity between the individual risks. D.f. = degrees of freedom for χ^2_k. -95 and + 95 are the 95% limits for the combined risk.

Caucasian patients, in American Blacks, but not significantly in African Blacks or Japanese. An increase of DRW8 in Japanese patients may substitute for the increase of DR3 in Caucasian patients. It should be pointed out, however, that the definitions of DR4 and DRW8 in Japanese are not as clear as in Caucasians. Finally, the decrease of DR2 is a characteristic of all populations studied. However, a number of DR2 positive IDDM patients were observed, but about 75% of these were either DR3 or DR4 positive. The frequency of patients lacking both DR3 and 4 ranged from 2.4 to 20.8% in Caucasians, 20.0 to 30.8% in Blacks, and was 37.5% in Asian Indians. In Japanese, 6.9 to 21.8% of the patients lacked both DR4 and DRW8. The associations were the same in the two sexes.

The relative risk for some DR phenotypes (Table 8). 'X' indicates antigens DR1, 2, 5, 7, and DRW8, and '0' indicates absence of detectable DR antigens. The relative risks were calculated against the absence of DR3 and 4 in patients and controls. It can be seen that the DR3,4 phenotype has the highest risk in Caucasians and DR4,DRW8 the highest in Japanese. However, when calculating the relative risks for the DR3 and DR4 phenotypes, it should be noted that the DR3 and DR4 phenotypes comprise both homozygotes (DR3,3 and DR4,4) and heterozygotes (DR3,0 and DR4,0). It seems likely that the relative risks for these heterozygotes are of the same order of magnitude as those for DR3,X and DR4,X, which are much lower than for the DR3 and DR4 phenotype, while the relative risks for the true homozygotes, DR3,3 and DR4,4, probably are higher. Nevertheless, when the HLA-DR genotype distribution of propositi who had been reliably HLA-DR genotyped by family studies (mainly familial cases) was analyzed, the picture seen in Table 9 emerged. Here patients who might be DR3,0 and DR4,0 heterozygous were classified as DR3,3 and DR4,4 homozygotes. When comparing these patient genotype frequencies with those expected in Caucasian controls on the basis of gene frequencies obtained from the analysis by Baur and Danilovs it appears that the relative risk for the DR3,4 heterozygotes by far exceeds that for each of the two homozygotes. Unfortunately, significant testing of the relative risks in Table 9 was difficult because the control frequencies were obtained indirectly via gene frequencies. Another weakness is that the patient samples were obtained from different populations showing considerable variations in their associations between HLA-DR and IDDM.

Affected sibpairs. It can be seen from Table 10 that there were 134 families with at least two affected sibpairs; in 12 families there were more than two affected sibpairs and in these cases we selected the two eldest affected sibs

Table 9. HLA-DR genotype distribution of propositi with familial IDDM

DR Genotype	IDDM Number	IDDM Per cent	Controls Per cent	Relative Risk
3/3	4	10.7	1.2	97.9
3/3 or 3/0	8			
3/4	46	41.1	2.6	173.6
4/4 or 4/0	4	9.8	1.4	76.9
4/4	7			
3/X or 3/0	8	7.1	16.9	4.6
4/X or 4/0	29	25.9	18.5	15.4
X/X, X/0, and 0/0	6	5.4	59.3	(1.00)
Total	112			

All patients who might be DRw3/3 or 4/4 homozygous have been classified as if they were homozygous. In contrast, the corresponding frequencies for controls involves only homozygotes. Control genotype frequencies were estimated from control gene frequencies assuming Hardy-Weinberg equilibrium. These gene frequencies are "averages" between European and North American values (Baur & Danilovs, this volume): p3=.110, p4=.120, px+po=.770. The relative risks were calculated against the genotypes not involving DRw3 or 4.

for analysis; in nine families one of the parents was also affected. When HLA-DR typing failed, sibpairs were classified by ABC antigens. Not all affected sibpairs could be unequivocally assigned as sharing 2, 1, or 0 haplotypes, and the doubtful pairs were divided with weights according to the proportions between the unequivocal pairs sharing 2, 1, or 0 haplotypes. The 'adjusted total' was used in the analysis according to the formula of Thomsen and Bodmer (9). The results of this analysis are shown in Figure 1, where we have also included the results of previously published data. There is to our knowledge no overlap between these published data and the Workshop material. It appears that the Workshop material shows almost precisely the same distribution between pairs sharing 2, 1, and 0 haplotypes as that seen in the published data. In the combined material, 59% share both, 37% share one, and 5% share no haplotypes. The abscissa of the figure is the frequency of the putative 'diabetes gene' while the ordinate is the chi square (with 2 df) for the goodness of fit between the observed distribution of the three classes (sharing 2, 1, and 0 haplotypes) and those expected according to the two simple hypotheses, dominant and

Table 10. Affected sibpair: number of haplotypes shared (IBD)*

LAB.code	2	(1-2)	1	(0-1)	0	N
BER	5	1	6	2		14
BRA	5				2	7
BRG	4	2	4	1		11
BRT	1					1
BSH	8	1	2		1	12
CEP	4	3	2		1	10
DEM	1		1			2
GUT	1		1			2
HAN	1					1
KAS	4		3		1	8
KRE	5	2	1		1	10
MOL	1					1
MYR	1					1
RAF	5	5	6	1		17
SUF	1					1
SVE	11		8	1		20
TII	3		3			6
VIL	6	1	2	1		10
A: no other first degree rel. affected	54	14	36	6	3	113
B: additional sib's affected (first pair included)	9	0	1	0	2	12
C: affected parent(s)	5	1	2	0	1	9
Total	68	15	39	6	6	134
Adjusted total	78	-	49	-	7	134

* IBD = Identical by descent

Explanation:

Not all sibpairs could unambitiously be classified as sharing 2, 1 or zero of the parental haplotypes (IBD), and thus two subgroups for doubtful cases had to be considered.

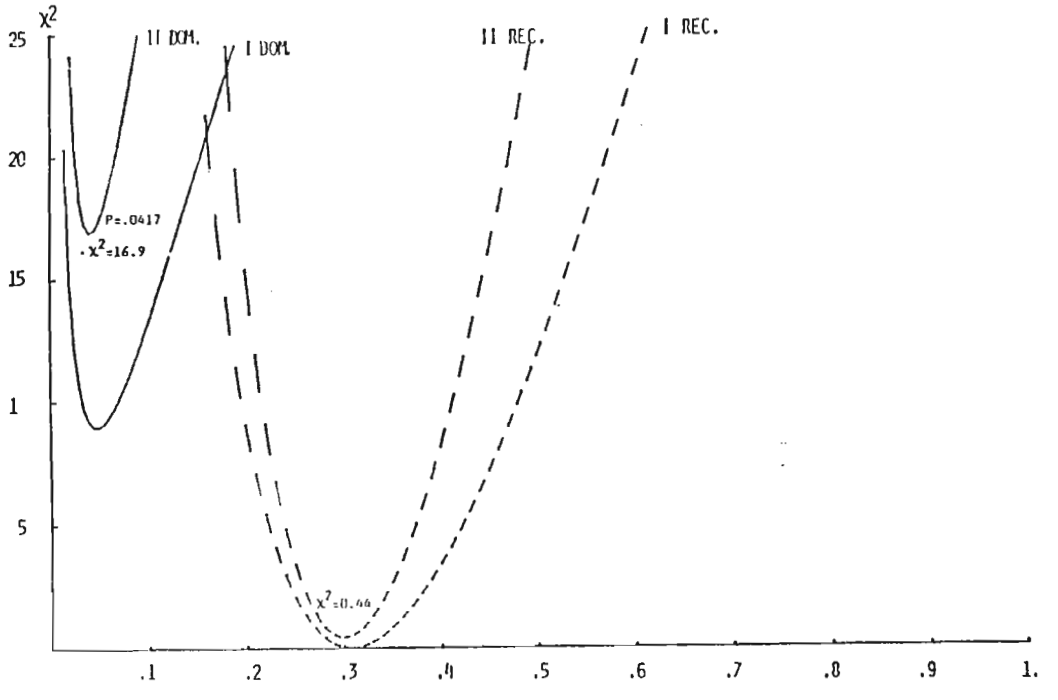
A typical example is that one of the parents carries only A1, B8, DR3 and can have given two indistinguishable haplotypes to the children.

The two doubtful subgroups were later divided with weights according to the proportions between the definite cases sharing 2, 1 and zero haplotypes, thus giving the "adjusted total".

recessive (both with or without complete penetrance). It appears that the minimum X^2 for the dominant model is 16.9 ($P<0.001$) which makes this model unlikely. The minimum $X^2=0.44$ for the recessive model does not correspond to significant P values, but if a X^2-value of 5.99 ($\sim P=0.05$) is used, the lowest acceptable frequency of the 'diabetes gene' is 0.234.

A number of HLA recombinants was seen in the family material. However, because we were informed by one investigator that at least one family was included because it contained a recombinant child and because attempts to rule out extrapaternity (by typing for other genetic markers) had generally not been done, we did not estimate the recombination fraction(s).

Figure 1. Analysis of haplotype distribution of affected sibpairs.



Number of shared haplotypes	2	1	0
8th Workshop study number (I)	78	49	7
Other data*	76	48	5
Total (II)	154	97	12
Percent	58.6	36.9	4.6

Abscissa Gene frequency for the putative 'diabetes susceptibility gene' (D).

Ordinate: X^2 (2 d.f.) for the goodness of fit between the observed distribution (58.6, 36.9, and 4.6%) with those expected to the dominant (DOM) and recessive (REC) models.

Curves 'I' are for the Workshop data alone and curves 'II' are for all available data.

The minimum X^2 for curve 'II DOM' is 16.9 at a gene frequency of 0.04 while the minimum X^2 for 'II REC' is 0.44 at a gene frequency of 0.30.

*Other data: Barbosa et al (19) (18 pairs), Cudworth (20) (40 pairs), Moller and Persson, personal communication (8 pairs), Ryder et al (3) (28 pairs), Spielman et al (11) (15 pairs + nonpublished pairs), Suciu-Foca et al (21) (14 pairs).

Finally, the segregations of the A1-B8 and A2-B15 haplotypes were studied: both male and female patients received each of these haplotypes equally frequently from the fathers and the mothers. The findings of Cudworth et al (16) could thus not be confirmed in the Workshop study.

Age-at-onset and HLA-DR Earlier studies (15) have indicated that DR4 positive patients tend to have an earlier onset of IDDM than other patients and, accordingly, we analyzed the frequencies of various HLA-DR phenotypes for patients with various ages-at-onset (Table 11). Only Caucasian patient samples of reasonable size have been included. When testing the six different DR phenotypes in the four age-at-onset groups (0 to 10, 11 to 20, 21 to 30, and >30 years) in a 6x4 contingency table, highly significant ($P<10^{-4}$) heterogeneity was found, indicating that the DR associations vary between the four groups. There is no significant difference between the two groups with onset before 21 years or between the two groups with onset over 20 years, but there is significant ($P<0.025$) difference between the groups with onset 11 to 20 and 21 to 30 years, respectively, and a highly significant ($P<0.001$) difference between onset less and more than 20 years. Accordingly, an age-at-onset about 20 years divides the entire material into two groups with different phenotype distributions: the DR3,X, DR3, and DRWX,X phenotypes show increased frequencies with increasing age-at-onset, whereas the DR3,4, DR4, and perhaps the DR4,X phenotypes decrease. It may be noted that the differences between the two groups with onset before and after 20 years is unlikely to be due to a decreasing frequency of DR3,3 and DR4,4 homozygotes because the DR3 phenotype frequency actually showed an increase whereas the DR4 phenotype frequency did not change notably until onset after 30 years. One criticism which may be raised against this analysis concerns the fact that we have had to pool different patient samples in order to get sufficient numbers in each group. This may explain some of the heterogeneity because the different samples were truncated differently in terms of age-at-onset, but it seems unlikely that this should explain all the heterogeneity.

Age-at-onset and BfF_1 type. Seven laboratories (BER, BSH, DEM, KAS, RUB, SVE, TII) provided information on 258 Bf typed propositi (22.5% were F_1 positive). There was a trend that the age-at-onset was lower for the F_1 positive patients than for the F_1 negatives but the difference was not significant.

Month-at-onset and HLA-DR. Two laboratories (BER and SVE) provided information about month-at-onset for all patients studied and Figure 2 shows the distribution of month-at-onset for various HLA-DR3 and 4 phenotypes. It appears that DR4 positive patients significantly more frequently had onset in the last three months of the year

Table 11. HLA-DRW phenotype frequencies (/) in four age-at-onset groups.

Age at Onset:0-10

DRw:	3,x	3	3,4	4	4,x	x,x	N
BER	20.0	40.0	0.0	40.0	0.0	0.0	5
BSH	0.0	22.2	55.6	22.2	0.0	0.0	9
KAS	24.1	6.9	48.3	6.9	10.3	3.4	29
MOL	0.0	14.3	28.6	35.7	14.3	7.1	14
MYR	0.0	12.5	12.5	50.0	25.0	0.0	8
SVE	12.5	4.2	37.5	37.5	8.3	0.0	24
TII	0.0	3.1	43.8	18.8	18.8	15.6	32
VIL	0.0	11.1	33.3	11.1	38.9	5.6	18
sum	7.9	9.4	38.1	23.0	15.8	5.8	139

Age at Onset:11-20

DRw:	3,x	3	3,4	4	4,x	x,x	N
BER	13.5	13.5	21.6	16.2	21.6	13.5	37
BSH	0.0	0.0	69.2	15.4	0.0	15.4	13
KAS	16.7	16.7	41.7	0.0	25.0	0.0	12
MOL	0.0	0.0	33.3	33.3	16.7	16.7	6
MYR	5.0	0.0	30.0	20.0	25.0	20.0	20
SVE	5.0	12.5	45.0	20.0	10.0	7.5	40
TII	0.0	0.0	27.3	36.4	27.3	9.1	11
VIL	10.0	20.0	50.0	10.0	0.0	10.0	10
sum	7.4	9.4	37.6	18.1	16.1	11.4	149

Age at Onset:21-30

DRw:	3,x	3	3,4	4	4,x	x,x	N
BER	11.5	26.9	7.7	15.4	19.2	19.2	26
BSH	12.5	37.5	25.0	12.5	12.5	0.0	8
KAS	0.0	0.0	0.0	0.0	100.0	0.0	1
MOL							0
MYR	12.5	12.5	12.5	18.8	25.0	18.8	16
SVE							0
TII	0.0	0.0	100.0	0.0	0.0	0.0	1
VIL	25.0	0.0	25.0	50.0	0.0	0.0	4
sum	12.5	21.4	14.3	17.9	19.6	14.3	56

Age at Onset:>=31

DRw:	3,x	3	3,4	4	4,x	x,x	N
BER	0.0	33.3	16.7	33.3	16.7	0.0	6
BSH	0.0	25.0	25.0	25.0	0.0	25.0	4
KAS							0
MOL							0
MYR	0.0	50.0	0.0	0.0	0.0	50.0	8
SVE							0
TII							0
VIL	33.3	8.3	8.3	0.0	8.3	41.7	12
sum	13.3	26.7	10.0	10.0	6.7	33.3	30

For the comparison between sums (4x6 table):
$\chi^2 = 47.13$ with 15 d.f., $p = 4 \times 10^{-5}$.

If the DRwX,X phenotype is excluded from the analysis: $\chi^2_{12}=32.0$ ($p<.005$) indicating that the overall heterogeneity is not solely due to an increase of the DRwX,X phenotype with increasing age-at-onset. If phenotypes involving DRw4 are excluded: $\chi^2_6=4.1$ (n.s.) indicating that the DRw3,X and DRw3 have constant frequencies in all four age-at-onset groups when DRw4 is excluded.

compared to DR4 negatives. When less complete data from other laboratories (MOL, MYR, KAS, and VIL) were analyzed in a similar way, the same trend was observed, but it was not significant (P = 0.21). It should be noted that the difference seen in Figure 2 may well be a chance deviation because many different combinations of months can be made and, accordingly, we only consider this observation a lead for further studies: it definitely needs confirmation.

Anti-Islet cell antibodies (ICA). Four laboratories (BER, DEM, MYR, RUB) provided information on 172 patients investigated for ICA, usually several years after diagnosis. Fifty-six patients had ICA. The frequencies of DR3 and 4 did not differ between patients with and without ICA. However, these data are probably not suited for testing possible differences.

Hardy-Weinberg equilibrium in patients and controls. It can be shown by algebra that Hardy-Weinberg structure for HLA antigens may be present in a patient sample if a disease is recessive and if the patients are ascertained by their disease from a background population in Hardy-Weinberg equilibrium with respect to HLA and the disease locus (3). Moreover, there should not be an excess of DR3,4 heterozygotes if the inheritance is intermediate (Ryder, unpublished data). All patients and control samples of reasonable sizes were tested for Hardy-Weinberg equilibrium using the gene counting method of maximum likelihood, and it appears from Table 12 that in almost all samples there is a slight excess of DR3,4 (Caucasians and Blacks) or DR4,DRW8 (Japanese) heterozygotes among the patients, whereas the controls show no such excess.

Discussion

This study has confirmed earlier observations of association between IDDM and DR3 and 4 in Caucasians including Jews, Basques, and Asian Indians. Moreover, an association with DR4 has been demonstrated for the other ethnic groups studied: African and American Blacks and Japanese. The association between DR4 and IDDM thus seems to be universal. In contrast, the association with DR3 seems only to hold for Caucasians and Blacks whereas this antigen appears to be substituted by DRW8 in Japanese IDDM patients. A limited number of DR2 positive patients were observed but this antigen still shows the strongest negative association with IDDM. Most DR2 positive patients were either DR3 or DR4 positive. In Caucasians, only about 10% of the patients carry neither DR3 nor 4.

It appeared that the deviations seen for HLA-B7, B8,

Figure 2. Month-at-onset for various DR phenotypes.

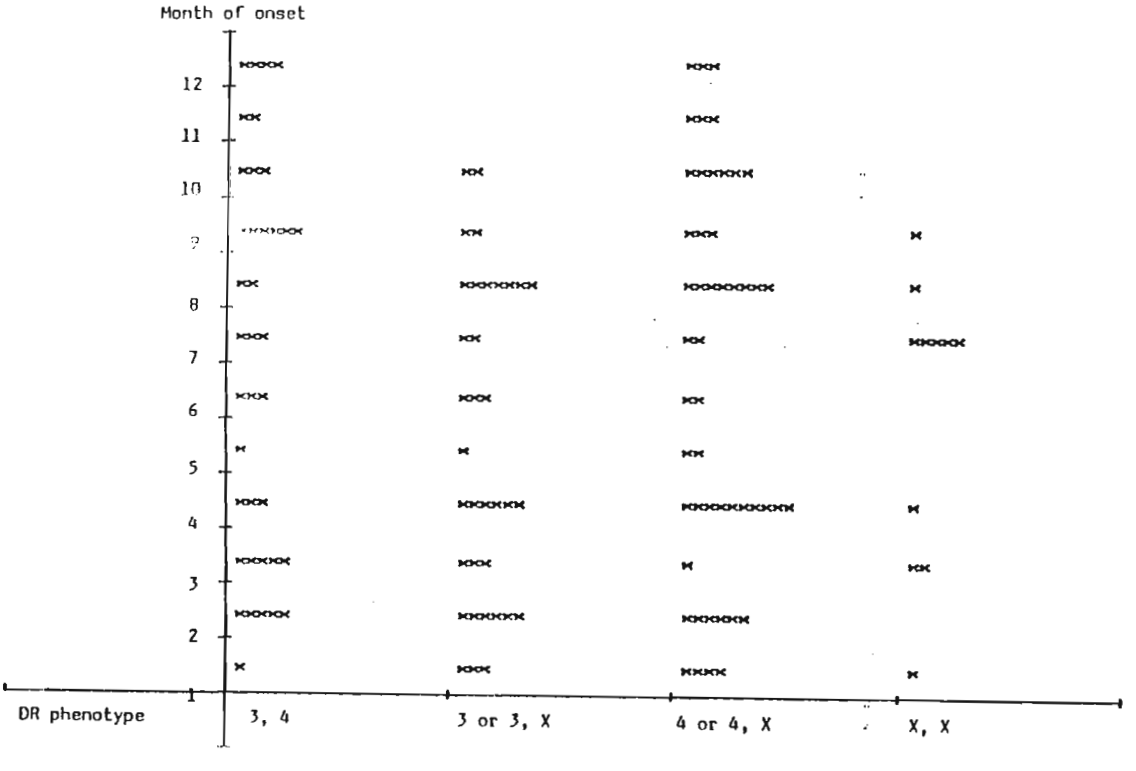


Figure 2. Month-at-onset for various DR phenotypes

Data from the two only complete sets of data (BER and SVE) are shown. 1=January, 2=February etc. 24% of 88 DR4-positive patients and 4% of 46 DR4-negative patients had onset during the last three months of the year (P=0.003).

Table 12. Hardy-Weinberg (DR3,4)

	Patients						Controls		
	local assign.			L.A. assign.			local assign.		
Lab.	obs	exp.	N	obs.	exp.	N	obs.	exp.	N
BER	11	13.5	74				1	1.2	53
BOD	3	3.3	17	4	2.8	17	1	1.8	44
BRA-Ashk.jew	10	8.0	35	5	4.5	17	2	1.3	46
BRA-Non-A.	17	12.4	33	16	11.5	30	1	.5	35
BRG-Am.Bl.	4	3.9	25	4	3.1	30	0	.2	36
BRI	3	2.1	13	2	1.3	13	4	1.9	74
BSH	17	11.9	34	17	10.7	34	2	4.1	58
CEP	13	10.1	34				1	1.2	57
DEH	15	11.8	49				0	.6	44
HAH-As.Ind.	3	1.3	16	3	0.9	11	0	.1	41
HAH-Afr.Bl.	4	2.9		2	1.9	11	1	.7	54
KAS	19	12.9	42	6	4.4	18	0	1.3	63
KRE	11	6.8	37	10	4.2	39	0	1.0	52
MOL	6	5.3	19				7	4.0	83
MYR	9	7.9	53				5	4.4	150
RAF							4	2.1	133
RUB-Sp.	3	3.8					1	1.0	26
RUB-Non-Sp.							-	-	25
SVE	27	22.2	64	27	21.2	64	7	9.6	174
TII	10	5.0	29				4	1.4	49
VIL	13	9.4	43	6	5.3	44	6	1.5	75
Total Cauc.	187	146.4		93	65.9		46	38.9	
χ^2		11.26			11.14			1.30	
Grand total	198	154.5		102	71.8		47	39.9	
χ^2		12.25			12.70			1.26	
Sign test	16+/3-; p=.002						7+/10-; n.s.		
DR4, 8 Japanese									
JUJ				11	8.0	29	1	2.8	104
TSU				12	8.9	61			
SAJ				9	6.0	55	6	2.7	116
Total Jap.				32	22.9		7	5.5	
χ^2					3.62			.41	

Table 13. Genetic models for IDDM

HLA Genes alone

A) One Locus a) two alleles: D = 'diabetes' gene

d = normal allele

Genotypes: D/D D/d d/d

Penetrance: f_2 f_1 f_o

1) dominant $f_2 = f_1 > 0$, $f_o = 0$

2) recessive $f_2 > 0$, $f_1 = f_o = 0$

3) intermediate $f_2 > f_1 > 0$, $f_o = 0$

b) three (or more) alleles: more complicated models which may involve overdominance, etc.

B) Two or more loci: Complicated models which may involve complementation, epistasis, etc.

HLA and Non-HLA Genes

A) HLA genes may be necessary (a sine qua non)

B) HLA genes may not be necessary in all cases

B15, and B18 are entirely secondary to deviations of DR2, 3, and 4 (Table 6).

There were rather small differences between familial and nonfamilial IDDM concerning DR associations - in fact, statistical significance was only obtained when pooling patients having only DR3, DR4, or both. The small magnitude of these differences indicates that HLA plays approximately the same role in nonfamilial as in familial IDDM. In both cases, HLA seems to play a major role in the predisposition.

Table 13 lists various genetic models which may explain the genetics of IDDM (and of other HLA related disorders). The models have been listed in increasing order of complexity. The first three models (dominant, recessive, and intermediate) involve only two alleles at one locus within the HLA system: a 'diabetes' susceptibility allele, D, and a normal allele, d. The differences between these three models are due to different penetrances for the three genotypes: D/D, D/d, and d/d. Models involving three (e.g., two different susceptibility genes and one normal allele) or more alleles at one locus give rise to more complicated situations which become even worse if two or more loci are involved. When clarifying the genetics of a disorder it seems rational first to exclude the simpler models before attempting to fit the more complicated ones. The major problem with the complicated models is that they involve so many variables that it is possible to fit almost every model by changing one or more of these variables.

The Workshop more than doubled the number of HLA-typed affected sibpairs available. Although the distribution of pairs sharing two, one, or no HLA haplotypes did not change, the increasing number made it possible to exclude the dominant model and provided strong indirect evidence against the recessive one because the minimal 'diabetes' gene frequency compatible with the observations was 0.234. This would correspond to a minimum frequency of $(0.234)^2=0.055$ of homozygotes in the population and, since the frequency of IDDM is 0.003 (17), the penetrance for these homozygotes would be only 0.003/0.055= 0.055 or 5.5% which is much too low. Indeed, it is not higher than the frequency of IDDM among siblings of all IDDM propositi (18), which it obviously should be because only a fraction of these sibs would be homozygous and susceptible. Accordingly, we think that these results are incompatible both with a dominant and with a recessive mode, which confirms earlier analyses using the same approach (3). However, the results in Figure 1 do not rule out the possibility that the 'diabetes' gene (D) may act in an intermediate way with a dose effect giving higher penetrance for homozygotes than for heterozygotes (i.e., the third model in Table 13).

The intermediate model involves certain predictions which may be used to test its validity. Firstly, it may be predicted that if heterogeneity exists within IDDM then this heterogeneity should reflect differences between the two 'diabetes' genotypes, D/D and D/d. Although we cannot determine these genotypes, it is inherent in the intermediate model that the 'diabetes' gene (D) must be positively associated with HLA-DR3 and DR4 (and negatively associated with DR2), and most D/D homozygotes would be either DR3/3 or DR4/4 homozygous or DR3/4 heterozygous. Accordingly, these DR genotypes may be used as markers for homozygosity on the postulated 'diabetes' locus. As pointed out by W.F. Bodmer (personal communication), an intermediate model with a high penetrance in homozygotes and a low penetrance in heterozygotes would imply an overweight of homozygotes among familial cases and an overweight of heterozygotes among nonfamilial cases. Evidence that this is so can be seen from Table 5 which shows that there is an excess of the phenotypes DR3, DR4, and DR3,4 in familial cases as compared with nonfamilial cases. However, the excess is not striking and barely significant.

A more pronounced heterogeneity was found when the DR phenotypes were analyzed for patients in various age-at-onset groups (Table 11). However, this heterogeneity is probably not due to differences between D/D homozygotes and D/d heterozygotes (i.e., to a dose effect according to the intermediate model) because patients who had only DR3 or DR4 did not have earlier onset than DR3,X or DR4,X patients, respectively. Accordingly, we think that the age-at-onset heterogeneity may reflect different etiological mechanisms for the different DR phenotypic groups. It is possible that the high frequency of DR3 and 4 negative patients in the older age-at-onset groups to some extent may reflect the existence of phenocopies (e.g., misclassification of non-IDDM patients as IDDM patients) but we do not think that this can explain all the heterogeneity, mainly because the DR3 and DR3,X phenotypes show steady increases with increasing age-at-onset. In fact, when disregarding DR4 positive patients, DR3 is equally increased in all age-at-onset groups. It seems likely to us that the heterogeneity observed may be due to the possibility that two different HLA factors, one associated with DR3 and one with DR4, each confers susceptibility to IDDM by its own mechanism. The DR3-associated factor may exert its effect 'throughout life' while that associated with DR4 may act mainly in young individuals. The main reservation concerning this conclusion is that the different patient samples on which Table 11 was based were truncated in different ways, and we think it necessary that the hypothesis should be tested in a few homogeneous populations.

The analysis of ICA did not provide any evidence for heterogeneity in this material.

Other predictions which can be made on the basis of the Intermediate model relate to the distribution of DR phenotypes among the patients. Firstly, it can be shown by

algebra (Ryder, unpublished) that under this model there should be no excess of DR3,4 heterozygotes among the patients. It appears from Table 12 that there was a small but almost universal excess of DR3,4 heterozygotes. Secondly, it can also be shown by algebra that the intermediate model should not lead to a relative risk for DR3,4 heterozygotes which is higher than both the relative risk for DR3,3 homozygotes and the relative risk for DR4,4 homozygotes (Svejgaard, unpublished). It appears from Table 9 that the relative risk for DR3,4 heterozygotes is almost twice as high as that for the two homozygotes. However, both of these statements are based on the assumption that the patients are drawn from a background population which is in Hardy-Weinberg equilibrium both for DR and for the 'diabetes' locus. Whereas this may be true for DR it may not be the case for the 'diabetes' locus because the fertility of IDDM patients is probably reduced. Nevertheless, these findings, together with the age-at-onset heterogeneity, make us reluctant to accept the intermediate model. However, before leaving all three two-allele models and accepting more complicated ones (Table 13), we feel that more HLA studies focusing on possible heterogeneity of IDDM in homogeneous populations are indicated because the weakness of the 8th Workshop data is the cause for the possible heterogeneity between the various populations studied.

Finally, we wish to stress that the analyses performed are not exhaustive because the time available was rather limited.

Conclusions

IDDM is associated with DR4 in all populations studied (Caucasian, Black, and Japanese) and with DR3 in most populations. About 90% of Caucasian IDDM patients are either DR3 and/or DR4 positive. In Japanese, DRW8 may substitute for DR3. IDDM may occur in DR2 positive individuals, but usually only when DR3 or 4 is present, too.

The associations observed for HLA-B8, B15, B18, and B7 are secondary to the DR associations.

Some DR phenotype associations may be stronger in familial than in nonfamilial IDDM, but the differences are minor.

The distribution of haplotype sharing (two, one, or none) among affected sibpairs is incompatible with a dominant mode of inheritance for IDDM susceptibility and leads to an unacceptable, high gene frequency for the recessive model but does not rule out an intermediate model.

The DR phenotype associations show significant heterogeneity between groups of patients with different ages-at-onset: DR4 is mainly associated with early age-at-onset IDDM whereas DR3 is equally associated with IDDM at all ages. This observation argues against the intermediate model.

The relative risk for DR3,4 heterozygotes is higher than for DR3,3 and DR4,4 homozygotes and there is an excess of DR3,4 heterozygotes when the patient samples are tested for Hardy-Weinberg equilibrium. These observations are also incompatible with the intermediate model.

More studies concerning possible heterogeneity of IDDM in homogeneous populations are warranted before the intermediate model may be finally disproved and before more complicated models are accepted. It is apparent from the Workshop study that the method of ascertainment should be very clearly defined in future studies.

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REFERENCES

1. Singal DP, Blajchman MA. Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes 1973, 22:429.
2. Nerup J. Platz P, Anderson OO, et al. HL-A antigens and diabetes mellitus. Lancet 1974, 2:864.
3. Ryder LP, Christy M, Nerup J, Platz P, Svejgaard A, Thomsen M. HLA studies in diabetes. In *Treatment of Early Diabetes 1979,* Camerini-Davalos RA, Hanover B, eds, Plenum Press, New York, 1979, 41.
4. Christy M, Green A, Christau B, et al. Studies of the HLA system and insulin-dependent diabetes mellitus. Diabetes Care 1979, 2:209.
5. Thomsen M, Platz P, Anderson OO, et al. MLC typing in juvenile diabetes mellitus and idiopathic Addison's disease. Transplant Rev 1975, 22:125.
6. Nerup J, Cathelineau C, Seignalet J, Thomsen M. HLA and endocrine diseases. In *HLA and Disease,* Dausset J, Svejgaard A, eds, Munksgaard, Copenhagen, 1977, 149.
7. Batchelor JR, Morris PJ. HLA and disease. In *Histocompatibility Testing 1977,* Bodmer WF, et al, eds, Munksgaard, Copenhagen, 1978, 205.
8. Cudworth AG, Woodrow JC. Evidence for HL-A linked genes in 'juvenile' diabetes mellitus. Brit Med J 1975, 2:133.
9. Thomsen G, Bodmer W. The genetic analysis of HLA and disease associations. In *HLA and Disease,* Dausset

J, Svejgaard A, eds, Munksgaard, Copenhagen, 1977, 84.
10. Rubinstein P, Suciu-Foca N, Nicholson JF. Genetics of juvenile diabetes mellitus. A recessive gene closely linked to HLA-D and with 50 percent penetrance. N Engl J Med 1977, 297:1036.
11. Spielman RS, Baker L, Zmijewski CM. Inheritance of susceptibility to juvenile onset diabetes. In *Genetic Analysis of Common Diseases. Applications to Predictive Factors in Coronary Disease,* Sing CF, Skolnick M, eds, Alan R. Liss, New York, 1979, 567.
12. Svejgaard A, Platz P, Ryder LP, Nielsen LS, Thomsen M. HL-A and diseases associations - a survey. Transplant Rev 1975, 22:3.
13. Platz P, Jakobsen B, Dickmeiss E, et al. Ia and HLA-D typing of patients with multiple sclerosis (MS) and insulin-dependent diabetes (IDD). Tissue Antigens 1977, 10:192.
14. Ilonen J, Herva E, Tiilikainen A, Aakerblom HK, Koivukangas T, Kouvalainen K. HLA-DW2 as a marker of resistance against juvenile diabetes mellitus. Tissue Antigens 1978, 11:144.
15. Svejgaard A, Christy M, Nerup J, Platz P, Ryder LP, Thomsen M. HLA and autoimmune disease with special reference to the genetics of insulin-dependent diabetes. In *Genetic Control of Autoimmune Disease,* Rose NR, et al, eds, Elsevier, North Holland, New York 1978, 101.
16. Cudworth AG, Wolf E, Gorsuch AN, Festenstein H. A new look at HLA genetics with particular reference to type-1 diabetes. Lancet 1979, 2:389.
17. Green A. 1980, In preparation.
18. Degnbol B, Green A. Diabetes mellitus among first and second degree relatives of early onset diabetics. Ann Hum Genet 1978, 42:25.
19. Barbosa J, King R, Noreen H, Yunis EJ. The histocompatibility system in juvenile, insulin-dependent diabetic multiple kindreds. J Clin Invest 1977, 60:989.
20. Cudworth AG. Type I diabetes mellitus. Diabetologia 1978, 14:281.
21. Suciu-Foca N, Rubinstein P, Nicholson J, et al. Juvenile diabetes mellitus and the HLA system. Transplant Proc 1979, 11:1309.

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The association between IDDM and HLA is well established although the mode of inheritance of the disease remains far from clear. Extensive studies have been carried out in European Caucasians, in Japanese and in American Blacks but studies in other populations and ethnic groups have been much less extensive. A number of interesting facts emerged from the 8th International Workshop. It appeared that the association with DR4 was universal, that with DR3 not so and DRw8 appeared to substitute in Japanese. The increase in frequency of the B locus alleles appeared to be secondary to those of the DR locus, but they were of interest because of their heterogeneity.

The 2nd AOHWS provided a unique opportunity to document the HLA associations with IDDM widely in the region.

Reports of associations between NIDDM and HLA have been isolated and in general have been confined to special forms of diabetes in selected populations. It was thought that significant data could emerge from the study of selected patient groups which would not fulfil the criteria for IDDM, and thus it was decided to include such groups in the study.

The aims of the study were:

1. To document the association of HLA antigens and IDDM in populations within the Asia-Oceania region. It was decided that in this workshop, Caucasian patients would not be studied to maximise the availability of serum for non-caucasoid studies.
2. To examine the heterogeneity of the disease by careful documentation of clinical features, age of onset and complications and the correlation of these with HLA and other markers.
3. To study other specially selected groups of diabetic subjects. Criteria for acceptance of patients was as follows:

(a) IDDM - onset <40
idiopathic
ketosis-prone
non-obese
insulin-dependent

(b) NIDDM - no criteria were established except that patients studied should be relatively homogeneous.

In both cases, it was requested that sufficient patients with age and sex matched controls be typed for the study to be self-contained.

Clinical data were requested as outlined in the Disease Card (Table I) for patients with IDDM; a further special card was generated for NIDDM.

Results

IDDM. No families were studied. The number of unrelated patients and controls typed in each laboratory is indicated in Table 2. In almost all laboratories the numbers studied were small and sampling error is inevitable. Despite some heterogeneity it was decided to pool the data from the two laboratories typing Chinese, the two laboratories typing Japanese and the two laboratories typing Indians and to analyse this as well as the data from individual laboratories. In this way, all possible associations would be visualised. In some cases associations were gained by pooling, in some, lost. In Tables 3 to 7 the phenotype frequencies of all antigens showing any significant change in frequency in any patient group are shown and the corresponding Relative Risk and probability values are shown in Table 7. Phenotypic frequencies of C_2, Bf and GLO are shown in Table 8. Gm studies will be reported elsewhere.

Chinese. Positive associations were shown with DR3 (RR 3.5) and DRw9 (RR 9.3) in the Shanghai Chinese (CHE) with BfF in the Peking Chinese (YGE), and DR3 (RR 3.1) and DRw9 (RR 6.7) in the combined Chinese. DR4 was not increased. Some of the observed differences between the two Chinese populations may be due to the small samples studied and it will be of interest to extend the observations to larger samples in both centres. The association of DR3 with IDDM in Peking confirms the observation of Maeda et al. in Taiwanese Chinese (1), and marks a distinctive difference between the Chinese and Japanese IDDM patients.

Japanese. There were no positive associations in the Nagasaki patients (HIR) but the Tokyo (SAS) study showed positive associations with Aw24 (RR 8.7), B40 (RR 4), Bw54 (RR 4.8) and with DR4 (RR 4). In the combined Japanese, the association was sustained with Aw24 (RR 6.1) and demonstrated with DRw9 (RR 2.96).

Indians. In the North Indians, strong positive associations were found with Bw49 (RR 12.7), DR3 (RR 19.5) and BfS, (RR 9.2), an extremely rare allele. These findings are striking and identify a susceptible haplotype in this population. DR4 was also significantly increased in the patients (RR 4.5).

The positive associations in the South African Indians were with Bw60 (RR 6.6), Cw3 (RR 10) and DR3 (RR 5.2). Although B8 was increased, the increase did not reach significant levels. There was no difference in the frequency of the B5 splits, Bw51, Bw52 and Bu in patients and controls. This was an unexpected finding but may have been due to the lack of discriminatory antisera or to the selection of subjects from predominantly Aryan rather than Dravidian stock. Hammond has previously reported significant associations with B8, Bw52 and Bu in Dravidian Indians (2).

Thais. There were no significant associations in the Thais although both DR4 and DRw9 were slightly increased. This small study should be regarded as preliminary and a large sample will need to be typed before definite conclusions can be reached.

Maoris. IDDM is excessively rare in Polynesians who are prone to develop the insulin independent form of the disease. Nevertheless six patients were found for this study. Although it would be inappropriate to report antigen frequencies in this small sample, it is of special interest. Three of the six patients were DR3 positive, another two DR4 positive and the sixth DR9 positive. Two of the DR3 positive individuals were also B8 positive, and both the DR4 positive individuals were B40 positive. It is possible that, as in the Chinese, IDDM is associated in the Maoris with DR3, however these patients may reflect Caucasoid admixture and a larger study to clarify this will be of interest.

Clinical Studies

An attempt was made to analyse the clinical data with regard to severity and the occurrence of the complications of diabetes, even though numbers of patients were small and data often incomplete. No significant associations with ketonuria, proteinuria, retinopathy or neurological signs were found with any antigen in any population.

Furthermore, no correlations could be found with the level of control as assessed by the attending physician.

Age of onset was analysed in all groups, and the only significant association found was for DR4 and onset below twenty years of age in the Peking Chinese.

Patients from three laboratories VAI, HIR, CHI were screened for auto-antibodies. The screen comprised the following antibodies: anti nuclear factor, smooth muscle, striational muscle, mitochondria, heart, thyroid and thyroglobulin.

Three patients only were positive for any of these, all were North Indians and their relevant details are as follows:

	Antibody Detected	Titre	Age at Onset	Years of Treatment Since Diagnosis	B8	DR3
VAI 111	Parietal	1/125	29	8	+	+
VAI 120	Striational	1/5	15	2	+	+
VAI 134	Parietal	1/25	13	1	+	+

Islet-cell antibody tests were carried out on the Japanese, Indian and Thai patients and all were negative.

Mature Onset Diabetes

Two populations were studied, Pima Indians and Maoris. In both cases, the disease was associated with obesity and inappropriate diet. It was not insulin dependent and age of onset varied. The incidence of MOD in the Maori is now known, but it is extremely high in the Pima, reaching 69% in women between the ages of 55 and 64 years (3).

There were no significant associations with any HLA antigens in the Pima. However, the extremely high frequency of DR3 in both patients (80%) and controls (70%) is of considerable interest and raises a number of important questions about the contribution of this antigen to the extreme propensity of the Pima to develope diabetes. It will be of interest to study other North American Indian Tribes which do not have this susceptibility, for their DR status, especially those which may be ethnically close neighbours of the Pima.

There were no significant associations in the Maori either but only eleven patients were studied and definite conclusions cannot be reached. However it was of interest that eight of the eleven individuals were either DR3 or DR4 positive, two of the three remaining were DRw9 positive and one DRw8 positive. B8 and B15 were not detected in any of the patients. B40 (Bw 60 and 61) were slightly increased.

No clinical details were supplied for either group both of which are involved in on going studies.

Conclusions

Although the majority of patient groups studied was too small for definite conclusions to be drawn, a number of significant or suggestive findings emerge which warrant further study. These include:

1. The association of IDDM with DR3 and DRw9 in Chinese.
2. The possible association of IDDM in Japanese with DRw9.
3. The association of Bw49 and DR3 and the rare allele BfS, in the North Indians, and of DR3, Bw60 and Cw3 in the Asian Indians.
4. In all ethnic groups except the Thais DR2 was decreased although this was significant in only three laboratories.
5. No associations were found for mature onset diabetes, but the very high frequency of DR3 in the Pima and their extreme susceptibility to diabetes raises a number of interesting questions.

References

1. Maeda H., Takekuchi F., Juji T., Akanuma Y., Kasuga Y.E., Lee K. Kosaka K. and Tsai S.H. HLA DRw3 in juvenile onset diabetes mellitus in Chinese. Tissue Antigens. 15, 173. 1980.
2. Hammond M.G. and Asmal A.C. HLA and insulin dependent diabetes mellitus in South African Indians. Tissue Antigens. 15, 244. 1980.

3. Speos E.K., Kostyu D.D., [illegible]
Anzma in Histocompatibility Testing 1972 (Dausset J and Columbani J. eds). Munksgaard, Copenhagen. 345. 1972.

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Table I — PATIENT CODING FORM (DM) — AOHWC II

01–02	Card number		
03–05	Typing laboratory	Use Workshop Code	
06–08	Individual ID-number		
09–10	Pedigree number		
11	Ascertainment: proband = 1, other = 0		
12	IDDM in first degree relatives = 1, isolated case = 0		Family history
13	Other types of DM in first degree relatives = 1, no = 0		
14	IDDM in other relative, yes = 1, no = 0		
15–16	Age at onset (years)		
17–18	Month of onset (01 = January, ..., 12 = December)		
19–21	Present weight in kilograms		
22–24	Present height in centimeters		
25	Episodes of ketoacidosis no = 0, yes = 1		
26–27	Duration of insulin treatment (years)		
28–31	Present insulin requirement (IU pr. kg. day, two decimal places)		
32–34	Type of insulin (fill out name(s):		
35	Species of insulin (Pork = P, Beef = B, Mixed = M, Other = X)		
36–38	Present fasting blood glucose (mg pr. 100 ml)		
39–41	Present postprandial blood glucose (mg pr. 100 ml)		
42–44	Present daily glucose excretion in urine (g pr. 24 hours)		
45	Ketonuria presently	No = 0, Yes = 1	
46	Retinopathy presently		
47	Abnormal tendon reflexes presently		
48	Decreased vibration sense presently		
49	Proteinuria presently		
50–51	Hb A1c (present)		
52	Physician's assessment of control 1 = good, 2 = moderate, 3 = poor		
53–55	Present serum-creatinine (m-mol pr. litre)		
56–59	Fasting C-peptide level (pico-mol pr. litre, two decimal places)		

Table I continued

60	ICA never detected = 0, present = 1, earlier = 2, not investigated = blank
61 62	ICA first investigated (years after diagnosis)
63 64	ICA persisted (years after diagnosis, if still present = 99)
65	PCA never detected = 0, present = 1, earlier = 2, not investigated = blank
66 67	PCA first investigated (years after diagnosis)
68 69	PCA persisted (years after diagnosis, if still present = 99)
70	AThA never detected = 0, present = 1, earlier = 2, not investigated = blank
71 72	AThA first investigated (years after diagnosis)
73 74	AThA persisted (years after diagnosis, if still present = 99)
75 76	Other endocrine disorders,

Give comments, name(s) of other chronic diseases and name, full address and phone number of the person who filled out this form: (use reverse side)

00 = no
01 = Graves' disease
02 = Hypothyroidism
03 = Addison's disease
04 = Pernicious anaemia
05 = Hypergonadotropic hypergonadism
06 = Idiopathic hypoparathyroidism
07 = Other

example: 13 = both Graves' and Addison's disease

Table 2

2nd AOHWS - Diabetic Study

Participating Laboratories

Laboratory	Ethnic Group	Type of Diabetes	No. of Patients	No. of Controls	Code
Chen - Shanghai	Chinese	IDDM	35	53	CHE
Ye - Peking	Chinese	IDDM	15	15	YGY
Sasazuki - Tokyo	Japanese	IDDM	15	75	SAS
Hirota - Nagasaki	Japanese	IDDM	14	36	HIR
Chiewsilp - Bangkok	Thai	IDDM	13	20	CHI
Hammond - Durban	Asian Indians	IDDM	20	35	HAM
Vaidya/Mehra - N. Delhi	North Indians	IDDM	36	40	VAI
Woodfield - Auckland	Polynesians - Maori	IDDM	6		WOO
Fong - Wellington	Polynesians - Maori	MOD	11	79	FON
Amos/Kostyu - Durham	N.A. Indians - Pima	MOD	39	53	AMO

Table 3

2nd AOHWS - Diabetic Study

Antigen Frequencies

A Locus

	A1		A2		A11		Aw24	
	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts
IDDM								
YGY Chinese	.067	.067	.667	.733	.133	.133	.133	.533
CHE Chinese	0	.019	.516	.423	.091	.365	.485	.404
Combined Chinese	.021	.029	.563	.493	.104	.313	.375	.433
SAS Japanese	0	.013	.400	.387	0	0	.867	.427
HIR Japanese	0	0	.142	.500	.071	.111	.786	.472
Combined Japanese	0	.009	.276	.432	.034	.036	.828	.441
CHI Thai	0	0	.460	.600	.460	.300	.460	.350
HAM Asian Indians	.100	.400	.300	.286	.250	.286	.350	.200
VAI North Indians	.139	.350	.444	.225	.083	.300	.222	.250
Combined Indians	.125	.373	.393	.253	.143	.293	.268	.227
MOD								
PON [illegible]	0	.076	.455	.430	.455	.329	.545	.557
AMO [illegible] Indians	0	0	.700	.808	0	0	.600	.462

Table 4

2nd NOHWS - Diabetic Study

Antigen Frequencies

B Locus

	B8		B15		B40		Bw46		Bw49		Bw51		Bw52		Bw54	
	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts
IDDM																
YGY Chinese	.067	0	.333	.533	.333	.133	0	0	0	0	.067*	.133	*		.200*	.067
CHE Chinese	.063	0	.156	.300	.313	.360	.031	0	0	0	.125	.080			.188	.120
Combined Chinese	.064	0	.213	.354	.319	.308	.021	0	0	0	.106	.092			.191	.108
SAS Japanese	0	0	0	.200	.666	.333	0	.053	0	0	.067	.133	.067	.186	.333	.103
HIR Japanese	0	0	.071	.111	.429	.389	0	.056	0	0	0	.111	.071	.111	.286	.306
Combined Japanese	0	0	0	.180	.552	.351	0	.054	0	0	.034	.126	.069	.162	.310	.162
CHI Thai	0	0	0	0	.300	.105	.300	.263	0	0	.100	.105	0	0	0	0
HAM Asian Indians	.250	.057	0	.058	.450	.200	0	0	0	0	.150	.250	.100	.114	0	0
VAI North Indians	.306	.125	.056	.200	.167	.175	0	0	.222	0	.056	.257	.056	.125	0	0
Combined Indians	.286	.093	.036	.133	.268	.187	0	0	.143	0	.089	.253	.071	.120	0	0
MOD																
FON Poly. Maori	0	.101	0	.076	.728	.442	0	0	0	0	0	.013	0	0	.455*	.139
AMO N. Am. Indians	0	0	0	0	0	0	0	0	0	0	.125	.275	0	0	0	0

* = Supertypic antigen B5 or Bw22

Table 5

2nd AOHWS - Diabetic Study

Antigen Frequencies

C Locus

	Cw1		Cw2		Cw3		Cw4	
	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts
IDDM								
YGY Chinese	.133	.067	.067	0	.467	.333	.067	.267
CHE Chinese	.143	.133	.057	.033	.371	.415	0	.208
Combined Chinese	.140	.103	.060	.029	.400	.397	.020	.221
SAS Japanese	.400	.227	0	0	.733	.507	0	.053
HIR Japanese	.071	.305	0	0	.429	.583	0	.028
Combined Japanese	.241	.243	0	0	.586	.532	0	.045
CHI Thai	.200	.182	0	0	.800	.727	.200	.182
HAM Asian Indians	0	.057	0	0	.200	0	0	.143
VAI North Indians	0	.075	.056	.075	.111	.050	.139	.200
Combined Indians	0	.067	.036	.040	.143	.027	.089	.173
MOD								
PON Poly. Maori	.455	.367	0	0	0	.127	.273	.114
AMO N. Am. Indians	0	0	.125	.173	.475	.423	.250	.212

Table 6

2nd AOHWS - Diabetic Study

Antigen Frequencies

DR Locus

	DR2		DR3		DR4		DR8		DR9		DR10	
	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts	Pts	Conts
IDDM												
YGY Chinese	.333	.133	.467	.267	.267	.267	.200	.200	.333	.133	0	0
CHE Chinese	.094	.366	.375	.146	.281	.366	.188	.220	.563	.122	0	0
Combined Chinese	.170	.304	.404	.179	.277	.339	.234	.214	.489	.125	0	0
SAS Japanese	0	.333	0	0	.750	.333	.200	.213	.533	.280	0	0
HIR Japanese	.071	.277	0	0	.214	.417	.071	.444	.429	.194	0	0
Combined Japanese	.034	.317	0	0	.448	.366	.138	.275	.483	.242	0	0
CHI Thai	.500	.267	.125	.133	.125	.067	0	0	.125	.067	0	0
HAM Asian Indians	.200	.343	.400	.114	.350	.371	.250	.086	.050	.086	.050	.029
VAI North Indians	.111	.375	.806	.175	.389	.125	.056	0	.028	.075	.028	.075
Combined Indians	.143	.360	.661	.147	.375	.240	.125	.040	.036	.080	.036	.053
MOD												
FON Poly. Maori	0	.074	.182	.162	.636	.427	.090	.250	.182	.118	0	0
AMO N. Am. Indians	.086	.133	.800	.696	.061	.182	.114	.111	.029	0	0	0

Table 7

Significant Relative Risk Estimate (p values†)

	Chinese			Japanese			Indian		
	YGY	CHE	Combined C.	SAS	HIR	Combined J.	VAI	HAM	Combined I.
A 1							.30*		.24**
2					.17*		2.8*		
3									
11		.17**	.25**				.21*		.40*
w24	.13*			8.7**		6.1***			
B 8									3.9**
15									
40				4.0*				†3.3*	2.7*
w49							12.7***		14.0***
w51							.18*		.29*
w54				4.8*					
C w3								10.6**	
w4		.10**	.07***						
DR 1									
2		.18*		.12**		.08***	.21**		.30**
3		3.5*	3.1*				19.5***	5.2**	11.3***
4				4.0*			4.5**		
7							.22**		.28**
8					.10*				
9		9.3***	6.7***			3.0*			
Bf F	7.43*					.29*			
S1							9.2*		8.4***

† = p value estimated from 2 x 2 contingency table

* = <.05, ** = <.01, *** = <.001

† = Relative risk for Bw60 is 8.9**

Table 8

Distribution of Other Chromosome 6 Markers in IDDM and Controls

		GLO			C2			Factor B				
		1	2	other	a	b	c	BfS	BfF	BfS1	BfF1	other
Chinese												
YGY	Pts.	.333	1.000	0	0	0	1.000	.800	.533	.067	0	0
	Conts.	.154	1.000	0	0	0	1.000	.933	.133	.067	0	0
CHE	Pts.	.257	.971	0	.029	.057	.971	.971	.206	.029	0	0
	Conts.	.151	1.000	0	.038	.038	1.000	.981	.245	.038	0	0
Combined C.	Pts.	.280	.980	0	.020	.040	.980	.918	.306	.041	0	0
	Conts.	.152	1.000	0	.029	.029	1.000	.971	.221	.044	0	0
Japanese												
SAS	Pts.	.200	1.000	0	0	.067	1.000	1.000	.200	0	0	0
	Conts.	0	1.000	0	0	.100	1.000	1.000	.600	0	0	0
HIR	Pts.	.143	.929	0	0	.071	1.000	.923	.154	0	0	0
	Conts.	.172	1.000	0	0	.033	1.000	1.000	.367	0	0	0
Combined J.	Pts.	.172	.966	0	0	.069	1.000	.964	.179	0	0	0
	Conts.	.07	1.000	0	0	.050	1.000	1.000	.425	0	0	0
Indians												
HAM	Pts.	.474	.895	0	0	.053	.105	.684	.632	.053	0	0
	Conts.	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
VAI	Pts.	.458	.875	0	0	.174	1.000	.760	.600	.240	0	0
	Conts.	1.000	1.000	0	0	.167	1.000	.767	.500	.033	0	0
Combined I.	Pts.	.465	.884	0	0	.119	.595	.727	.614	.159	0	0
	Conts.	1.000	1.000	0	0	.167	1.000	.767	.500	.033	0	0
Thais												
CHI	Pts.	.417	1.000	0	0	.077	1.000	1.000	.231	0	0	0
	Conts.	.263	1.000	0	0	.100	1.000	1.000	.050	0	0	0

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HLA-A, B, C and DR antigens in young South African blacks with Type 1 (insulin-dependent) diabetes mellitus

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Summary. The HLA status of South African black Type 1 (insulin-dependent) diabetic patients with age of onset under 35 years was compared with that of healthy black control subjects. HLA-A, B and C antigens were determined in 94 patients and 995 control subjects, while DR typing was carried out on 56 patients and 195 control subjects. There was a significant increase in the frequency of DR4 in patients as compared with control subjects ($p < 0.01$; relative risk 3.4). DR3/DR4 heterozygosity was associated with a greater relative risk for developing Type 1 diabetes mellitus (3.7) than the presence of DR3 alone (relative risk 1.6). A significant negative association was observed between the presence of BW42 and Type 1 diabetes in this population sample ($p < 0.04$; relative risk 0.3). A similar trend was observed with regard to DR2, the corrected p value just attaining statistical significance ($p < 0.05$; relative risk 0.1).

Key words: HLA-A, B, C DR antigens, Type 1 diabetes, South African blacks, B8/B14, DR4, BW42, DR2, DR3/DR4.

The association between Type 1 (insulin-dependent) diabetes mellitus and the HLA system has been documented in many studies involving different population groups. HLA antigens associated with Type 1 diabetes in White Caucasoids include CW3, CW4, B8, B15, DW3, DW4, DR3 and DR4 [1]. In the Japanese, the disease has been associated with HLA-DYT and BW54 [2, 3] while in South African Indians an association with B8 has been shown [4]. Other studies have shown a relationship with DR3 and DR4 in American blacks [5] and with either B8 or B14, which are cross-reacting antigens, in South African blacks [6]. It is thus evident that there are differences in the specific allelic associations among various ethnic groups.

There is little information on the relationship between Type 1 diabetes mellitus and antigens at the D locus of the HLA systems in populations other than Caucasoids. Therefore a group of South African blacks with the disease was studied to evaluate the frequencies of HLA-A, B, C and the recently-discovered serologically-detected DR antigens, which appear to be controlled by genes located at the same locus as the HLA DW antigens [7]

Patients and methods

All the patients and control subjects were blacks of Zulu descent. HLA-A, B, and C antigens were determined in 94 patients with Type 1 diabetes and 995 control subjects, whilst HLA-DR antigens were determined in 56 patients with Type 1 diabetes and 195 controls. Classification of patients as having Type 1 diabetes was based on the revised criteria recommended by the National Diabetes Data Group and the WHO: all had always been dependent on insulin for control of symptoms and prevention of basal ketosis [8, 9].

A total of 180 antisera were used in a two-stage microlymphocytotoxicity test to determine HLA-A, -B, and -C specificities. Lymphocytes were isolated on a Ficoll-Hypaque density gradient [10]. HLA-DR specificities were determined in an extended incubation microlymphocytotoxicity test, using T-cell-depleted, B-cell-enriched lymphocytes. The frequency differences between the patients and controls were tested for significance by means of the chi-squared test (without Yates' correction). The resulting probabilities were multiplied by the number of specificities tested in order to determine the corrected value [11].

Relative risk was calculated according to the method of Woolf [12].

Results

At the A and C loci there was no difference in the frequency of any of the antigens between patients and control subjects. The lower frequency of A30 in patients (23.4% versus 38.4%) was not significant after correcting for the number of antigens being tested (Table 1).

The frequency of B14 was increased in patients as compared with control subjects (12.8% versus 5.2%), but this was not significant after correcting the p value (Table 2). Similarly the frequency of B8, although increased in patients (22.3% versus 12.8%), did not attain significance level after correction for the number of an-

Table 1. Percentage frequencies of HLA-A and -B antigens in patients and control subjects

HLA antigen	Percentage frequency in		
	Control subjects ($n=995$)	Diabetic patients ($n=94$)	
A1	6.5	7.5	
A2	21.1	25.5	
A3	12.5	13.8	
A11	0.1	0	
AW23	17.5	24.5	
AW24	5.1	4.3	
A25	14.3	8.5	
A26	9.2	7.5	
A28	20.6	23.4	
A29	18.1	18.1	
AW30[a]	38.4	23.4	rr 0.5
AW31	7.1	8.5	
AW32	2.3	6.4	
AW33	1.6	1.1	
One antigen	25.5	27.7	
B5	1	0	
B7	19.9	18.1	
B8[b]	12.8	22.3	
B14	5.2	12.8	rr 2.7
B8/B14[c, d]	17.7	34.0	rr 2.4
B13	4.1	5.3	
B15	5.0	6.4	
B16	3.1	1.1	
B17	38.6	36.2	
B18	4.2	6.4	
BW21	1.2	1.1	
BW22	0.1	0	
B27	0.4	0	
BW35	6.2	3.2	
B37	0	0	
BW40	0.8	0	
BW41	2.1	9.6	
BW42	24.8	9.6	rr 0.3
BW44	16.0	11.7	
BW45	7.7	9.6	
BW53	1.3	1.1	
BW	19.1	14.9	
One Antigen	36.1	30.9	

[a] p uncorrected <0.005; [b] p uncorrected <0.05; [c] p uncorrected <0.001; [d] p corrected <0.04; rr = relative risk

Table 2. Percentage frequencies of DR antigen in patients and control subjects

HLA antigen	Percentage frequency in		
	Control subjects ($n=195$)	Diabetic patients ($n=56$)	
DR1	2.6	7.1	
DR2[a]	21.0	3.6	rr 0.1
DR3	34.4	42.9	rr 1.3
DR4[b]	12.3	32.1	rr 3.4
DR5	33.9	17.9	
DR6	15.9	10.7	
DR7	12.3	23.2	
DR8	1.0	3.6	
DR9	0.5	1.8	
DR10	2.6	1.8	
One antigen	63.6	55.4	
DR3/DR4	2.5	8.9	rr 3.7
DR3/any other antigen	14.3	16.2	
DR3/DR blank	21.4	16.2	
DR4/any other antigen	8.9	4.6	
DR4/DR blank	12.5	5.5	

[a] p corrected <0.05; [b] p corrected <0.01; rr relative risk

Table 3 Linkage disequilibrium between HLA-B locus antigens and HLA-DR locus antigens

		Control subjects			Diabetic patients		
		Haplotype frequency $X10^3$	$\Delta X10^3$	Δ/SE	Haplotype frequency $X10^3$	$\Delta X10^3$	Δ/SE
DR3	BW42	72	51	3.4[a]	3.1	18	0.7[c]
DR2	B7	48	35	2.7[b]	8	6	0.6[c]
DR3	B8	34	21	1.7[c]	65	37	1.2[c]
DR5	B7	34	13	0.8[c]	53	45	1.9[c]
DR5	B17	15	−27	1.2[c]	50	36	1.4[c]

Δ/SE = delta/standard error.
[a] $p<0.01$; [b] $p<0.05$; [c] not significant

tigens tested (Table 1). Since HLA-B8 and -B14 form part of a cross-reacting group of antigens, the presence of either of these antigens in the patients was compared with that in the control subjects. The difference is highly significant (34% versus 17.7%, $p<0.04$, relative risk 2.4; Table 1).

There was a lower frequency of HLA-BW42 in patients as compared with control subjects (9.6% versus 24.8%, relative risk 0.3), the difference being significant even after correction for the number of antigens tested (Table 1).

At the DR locus, Type 1 diabetes mellitus in the black patients was associated with a significant increase in the frequency of DR4 (32.1% versus 12.3%; relative risk 3.4) even after correcting the p value (Table 2). The frequency of DR2 is lower in patients than in control subjects (3.6% versus 21%), the difference just attaining a level of statistical significance after correction for the number of antigens being tested (Table 2). The frequency of DR3 is only slightly higher in patients than in control subjects, there being no significant difference.

HLA-DR3 and DR4 were found together in 8.9% of patients and in only 2.5% of control subjects (relative risk 3.7; Table 2). Thus the relative risk of DR3/DR4 heterozygosity was much greater than that for DR3 alone (relative risk 1.3) but only slightly higher than that for DR4 alone (relative risk 3.4).

The occurrence of specific DR antigens together with certain B locus antigens in the same haplotype is shown in Table 3. Whereas there are significant linkage disequilibra between DR2 and B7 ($\Delta \times 1000 = 35$, $p < 0.05$ and between DR3 and BW42 ($\Delta \times 1000 = 51$; $p < 0.01$) in control subjects, these phenomena are not seen in the patients ($\Delta \times 1000 = 18$, $p > 0.05$ and $\Delta \times 1000 = 6$, $p > 0.05$ respectively; Table 3). The HLA-DR3/B8 haplotype is present in a greater proportion of

diabetic patients (6.5%) than control subjects (3.4%) but there is no significant linkage disequilibrium in either group (Table 3).

Discussion

In white Caucasoids two distinct forms of Type 1 diabetes have been recognised and these may be distinguishable on the basis of HLA studies [13]. There is an autoimmune variety which is associated with DR3 and DW3 and less strongly with B8 [13]. The other type has an earlier age of onset and tends to be associated with DR4 and DW4, but less strongly with B15 and CW3 [1].

The present study has demonstrated a significant association between Type 1 diabetes in South African blacks and the presence of HLA-DR4. Such an association has been observed in virtually all the ethnic groups studied thus far [14]: However, an association with DR3 could not be shown among the Zulu patients here, unlike the findings in European Caucasoids [1] and American blacks [5]. It is possible though that such a relationship is still present but masked by the relatively small sample size.

The presence of DR3/DR4 heterozygosity in South African blacks was associated with a much greater susceptibility to Type 1 diabetes than that associated with possession of DR3 alone, but in comparison with DR4 alone, DR3/DR4 did not greatly increase the risk. In white Caucasoids, however, the relative risk associated with possession of both DR3 and DR4 has been found to be much greater than that associated with DR3 alone or DR4 alone [1]. Studies in white Caucasoids have established a negative correlation between Type 1 diabetes and the presence of DR2. Such a trend was also observed among the South African blacks with Type 1 diabetes, the corrected p value being significant at the 0.05 level. In addition, there was a significant negative correlation with BW42 in these patients. It is difficult to gauge the significance of such findings at present, since a decreased frequency of an antigen as opposed to an increased frequency requires a much larger sample size to become evident [15]. The negative correlation between B7 and Type 1 diabetes shown in white Caucasoids [1] was not seen in the black patients described here, nor has it been observed in American blacks [5].

Previously it had been shown that there was a close correlation between Type 1 diabetes in South African blacks of Zulu origin and the presence of either B8 or B14, which are cross-reacting antigens, thus raising the possibility that the same susceptibility gene might be associated with either of these antigens in this population group [6]. The findings in this study, which was extended to involve a larger number of patients, confirmed such a relationship.

The black patients with Type 1 diabetes did not show any increase in the frequencies of CW3, B15, and B18 as has been found in European Caucasoids, nor of BW54 and B12 as observed in Japanese [1–3]. Studies in American blacks or Nigerians have not shown any significant association at the B locus [15–17]. Patel et al. did find an increased frequency of B8 in the former, but the corrected p value was not significant [18], as has been the case with the black patients reported in this study.

In a study on a small number of Nigerians with Type 1 diabetes none of the patients had A30, whereas it was present in 15% of the 226 controls [17]. Such a trend has also been observed in this study done on patients who are ethnically related to Africans in the rest of Africa [19].

Linkage disequilibrium between antigens of the B locus and those of the DR locus was observed in this study, but the degree to which this phenomenon occurred was different in control subjects and patients. DR2 and B7 were found together far more frequently in the former. However, the frequency of B7 if present alone does not differ much between patients and control subjects, thereby supporting the well-known conclusion that the relationship between Type 1 diabetes and the HLA system is stronger at the D locus than the B locus [1].

Linkage disequilibrium involving the DR3 – B8 haplotype, which has been a constant finding in white Caucasoids [1], was not a significant finding in the black patients studied here. However, a significant association was seen between DR3 and BW42 in the black control subjects, whilst the frequency of this haplotype was much lower in the patients.

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References

1. Christy M, Green A, Chuistau B, Kromann H, Nerup J, Platz P, Thomsen M, Rydēn LP, Svejgaard A (1979) Studies of the HLA system and insulin-dependent diabetes mellitus. Diabetes Care 2: 209–214
2. Kawa A, Nakazawa M, Sakaguchi S, Nakamuras S, Komo Y, Hazeki H, Kanehisa T (1977) HLA system in Japanese patients with diabetes mellitus. Diabetes 26: 591–595
3. Nakao Y, Fukunishi T, Koide M, Akasawa K, Ikeda M, Yahata M, Imura H (1977) HLA antigens in Japanese patients with diabetes mellitus Diabetes 26: 736–739
4. Hammond MG, Asmal AC (1980) HLA and insulin-dependent diabetes in South African Indians. Tissue Antigens 15: 244–248
5. Rodey GE, White N, Fraser TE, Duquesnoy RJ, Santiago JV (1979) HLA – DR specificities among Black Americans with juvenile onset diabetes. N Engl J Med 301: 810–812
6. Hammond MG, Asmal AC, Omar MAK (1980) HLA and insulin-dependent diabetes in South African Negroes. Diabetologia 19: 101–102
7. Platz P, Thomsen M, Svejgaard A, Cudworth AG, Woodrow JC, Nerup J (1978) More on the genetics of juvenile diabetes. N Engl J Med 301: 810–812
8. National Diabetes Data Group (1979) Classification and diagnosis of diabetes mellitus and other categores of glucose intolerance. Diabetes 28: 1039–1057

9. WHO Expert Committee Report on Diabetes Mellitus (1980) Second Report. Technical Report Series No 646, World Health Organization, Geneva
10. Boyum A (1967) Separation of leucocytes from blood and bone marrow. Introduction. Scand J Clin Lab Invest 21 (Suppl) 97: 7–108
11. Svejgaard A, Jersild L, Staub-Nielson L, Bodmer WF (1974) HLA antigens and disease. Statistical and genetical considerations. Tissue Antigens 4: 95–105
12. Woolf B (1955) On estimating the relation between blood group and disease. Ann Hum Genet 19: 251–253
13. Nerup J, Andersen OO, Christy M, Platz P, Ryder L, Thomsen M, Svejgaard A (1976) HLA, autoimmunity, virus and the pathogenesis of juvenile diabetes mellitus. Acta Endocrinol (Suppl 1) (Copenh) 83 (Suppl 205): 167–175
14. Svejgaard A, Platz P, Ryder LP (1980) Insulin-dependent diabetes mellitus In: Terasaki PI (ed) Histocompatibility testing 1980. UCLA Tissue Typing Laboratory, Los Angles, pp 653–689
15. Svejgaard A, Hansen M, Jersild C, Platz P, Ryder BP, Staub-Nielson L, Thomsen M (1975) The HLA system. An introductory survey. Monogr Genet 7: 1–100
16. Duquesnoy RJ, MacDonald MJ, Mullins P, Hackbarth SA, Trasman HS, Levitsy LL (1979) Increased frequency of HLA-DW3 in North American Black patients with juvenile-onset diabetes. Tissue Antigens 13: 369–372
17. Famuyiwa OO, Nwabuebo IE, Abioye AA (1982) Pattern of histocompatibility (HLA) distribution among Nigerian (West African black) diabetics. Diabetes 31: 1119–1122
18. Patel R, Ansari A, Covarrubias (1977) Leucocyte antigens and disease III. Association of HLA-B8 and HLA-BW15 with insulin-dependent diabetes in three different population groups. Metabolism 26: 487–492
19. Tobias PV (1971) The biological invalidity of the term Bantu. S Afr J Sci 67: 517–520

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study is short, non-invasive and inexpensive and can be repeated many times during the average stay in hospital. The radiation dose is well within the safety limits prescribed by most hospital radiation safety committees. Previous reports have shown a strong negative correlation between the degree of steatorrhoea measured by 4-day stool fat collections and the peak excretion rate of breath $^{14}CO_2$ after the ^{14}C-labelled fat test.[2]

This test made it possible to demonstrate a general significant improvement (2½ times) in fat absorption after supplementation with 8 tablets of pancreatic extract (equivalent to 96 000 U lipase). It is generally accepted that antacids and histamine-2 blockers reduce steatorrhoea when given with pancreatic enzymes containing in excess of 30 000 U lipase.[4] The failure of such manipulations to increase absorption in our patients could be explained by the unique design of the encapsulated enzymes used. In addition to pancreatic enzymes, they have an outer coat of bromelin — a proteolytic enzyme — designed for release in the acid medium of the normal stomach. The enteric-coated pancreatic enzymes are then released into the duodenum where they are activated by the relatively higher pH. This would also explain why in the patient with achlorhydria the simultaneous administration of acid actually increased fat absorption.

In conclusion, our findings would suggest that in order to optimize control of pancreatic steatorrhoea, gastric acid studies should always be performed initially and enteric-coated preparations should be reserved for those patients with normal or high secretion rates. The use of the ^{14}C fat test permits a rapid assessment of the adequacy of therapy. The resulting improvement in fat absorption might then be expected to improve the depleted nutritional state of patients with chronic pancreatitis.

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REFERENCES

1. Abt AF, von Schuching SL. Fat utilisation test in disorders of fat metabolism. *Bull Johns Hopkins Hosp* 1966; **119:** 316-330
2. Kaihara S, Wagner HN. Measurement of intestinal fat absorption with ^{14}C labelled tracers. *J Lab Clin Med* 1968; **71;** 400-411.
3. Goff JS. Two-stage triolein breath test differentiates pancreatic insufficiency from other causes of malabsorption. *Gastroenterology* 1982; **83:** 44-46.
4. DiMagno EP. Controversies in the treatment of exocrine pancreatic insufficiency. *Dig Dis Sci* 1982; **27:** 481-484.
5. Newcomer AD, Hofmann AF, DiMagno EP *et al.* Triolein breath test: a sensitive and specific test for fat malabsorption. *Gastroenterology* 1979; **76:** 6-13.
6. Jelliffe DB. *Assessment of the Nutritional Status of a Community* (WHO Monograph Series No. 53). Geneva: WHO, 1966.

HLA A, B, C and DR antigens in young South African Indians with insulin-dependent diabetes mellitus

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Summary

The HLA status of South African Indian insulin-dependent diabetic patients in whom the age of onset of diabetes was under 35 years was compared with that of a group of healthy Indian controls. HLA A, B and C antigens were determined in 68 patients and 760 controls, while DR specificities were determined in 35 patients and 235 controls. The diabetic patients showed a significant increase in the frequencies of HLA B8 (19,1% v. 6,8%; relative risk 3,2; corrected $P < 0,04$) and Aw24 (42,6% v. 26,8%; relative risk 2,2; corrected $P < 0,04$) antigens compared with controls. HLA DR3 was found to be much more common in patients than in controls (31,4% v. 12,8%; relative risk 3,1; corrected $P < 0,035$), but DR3/DR4 heterozygosity was associated with a much greater relative risk (7,25). Among patients of North Indian origin a strong association with DR4 was seen (45,5% v. 9,1% (controls); relative risk 8,3).

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Studies of the relationship between insulin-dependent diabetes mellitus (IDDM) and the HLA system have shown clear associations between the disease and certain HLA antigens. High frequencies of HLA B8, B15, B18, Cw3, Cw4, Dw3, Dw4, DR3 and DR4 have been found in Whites with the disease.[1] Studies in other population groups have shown an association with B12 and B54 among the Japanese,[2,3] and with DR3 and DR4 among American Blacks.[4] Thus it can be seen that there are differences in the specific allelic associations among the various ethnic groups.

In a previous article a strong association between IDDM and HLA B8 among South African Indians was reported.[5] The present study was undertaken to evaluate the relationship between IDDM in Indians and HLA A, B and C antigens and the serologically detected DR antigen which appear to be controlled at the same locus as the Dw antigens.[6]

Subjects and methods

The patients and controls were all Indians, descendants of Indians who migrated from the Indian subcontinent towards the latter half of the last century. They comprised North Indians of Aryan descent and South Indians of Dravidian descent. As far as could be ascertained none of them was of mixed descent. All the patients were diagnosed as having IDDM on the basis of the revised criteria recommended by the National Diabetes Data Group and the World Health Organization, i.e. they had always depended on insulin for the control of symptoms and prevention of basal ketosis.[7,8] Sixty-eight patients were typed for HLA A, B and C antigens, while DR specificities were determined in 35 of these. The frequencies of HLA A, B and C antigens in the patients were compared with those in a group of 760 healthy Indian controls, while the frequency of HLA DR antigen was compared with that determined in 235 healthy controls.

A total of 180 antisera was used in a two-stage microlymphocytotoxicity test to determine HLA A, B and C specificities, the lymphocytes being isolated on a Ficoll-Hypaque density gradient.[9] Typing for DR specificities was performed by means of an extended incubation microlymphocytotoxicity test using T-cell-depleted B-cell-enriched lymphocytes. The differences in frequencies in the patients and the controls were tested for significance by means of the chi-squared test (without Yates' correction). To determine the corrected P value the resulting probabilities were multiplied by the number of antigens tested.[10] Relative risk was calculated according to the methods of Woolf.[11]

Results

Results are shown in Tables I - III. At the A locus there is an increase in the frequency of Aw24 in patients compared with controls, even after the P value is corrected (42,6% v. 26,8%; corrected $P < 0,04$). The frequency of B8 antigen is higher in patients than in controls (19,1% v. 6,8%), the difference being significant even when the P value is corrected (corrected $P < 0,04$). There is no difference between patients and controls as regards the frequency of B7 antigen.

As regards the D locus, there is a significant increase in the frequency of DR3 in patients compared with controls (31,4% v. 12,8%; corrected $P < 0,035$; relative risk 3,1). The frequencies of DR4 and DR2 in patients and controls did not differ significantly. However, the presence of DR3/DR4 heterozygosity is associated with a much greater relative risk (7,25) than is the presence of DR3 alone.

Comparison of the two Indian subgroups, viz. North (Aryan) Indians and South (Dravidian) Indians, shows an increased frequency of HLA B8 in the former ($P < 0,01$), but the corrected P value was not significant. The increase in the frequency of B8 was particularly marked in the 29 North Indian patients in whom onset of IDDM was before the age of 30 years compared with controls (20,7% v. 6,1%; uncorrected $P < 0,005$), the corrected P value falling just short of significance. The 23 Dravidians in whom onset of IDDM was before the age of 20 years also showed an increase in the frequency of this antigen compared with controls (26,1% v. 7,3%), but the difference just failed to attain significance after correcting for the number of antigens tested (uncorrected $P < 0,005$).

HLA DR4 was strongly associated with IDDM among the Aryans compared with controls (45,5% v. 9,1%; corrected $P < 0,035$; relative risk 8,3). There was a similar although weaker association with DR3 in this subgroup (45,5% v. 12,1%; relative risk 6); however, the difference was not significant when correcting for the number of antigens tested (uncorrected $P < 0,05$). Although the frequency of DR7 appeared to be lower in patients than in controls as regards both Aryans and Dravidians, the difference was not significant.

TABLE I. PERCENTAGE FREQUENCY OF SELECTED HLA ANTIGENS IN INDIANS WITH IDDM AND IN CONTROLS

HLA antigen	% frequency	
	Controls ($N = 760$)	Patients ($N = 68$)
Aw24*	**26,8**	**42,6 (RR 2,2)**
A25	**2,1**	**2,9**
A26	**6,8**	**11,8**
A10	**8,9**	**14,7**
A29	**0,9**	**2,9**
B7	**12,6**	**16,2**
B8*	**6,8**	**19,1 (RR 3,2)**
B15	**9,1**	**13,2**
Bw60	**11,5**	**10,3**
Bw61	**17,9**	**20,6**
Bw40	**29,3**	**30,9**
Bw51	**16,3**	**14,7**
Bw52	**13,4**	**13,2**
B5	**29,7**	**27,9**

*Uncorrected $P < 0,001$; corrected $P < 0,04$.
RR = relative risk.

TABLE II. PERCENTAGE FREQUENCY OF HLA DR ANTIGENS IN PATIENTS AND CONTROLS

HLA antigen	% frequency	
	Controls ($N = 235$)	Patients ($N = 35$)
DR1	**5,1**	**0,0**
DR2	**41,3**	**28,6**
DR3*	**12,8**	**31,4 (RR 3,1)**
DR4	**20,9**	**37,1**
DR5	**17,5**	**20,0**
DR6	**9,4**	**14,3**
DR7	**29,4**	**14,3**
One antigen	**63,8**	**54,3**
DR3/DR4	**8,6**	**1,3 (RR 7,25)**
DR3/any other antigen	**20,0**	**8,6**
DR3/DR blank	**2,9**	**2,6**
DR4/any other antigen	**17,1**	**13,7**
DR4/DR blank	**11,4**	**5,6**

*Uncorrected $P < 0,05$; corrected $P < 0,035$.
RR = relative risk.

Discussion

In Whites two distinct forms of IDDM have been recognized and these may be distinguished on the basis of HLA studies.[1,12] There is an auto-immune variety associated with DR3 and Dw3 and less strongly so with B8.[12] In the other type of IDDM age of onset is earlier, and it tends to be associated with DR4 and Dw4 and less strongly so with B15 and Cw3.[1]

A close correlation has been shown between IDDM in South African Indians and the presence of HLA B8.[5] This study, extended to involve a larger number of patients, confirmed such a relationship. The significant association with the presence of DR3 observed was not surprising in the light of recent work suggesting that the relationship between IDDM and HLA B8 is secondary to the association with DR3. In Whites and American Blacks a relationship with DR3 has also been noted,[1,4] although

TABLE III. PERCENTAGE FREQUENCIES OF HLA DR ANTIGENS AND SELECTED A AND B ANTIGENS IN THE INDIAN SUBGROUPS

	% frequency in Aryans				% frequency in Dravidians			
		Patients with onset at:				Patients with onset at:		
HLA antigens	Controls	< 20 yrs	< 30 yrs	< 35 yrs	Controls	< 20 yrs	< 30 yrs	< 35 yrs
A and B antigens†	**N = 246**	**N = 17**	**N = 29**	**N = 31**	**N = 491**	**N = 23**	**N = 30**	**N = 33**
A1	21,1	23,5	27,6	25,7	34,0	13,0	16,7	18,2
Aw24	24,4	47,1	41,4	41,9	28,7	47,8	46,7	42,4
A29	0,8	11,8***	6,9	6,5	1,0	0,0	0,0	0,0
B7	9,8	17,7	17,2	16,1	14,5	17,4	16,7	18,2
B8*	6,1	23,5*[1]	20,7**[2]	19,4*[3]	7,3	26,1***[4]	20,0	18,2
B15	7,3	17,7	10,3	9,7	9,6	13,0	16,7	18,2
Bw60	11,0	5,9	10,3	9,7	12,0	8,7	10,0	12,1
Bw61	14,2	23,5	20,7	22,6	19,1	17,4	20,0	21,2
Bw40	25,2	29,4	31,0	32,3	31,1	26,1	30,0	33,3
DR antigen‡	**N = 66**	**N = 6**	**N = 11**	**N = 11**	**N = 166**	**N = 14**	**N = 18**	**N = 20**
DR1	12,1	0,0	0,0	0,0	2,4	0,0	0,0	0,0
DR2	37,9	50,0	36,4	36,4	42,8	21,4	27,8	25,0
DR3	12,1	33,3	45,5*[5]	45,5*	12,7	21,4	16,7	20,0
DR4	9,1	33,3	45,5**[6]	45,5**[6]	25,3	35,7	38,9	35,0
DR5	16,7	16,7	9,1	9,1	18,1	26,8	27,8	30,0
DR6	3,0	16,7	18,2	18,2	11,5	14,3	16,7	15,0
DR7	33,3	33,3	18,2	18,2	28,3	14,3	11,1	10,0
One antigen	75,8	16,7	27,3	27,3	59,0	64,3	61,1	65,0

* $P < 0.01$
** $P < 0.005$.
*** $P < 0.0001$.
†Four patients and 23 controls could not be grouped.
‡Four patients and 3 controls could not be grouped.
Relative risk: 1 = 3,6; 2 = 5,9; 3 = 3,7; 4 = 4,5; 5 = 6,0; 6 = 8,3.

in the latter group a significant association with B8 has not been found.[4,13]

Population studies so far have demonstrated a close correlation between IDDM and the presence of HLA DR4 in virtually all ethnic groups.[14] In contrast, such a relationship could not be established here if all the Indian patients studied were compared with controls. However, in the Aryan subgroup a significant association was seen, although the small number of patients studied calls for caution in reaching any definite conclusion. The relationship between the presence of DR3 and IDDM was also seen in Aryans.

In contrast, neither DR3 nor DR4 tended to be associated with the disease in Dravidians. Notwithstanding the relatively small number of patients studied, there was a trend towards a much greater relative risk in patients showing DR3/DR4 heterozygosity compared with those possessing DR4 alone or even DR3 alone. Such findings have been well documented in studies on Whites with IDDM.[1] The association between IDDM and HLA Aw24 seen in this study has not been observed in other populations. This appears to support the concept that the disease is heterogeneous also in terms of HLA associations.

A significant negative correlation between IDDM and the presence of HLA B7, DR2 and DR7, which has been observed in Whites,[1,15] was not seen in the Indian patients. Srikanta *et al.*,[16] however, have observed a significant decrease in the frequency of B7 in North Indians of India. Moreover, South African Blacks with IDDM also have a lower frequency of DR2.[17]

Indians with IDDM do not show increased frequencies of Cw3, B15 and B18, as has been observed in Whites,[1] or of Bw54 and B12, found in Japanese.[2,3] Such findings serve to emphasize the ethnic variability in the association between IDDM and the HLA system.

The demonstration of a close correlation between IDDM and the presence of DR3 and B8 antigens raises questions as to the importance of auto-immunity in the pathogenesis of IDDM in Indians. In this respect the determination of islet cell and other antibodies could provide useful clues. Studies are in progress to evaluate the presence of such antibodies and their relationship to HLA antigens.

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REFERENCES

1. Christy M, Green A, Christau B *et al.* Studies of the HLA system and insulin-dependent diabetes mellitus. *Diabetes Care* 1979; **2**: 209-214.
2. Kawa A, Nakazawa M, Sakaguchi S *et al.* HLA system in Japanese patients with diabetes mellitus. *Diabetes* 1977; **26**: 591-595.
3. Nakao Y, Fukunishi T, Koide M *et al.* HLA antigens in Japanese patients with diabetes mellitus. *Diabetes* 1977; **26**: 736-739.
4. Rodey GE, White N, Frazer TE, Duquesnoy RJ, Santiago JV. HLA-DR specificities among Black Americans with juvenile onset diabetes. *N Engl J Med* 1979; **301**: 810-812.
5. Hammond MG, Asmal AC. HLA and insulin-dependent diabetes in South African Indians. *Tissue Antigens* 1980; **15**: 244.
6. Platz P, Thompsen M, Svejgaard A *et al.* More on the genetics of juvenile diabetes. *N Engl J Med* 1978; **198**: 1200-1201.
7. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 1979; **28**: 1039-1057.
8. World Health Organization Expert Committee. Report on Diabetes. *WHO Tech Rep Ser* 1980; **646**: 8-14.
9. Boyum A. Separation of leucocytes from blood and bone marrow. Introduction. *Scand J Clin Lab Invest* 1968; **21**: suppl 97, 7.
10. Svejgaard A, Jersild L, Staub-Nielson L, Bodmer WF. HLA antigens and disease: statistical and genetical considerations. *Tissue Antigens* 1974; **4**: 95-105.
11. Woolf B. On estimating the relation between blood group and disease. *Ann Hum Genet* 1955; **19**: 251-253.
12. Nerup J, Andersen OO, Christy M *et al.* HLA, autoimmunity, virus and the pathogenesis of juvenile diabetes mellitus. *Acta Endocrinol (Suppl) (Copenh)* 1976; **83**: suppl 205, 167-175.
13. Duquesnoy RJ, MacDonald MJ, Mullins P, Hackbarth SA, Trasman HS, Levitsy LL. Increased frequency of HLA DW3 in North American Black patients with juvenile-onset diabetes. *Tissue Antigen* 1979; **13**: 369-372.
14. Svejgaard A, Platz P, Ryder LP. Insulin-dependent diabetes mellitus. In: Terasaki PI, ed. *Histocompatibility Testing 1980.* Los Angeles: UCLA Tissue Laboratory, 1980: 653-689.
15. Cudworth AG, Wold E. The genetic susceptibility to type 1 (insulin-dependent) diabetes mellitus. *Clin Endocrinol Metab* 1982; **11**: 389-408.
16. Srikanta NK, Mehra MC, Caidya AN, Malaviya AN, Ahuja MMS. HLA antigens in type I (insulin dependent) diabetes mellitus in North India. *Metabolism* 1981; **30**: 992-993.
17. Omar MAK, Hammond MG, Asmal AC. HLA A, B, C and DR antigens in South African Blacks with insulin-dependent diabetes mellitus. *Diabetologia* 1984; **26**: 20-23.

HLA antigens and non-insulin-dependent diabetes mellitus in young South African Indians

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Summary

HLA A, B and C antigens were determined in 84 South African Indian patients with non-insulin-dependent diabetes mellitus (NIDDM) in whom age of onset was under 35 years and in 760 healthy Indian controls.

Increased frequencies of Aw24, B15 and Bw61 were seen in the patients, but the corrected P value was not significant. Among Indians of North Indian origin, however, there was a significant association between B15 and NIDDM (corrected $P < 0,012$; relative risk 4,8). In Indians of South Indian origin no clear association with any specific HLA antigens was seen, although there was a slight increase in the frequency of Aw24 (uncorrected $P < 0,007$; corrected $P > 0,05$). The findings in this study serve to emphasize the heterogeneity of diabetes mellitus, since no association between NIDDM and HLA antigens has been noted in whites.

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It has recently become quite clear that the genetic mechanisms involved in the pathogenesis of insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) are quite distinct from each other.[1,2] Therefore, while an association between IDDM and certain HLA agents has been established in numerous studies involving various population groups,[3-6] the relationship between IDDM and the HLA system is not clear.

Studies confined to young Indians with IDDM have shown an increased frequency of HLA B8.[6] This study was undertaken to evaluate the relationship between NIDDM in young Indians and the antigens of the HLA system.

Subjects and methods

Eighty-four Indians with NIDDM were studied. They comprised 40 Aryans (North Indian origin) and 41 Dravidians (South Indian origin); 3 could not be classified as either Aryan or Dravidian. The age of onset of the disease in all the patients was under 35 years. Diagnosis of diabetes mellitus and classification as NIDDM were based on the revised criteria recommended by the National Diabetes Data Group and the World Health Organization's Expert Committee on Diabetes Mellitus.[7,8] In all the patients the condition was controlled by means of diet (with or without oral hypoglycaemic agents) and they had never shown ketosis at any time. HLA A, B and C antigens were determined in all 84 patients and in 760 healthy controls. The latter included 491 Dravidians and 246 Aryans; 23 could not be classified into these two subgroups.

A total of 180 antisera were used in a two-stage microlymphocytotoxicity test to determine HLA A, B and C specificities, the lymphocytes being isolated on a Ficoll-Hypaque density gradient.[9] The differences in frequencies in the patients and the controls were tested for significance by means of the chi-squared test (without Yates' correction). The resulting probabilities were then multiplied by the number of antigens tested in order to determine the corrected P value.[10]

Results

Results are shown in Tables I-III. Increased frequencies of HLA Aw24 and Bw61 are seen in the Indians with NIDDM. However, the differences were not significant when corrections were made for the number of antigens being tested. The increased frequency of B15 in patients compared with controls (19,0% v. 9,1%) just fails to attain statistical significance if the P value is corrected (uncorrected $P < 0,005$).

Among the Aryans there was a much higher frequency of HLA B15 in patients than in controls (27,5% v. 7,3%), the difference being significant even after correcting for the number of antigens tested (corrected $P < 0,012$). No such difference was found between Dravidian patients and controls. Although the frequency of Bw61 was also higher in Aryan patients than in Aryan controls ($P < 0,05$), the corrected P value fails to attain statistical significance. Dravidians with NIDDM showed a higher frequency of HLA Aw24 (48,8% v. 28,7%), but the difference was not significant once the P value was corrected.

Discussion

Studies in whites with NIDDM have so far been unable to establish a clear relationship between the disease and the HLA system.[3,11,12] In other population groups, however, such an association has been shown;[13-15] an increase in the frequency of B35 has been shown in a study involving a small number of Xhosas with NIDDM,[13] and in Pima Indians with the disease an association with HLA A2 has been shown (particularly in those in whom age of onset was under 35 years).[14]

Among the Indians reported in this study a higher frequency of Bw61 was found in patients than in controls (uncorrected $P < 0,05$). Serjeantson *et al.*[15] have shown the same thing in Fiji Indians with NIDDM.[15] However, the findings of their study were significant even after correcting for the P value, whereas this was not the case with the patients reported here. None the

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TABLE I. PERCENTAGE FREQUENCY OF HLA A AND B ANTIGENS IN INDIANS WITH NIDDM AND IN CONTROLS

HLA antigen	% frequency Controls ($N = 760$)	Patients ($N = 84$)
A1	29,6	15,5
A2	33,4	35,7
A3	10,9	15,5
A11	27,9	26,2
A23	1,6	0,0
*Aw24**	*26,8*	*40,5*
Aw25	2,1	2,4
Aw26	6,8	4,8
Aw28	12,6	7,1
Aw29	0,9	1,2
Aw30	3,7	3,6
Aw31	2,6	3,6
Aw32	4,0	4,8
Aw33	13,6	14,3
One antigen	23,4	25,0
B7	12,6	19,0
B8	6,8	10,7
B13	6,6	4,8
B14	0,5	0,0
*B15**	*9,1*	*19,0*
B16	3,7	3,6
B17	22,5	8,3
B18	3,2	2,4
Bw21	2,9	7,1
Bw22	3,8	1,2
Bw27	1,8	4,8
Bw35	20,3	20,2
Bw37	6,6	3,6
Bw41	0,4	0,0
Bw42	0,0	0,0
Bw44	13,3	10,7
Bw45	0,3	0,0
Bw51	16,3	17,9
Bw52	13,4	10,7
Bw53	0,9	0,0
B51	4,9	3,6
Bw60	11,5	7,1
*Bw61***	*17,9*	*27,4*
BU	1,8	1,2
One antigen	19,1	16,7

*Uncorrected $P < 0,01$.
**Uncorrected $P < 0,005$; relative risk 2,4.
***Uncorrected $P < 0,05$.

TABLE II. PERCENTAGE FREQUENCY OF HLA A AND B ANTIGENS IN NORTH INDIANS (ARYANS) WITH NIDDM AND NORTH INDIAN CONTROLS

HLA antigen	% frequency Controls ($N = 246$)	Patients ($N = 40$)
A1	21,1	17,5
A2	33,3	32,5
A3	12,2	12,5
A11	31,7	30,0
A23	1,6	0,0
Aw24	24,4	32,5
Aw25	2,4	2,5
Aw26	5,3	5,0
Aw28	13,0	12,5
Aw29	0,8	2,5
Aw30	3,7	5,0
Aw31	2,4	2,5
Aw32	4,9	5,0
Aw33	20,7	15,0
One antigen	22,4	25,0
B7	9,8	10,0
B8	6,1	7,5
B13	6,1	2,5
B14	1,6	0,0
*B15**	*7,3*	*27,5*
B16	4,9	2,5
B17	18,7	10,0
B18	3,3	2,5
Bw21	2,9	5,0
Bw22	4,5	0,0
Bw27	3,3	5,0
Bw35	25,6	22,5
Bw37	3,3	5,0
Bw41	0,4	0,0
Bw42	0,0	0,0
Bw44	20,7	7,5
Bw45	0,4	0,0
Bw51	13,0	17,5
Bw52	15,0	10,0
Bw53	0,4	0,0
B51	4,9	5,0
Bw60	11,0	2,5
*Bw61***	*14,2*	*30,0*
BU	1,6	0,0
One antigen	21,1	27,5

*Corrected $P < 0,012$; relative risk 4,8.
**Uncorrected $P < 0,05$.

TABLE III. PERCENTAGE FREQUENCIES OF HLA A AND B ANTIGENS IN SOUTH INDIANS (DRAVIDIANS) WITH NIDDM AND SOUTH INDIAN CONTROLS

HLA antigen	% frequency Controls ($N = 491$)	Patients ($N = 41$)
A1	34,0	12,2
A2	33,0	39,0
A3	10,2	19,5
A11	25,7	22,0
A23	1,4	0,0
*Aw24**	*28,7*	*48,8*
Aw25	1,8	2,4
Aw26	7,7	4,9
Aw28	12,6	2,4
Aw29	1,0	0,0
Aw30	3,9	2,4
Aw31	2,9	4,9
Aw32	3,3	4,9
Aw33	10,2	9,8
One antigen	23,6	26,8
B7	14,5	29,3
B8	7,3	14,6
B13	6,9	7,3
B14	0,0	0,0
B15	9,6	12,2
B16	3,1	4,9
B17	24,2	7,3
B18	3,1	2,4
Bw21	2,9	9,8
Bw22	3,3	2,4
Bw27	1,2	2,4
Bw35	17,5	19,5
Bw37	8,4	0,0
Bw41	0,4	0,0
Bw42	0,0	0,0
Bw44	9,4	12,2
Bw45	0,2	0,0
Bw51	18,1	19,5
Bw52	12,8	12,2
Bw53	1,2	0,0
B51	4,5	2,4
Bw60	12,0	7,3
Bw61	19,1	24,4
BU	2,0	2,4
One antigen	18,3	7,3

*Uncorrected $P < 0,007$; relative risk 2,4.

less, since both the Natal Indians and the Fiji Indians have similar origins, identical HLA associations are not unexpected. Unlike Fiji Indians, Natal Indians do not show any linkage disequilibrium between Bw61 and Aw24.

The significant relationship between HLA B15 and NIDDM in North Indians is somewhat unexpected, since the same antigen has been associated with IDDM in whites.[3] However, this finding serves to highlight the heterogeneity of diabetes mellitus. The fact that in Pima Indians a relationship with NIDDM has been shown at a different locus[13] serves to emphasize the heterogeneity of such associations, as has been shown in IDDM.

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REFERENCES

1. Barnett AH, Eff C, Leslie RDG, Pyke DA. Diabetes in identical twins: a study of 200 pairs. *Diabetologica* 1981; **20:** 87-93.
2. Gottlieb MS, Soeldner JS, Kyner JL, Gleason RE. Oral glucose stimulated insulin release in non-diabetic twin siblings of diabetic twins. *Diabetes* 1974; **23:** 684-692.
3. Christy M, Green A, Christau B *et al.* Studies of the HLA system and insulin-dependent diabetes mellitus. *Diabetes Care* 1979; **2:** 209-214.
4. Rodey GE, White N, Frazer TE, Duquesnoy RJ, Santiago JV. HLA-DR specificities among Black Americans with juvenile onset diabetes. *N Engl J Med* 1979; **301:** 810-812.
5. Hammond MG, Asmal AC, Omar MAK. HLA and insulin-dependent diabetes in South African Negroes. *Diabetologica* 1980; **19:** 101-102.
6. Hammond MG, Asmal AC. HLA and insulin-dependent diabetes in South African Indians. *Tissue Antigens* 1980; **15:** 244.
7. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 1979; **28:** 1039-1057.
8. World Health Organization Expert Committee. Report on diabetes. *WHO Techn Rep Ser* 1980; **646:** 8-14.
9. Boyum A. Separation of leucocytes from blood and bone marrow. Introduction. *Scand J Clin Lab Invest* 1968; **21:** suppl 97: 7.
10. Svejgaard A, Jersild L, Staub-Nielsen L, Bodmer WF. HLA antigens and disease: statistical and genetical considerations. *Tissue Antigens* 1974; **4:** 95-105.
11. Nelson PG, Pyke DA, Cudworth AG, Woodrow JC, Batchelor JR. Histocompatibility antigens in diabetic identical twins. *Lancet* 1975; **ii:** 193-194.
12. Platz P, Jakobsen BK, Thomsen BS *et al.* No evidence of linkage between HLA and maturity onset type of diabetes in young people. *Diabetologica* 1982; **23:** 16-18.
13. Briggs BR, Jackson WP, Du Toit ED, Botha MC. The histocompatibility (HLA) antigen distribution in diabetes in Southern African Blacks (Xhosa). *Diabetes* 1980; **29:** 68-71.
14. Knowles WC, Williams RC, Butler WJ, Petit DJ, Lisse JR, Mann DL. HLA-A2 and type II diabetes. *Diabetes* 1981; **30:** suppl 1, 219.
15. Serjeantson SW, Ryan DP, Ram P, Zimmet P. HLA and noninsulin dependent diabetes in Fiji Indians. *Med J Aust* 1981; **1:** 462-463.

Postpartum-sterilisasies en die private praktisyn

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Summary

A postpartum programme of sterilization was initiated by private practitioners at the Paarl Hospital in 1968. The unit was later taken over by the University of Stellenbosch and a total of 4 704 procedures had been completed by the end of 1983. Most of the doctors trained in the method are now in private practice. The sterilization-to-delivery ratio of 1 in 5 indicates that ± 200 000 postpartum sterilizations would be requested in South Africa, if the Paarl figures are projected to the rest of the country. The most effective method of mobilizing our medical manpower would be a fair fee per procedure. South Africa cannot afford the present continued burden of unwanted and unplanned births.

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Die Paarl-hospitaal is lank reeds gevestig as 'n sentrum vir postpartum sterilisasies.[1] Vanaf 1968 is hierdie klein operasie deur die algemene praktisyns van die Paarl beskikbaar gestel aan enige vrou wat hierdie ingreep vrywillig aangevra het. Sy het die basiese reg om permanente chirurgiese kontrasepsie aan te vra om sodoende te sorg dat sy en haar gesin teen enige verdere ongewenste en onbeplande swangerskappe beskerm word.[2] Waardevolle ondervinding in die uitvoer van die ingreep is oor die jare opgedoen, en verskillende basiese tegnieke is ondersoek.[3]

Onlangs is 'n nuwe tegniek (die Filshie-klemaanwending) ondersoek met die oog op spoed van die prosedure, permanensie en die beste omkeerbaarheid.[4,5] Die effek van buisafbinding op maandstondepatrone is al in 1975 deur die Paarl-hospitaal ondersoek en beskryf.[2]

Al hierdie bevindings is onlangs deur 'n groot multisentriese en meer wetenskaplik gefundeerde ondersoek bevestig.[6] Sterilisasie veroorsaak nie maandstondeafwykings nie, maar aangesien elke vrou se maandstondepatroon veranderlik is, kan nieverwante veranderings natuurlik in die individuele geval voorkom. Postpartum-sterilisasies is so 'n veilige prosedure[7] en het so 'n groot aanvraag in die Paarl geskep, dat 4 704 vrouens teen die einde van 1983 reeds die operasie ondergaan het (ongeveer 20% van alle vrouens in die Paarl verlos). As die prosedure op 'n jong gesonde vrou uitgevoer word, is die risiko van dood minimaal.

The HLA system and diabetes mellitus

The HLA system, also known as the major histocompatibility complex, constitutes a complex group of antigens determined by genes located on the short arm of the sixth chromosome where they are closely linked with genes controlling various immune responses and some components of the complement cascade. HLA A, B, C and D antigens are determined by four different loci, each with a large number of alleles. The recently described DR antigens appear to be controlled at the same locus as the HLA D antigens.[1-3]

The HLA system is characterized by extreme polymorphism at each locus. At the same time pronounced linkage disequilibrium occurs between the various loci, that is, certain pairs of HLA antigens are found together in a population at a greater frequency than would be expected from multiplying their individual frequencies together.

In recent years numerous studies have shown clear associations between the HLA systems and various diseases.[1] Although the mechanisms through which HLA antigens confer disease susceptibility are obscure, several hypotheses have been suggested: (*i*) through direct effects of the HLA antigens (e.g. interference with ligand-receptor interaction on all cell surfaces); (*ii*) through effects of different but closely linked or functionally related genes in the HLA region (e.g. immune response genes); and (*iii*) through effects of genes in linkage disequilibrium with HLA by pure coincidence, the HLA antigens here being 'inert' markers.

After Nerup *et al.*[4] had produced definite evidence showing an increased frequency of HLA B8 and B15 in patients with insulin-dependent diabetes mellitus (IDDM), many studies using two approaches (viz. population studies and family studies) have provided unequivocal evidence of an association between IDDM and the HLA system.[5]

The antigens associated with IDDM in white Caucasian populations include HLA Cw3, Cw4, B8, B15, B18, D3, DR3m, D4 and DR4.[4,5] In addition, a relationship has been found with complement factors Bf, C4 and C2, which are determined at loci closely linked with the HLA complex.[5] HLA B7 and D2, however, show a negative correlation with the disease.[5]

It has now become clear that the presence of certain D-locus antigens is far more important in determining susceptibility to IDDM than those at other loci, and that the latter associations, being secondary to those involving the D - DR antigens, could be explained on the basis of linkage disequilibrium.[4,5] Thus the relationships between IDDM and HLA B8 and B15 are secondary to the presence of D3 and D4 respectively. Similarly, the degree of negative correlation is greater with DR2 than with B7.

On the basis of the HLA studies two distinct forms of IDDM have been recognized in white Caucasoids.[1] There is an auto-immune variety, which is associated with Dw3 and less strongly so with B8, the presence of persistent islet antibodies, and an increased risk of micro-angiopathy. The other type which is associated with B15 and C3 appears to have an earlier age of onset and to show an increased antibody response to exogenous insulin. It shows a stronger association with Dw4, and is not associated with auto-immune disease or persistence of islet-cell antibody.[4,6] The presence of both B8 and D4 is characterized by an increased relative risk and an increased prevalence of the disease among twins, i.e. the presence of both allelic groups confers an additive risk of developing the disease.

Although little work has been done on non-Caucasoids, certain definite associations between IDDM and the HLA system have been established. The presence of DR4 or D4 appears to be a risk factor in virtually all ethnic groups studied so far.[7] In addition, among Japanese the disease has been associated with HLA DYT and B54, in American blacks with DR3 and DR4,[8-12] and in South African blacks of Zulu descent with HLA DR4 but not with DR3.[13] In neither the Japanese nor the black groups studied in South Africa, Nigeria and America has a relationship between IDDM and B8 been shown, although such a relationship has been a constant finding in white Caucasoids.[8-15]

In South African Indians with IDDM a strong association with HLA B8 is shown.[16] It is thus evident that there are differences in the specific allelic associations among various ethnic groups.

Since a decreased frequency of an antigen, as opposed to an increased frequency, requires a much larger sample size to become evident,[17] studies in non-Caucasoids so far have not shown any obvious negative associations between IDDM and HLA antigens. None the less, possession of HLA DR2 or Bw42 does appear to protect against the development of IDDM in South African blacks.[13] A negative correlation between the disease and HLA B7 has been shown in a group of Indians in India,[18] but not in South African Indians.

So far almost all studies on white Caucasoids have shown no association between non-insulin-dependent diabetes mellitus (NIDDM) and the HLA system.[4,5] In other population groups, however, such an association has been shown, although it is not as strong as with IDDM. In Fiji Indians with NIDDM a positive correlation with HLA Bw61 has been shown, as well as in Natal Indians in whom, however, the finding fails to reach statistical significance.[19] Of particular interest is the association between HLA B15 and NIDDM in Natal Indians of North Indian origin, since this antigen has been associated with IDDM in white Caucasoids.[20] Other antigens that have been found to be associated with the disease are A2 in young Pima Indians and B35 in a small group of Xhosas.[21,22] Among white Caucasoids only a Finnish group has shown an association between NIDDM and HLA antigens.[23]

In conclusion, there seems little doubt that diabetes mellitus is a heterogeneous entity even in terms of HLA associations.

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1. Platz P, Thompsen M, Svejgaard A, Cudworth AG, Woodrow JC, Nedrup J. More on the genetics of juvenile diabetes *N Engl Med J* 1978; **298:** 1200-1201.
2. Rubinstein D, Sucin-Foca N, Nicholson JF. Genetics of juvenile diabetes mellitus: a recessive gene closely linked to HLA D and 50 percent penetrance. *N Engl J Med* 1977; **297:** 1036-1040.
3. Rotter JI, Rimoin DL. Heterogeneity in diabetes mellitus — update 1978. Evidence for further genetic heterogeneity within juvenile-onset insulin-dependent diabetes mellitus. *Diabetes* 1978; **27:** 599-608.
4. Nerup J, Cathelneau C, Seignalet J, Thomsen M. HLA and endocrine diseases. In: Dausset J, Svejgaard A, eds. *HLA and Disease*. Copenhagen: Munksgaard, 1977: 149-167.
5. Christy M, Green A, Christau B *et al*. Studies of the HLA system and insulin-dependent diabetes mellitus. *Diabetes Care* 1979; 2: 209-214.
6. Morris RJ, Vaugh H, Irvine WJ *et al*. HLA and pancreatic islet cell antibodies in diabetes. *Lancet* 1976; **ii:** 652-653.
7. Svejgaard A, Platz P, Ryder LP. Insulin-dependent diabetes mellitus. In: Terasake PI, ed. *Histocompatibility Testing 1980*. Los Angeles: UCLA Tissue Typing Laboratory, 1980: 653-689.
8. Kawa A, Nakazawa M, Sakaguch S *et al*. HLA system in Japanese patients with diabetes mellitus. *Diabetes* 1977; 26: 591-595.
9. Nakao Y, Fukunishi T, Koide M *et al*. HLA antigens in Japanese patients with diabetes mellitus. *Diabetes* 1977; 26: 736-739.
10. Okimoto K, Juyi T, Ishiba S, Maruyama H, Tohyama H, Kosaka K. HLA BW54 (BW 22 -J, J-1) antigen in juvenile onset diabetes mellitus in Japan. *Tissue Antigens* 1978; **11:** 418-422.
11. Duquesnoy RJ, MacDonald MJ, Mullins P, Hackbarth SA, Tranman HS, Levitsky LL. Increased frequency of HLA DW3 in North American Black patients with juvenile-onset diabetes. *Tissue Antigens* 1979: **13:** 369-372.
12. Rodey GE, White N, Frazer TE, Duquesnoy RJ, Santiago JV. HLA-DR specifities among Black Americans with juvenile-onset diabetes. *N Engl J Med* 1979; **301:** 810-812.
13. Omar MAK, Hammond MG, Asmal AC. HLA A, B, C, and DR antigens in young South African Blacks with type I (insulin-dependent) diabetes mellitus. *Diabetologia* 1984; 26: 20-23.
14. Famuyiwa OO, Nwabuebo IE, Ahioye AA. Patterns of histocompatibility (HLA) among Nigerian (West African Black) diabetics. *Diabetes* 1982; **31:** 1119-1122.
15. Hammond MG, Asmal AC, Omar MAK. HLA and insulin-dependent diabetes in South African Negroes. *Diabetologia* 1980; **19:** 101-102.
16. Omar MAK, Hammond MG, Rajput MC, Asmal AC. HLA A, B, C and DR antigens in young South African Indians with insulin-dependent diabetes mellitus. *S Afr Med J* 1984; 66: 765-767.
17. Svejgaard A, Hansen M, Jersild C, Platz P, Ryder BP, Staub-Nielsen Thomsen M. The HLA System: an introductory survey. *Monogr Hum Genet* 1975; 7: 1-100.
18. Srikanta NK, Mehra MC, Vaidya AN, Malariya AN, Ahuja MMS. HLA antigens in type I (insulin-dependent) diabetes mellitus in North India. *Metabolism* 1981; 30: 992-993.
19. Serjeantson SW, Ryan DP, Ram P, Zimmet P. HLA and non-insulin-dependent diabetes in Fiji Indians. *Med J Aust* 1981; 1: 462-463.
20. Omar MAK, Hammond MG, Seedat MA, Asmal AC. HLA antigens and non-insulin-dependent diabetes mellitus in young South African Indians. *S Afr Med J* 1985; 67: 130-132.
21. Knowles WC, Williams RC, Butler WJ, Petit DJ, Lisse JR, Mann DL. HLA AZ and type II diabetes. *Diabetes* 1981; 30: supp 1, 219.
22. Briggs BR, Jackson WP, Du Toit ED, Botha MC. The histocompatibility (HLA) antigen distribution in diabetes in Southern African Blacks (Xhosa). *Diabetes* 1980; 29: 68-71.
23. Groop L, Koskimies S, Pelkonen R, Tolppanedn EM. Increased frequency of HLA-CW4 in type II diabetes. *Acta Endocrinol* 1983; **104:** 475-478.

Veertig jaar later

Dit is nou 40 jaar sedert die oorlogskanonne in Europa stil geword het en alhoewel elke beskaafde mens wil vergewe en vergeet, is dit nodig om oorsigtelik om te kyk en te put uit die toendertydse lyding van enkelinge aan, wat eers duidelik word wanneer die geneesheer van die pasiënt se oorlogsverlede weet.

'n Neweprobleem van die KZ-sindroom asook die sogenaamde oorlogstressindroom is 'n bykomende angs

6 Mohan, V., Snehalatha, C., Ramachandran, A., Jayashree, R., and Viswanathan, M.: Pancreatic beta cell function in tropical pancreatic diabetes. Metab. Clin. Exp. 1983; 32:1091–92.

Fat Atrophy in Human Insulin Therapy

Fat atrophy is generally considered to be an immunologic reaction to impurities contained in insulin preparations.[1] It was seen fairly frequently before the introduction of highly purified insulins, but in recent years the incidence seems to have decreased markedly, probably due to the increased purity of currently available insulins. To my knowledge, fat atrophy has never previously been reported in patients receiving human insulin.

A 24-yr-old woman developed insulin-dependent diabetes in April 1983. She was subsequently stabilized on a single morning injection of porcine monocomponent insulin (6 U Actrapid insulin and 15 U Monotard insulin, Novo, Johannesburg, South Africa) before breakfast with excellent diabetes control, as evidenced by intensive self-monitoring of blood glucose. She subsequently married and moved to another city but returned to see me in January 1985 when she was experiencing problems with staphylococcal skin infections. Her insulin regimen was unchanged and her diabetes control remained good, with a glycosylated HbA_k level of 6.9% (normal range 5.8–8.8%). At that stage she had noted small areas of fat atrophy on both thighs in areas distant from the skin infections. Examination revealed two shallow indentations, 1–2 cm in diameter, on the anterior aspect of both thighs. She was changed to the identical dose of semisynthetic human insulin (Novo Actrapid-HM and Monotard-HM).

She returned to see me in September 1985 and reported that the areas of fat atrophy had enlarged. Examination showed a large area up to 5 cm diameter and 1 cm deep on each thigh. Her diabetes control had remained good, with a glycosylated HbA_k of 5.9%. She was then changed to biosynthetic human insulin (Humulin-R and Humulin-N, Eli Lilly, Indianapolis, IN) and has returned home to see whether the areas of fat atrophy will continue to progress or start regressing. I am awaiting follow-up when she next visits Cape Town.

This must presumably be an extremely rare complication of human insulin therapy, and it would be interesting to know whether this has been noted elsewhere.

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REFERENCE

1 Huntley, A. C.: The cutaneous manifestations of diabetes mellitus. J. Am. Acad. Dermatol. 1982; 7:427–55.

HLA and NIDDM in the Young

In the South African Indian the presentation of diabetes in the young is atypical in that insulin-dependent diabetes mellitus (IDDM) is rare, whereas non-insulin-dependent diabetes mellitus (NIDDM) in the young is common.[1–3] This syndrome of NIDDM in the young is uniformly accepted to be a subset of NIDDM with the strongest genetic component and appears to segregate in an autosomal dominant fashion.[4] In the previous studies in which the HLA status of Caucasoid patients with NIDDM in the young were investigated, this syndrome does not appear to be associated or linked to the HLA system.[5–8] In an attempt to ascertain whether the HLA system was involved in a non-Caucasian population, the HLA antigens of four Indian families with NIDDM in the young (25 members) were determined.

Twelve patients belonged to families in which NIDDM was transmitted via one parent through three successive generations. NIDDM in the young was categorized according to the following criteria: age <30 yr at diagnosis, duration of diabetes >2 yr (as defined by WHO criteria[9]), aketonuric but symptomatic presentation, and prevention of ketonuria and control of symptoms without insulin therapy.

HLA-A, -B, and -C antigens of all family members were determined by the standard two-stage microlymphocytotoxicity test,[10] by use of 180 local and exchanged sera to define the specificities. HLA-DR antigens were defined by the long-incubation technique (Ninth International Histocompatibility Workshop) with 120 local and exchange sera. Lymphocytes were isolated on a Ficoll-Hypaque density gradient,[11] and T- and B-cells were separated by means of straws containing nylon wool.[12]

The HLA haplotypes, ages, 2-h plasma glucose levels (after 75 g oral glucose), and body mass indices of the families are shown in Table 1. It is evident that in none of the families did the diabetic state segregate with an HLA haplotype or a combination of haplotypes. In addition, it appears that no HLA type is more frequent in the diabetic than in the nondiabetic family members.

In 1976, Nelson and Pyke[5] studied 13 diabetic and 9 nondiabetic members of families with NIDDM in the young. They reported that the gene involved is not linked to the HLA-B locus. During the same year Barbosa[13] suggested that there was an association between the HLA haplotypes A3 and BW15 and the hyperglycemic trait. He later confirmed this suggestion.[14]

Faber et al.[6] HLA-typed a family with NIDDM in the young for A, B, C, and D antigens. They demonstrated that there was no association between specific HLA antigens and NIDDM in the young, whereas Platz et al.[7] performed HLA typing for A, B, and C antigens on 53 members of one family. They also concluded that there was no significant positive linkage of HLA type with NIDDM in the young. More recently, Barbosa[8] studied 10 large families with NIDDM in the young and found that the disorder was neither associated nor linked

TABLE 1
HLA haplotypes in families with three generations of NIDDM in the young

Glucose (mmol/L)	BMI (kg/m^{-2})	Age (yr)	Generation	Family member	Condition of subject				
Family 1									
			1.1	Grandmother	NIDDM*				
21,0	22	48	2.1	Mother	NIDDM	a	A2	C−	B51	DR−
						b	A28	C−	B8	DR3
7,9	25	52	2.2	Father	Normal	c	A2	Cw1	B37	DR10
						d	A1	Cw6	B57	DR7
7,6	22	23	3.1	Child 1 (M)	Normal	b	A28	Cw−	B8	DR3
						c	A2	Cw1	B37	DR10
5,7	20	21	3.2	Child 2 (M)	Normal	a	A28	Cw−	B8	DR3
						d	A1	Cw6	B57	DR7
14,0	21	17	3.3	Child 3 (F)	NIDDM	b	A28	Cw−	B8	DR3
						c	A2	Cw1	B37	DR10
4,9	19	13	3.4	Child 4 (M)	Normal	b	A28	Cw−	B8	DR3
						c	A2	Cw1	B37	DR10
3,9	19	12	3.5	Child 5 (M)	Normal	a	A2	Cw−	B51	DR−
						d	A1	Cw6	B57	DR7
5,9	20	10	3.6	Child 6 (F)	Normal	b	A28	Ce−	B8	DR3
						d	A1	Cw6	B547	DR7
Family 2										
			1.1	Grandmother	NIDDM*					
13,5	29	49	2.1	Mother	NIDDM	a	A1	Cw−	B62	DR−
						b	A1	Cw−	B57	DR7
14,2	25	46	2.2	Aunt	NIDDM	e	A33	Cw−	B61	DR2
						f	A−	Cw−	B−	DR−
4,6	26	56	2.3	Father	Normal	c	A33	Cw−	B61	DR2
						d	A1	Cw−	B17	DR7
4,9	22	24	3.1	Child 1 (F)	Normal	b	A1	Cw−	B57	DR7
						c	A33	Cw−	B61	DR2
12,8	29	23	3.2	Child 2 (F)	NIDDM	a	A1	Cw−	B62	DR−
						c	A33	Cw−	B61	DR2
5,7	22	21	3.3	Child 3 (F)	Normal	b	A1	Cw−	B57	DR7
						c	A33	Cw−	B61	DR2
5,8	22	15	3.4	Child 4 (M)	Normal	b	A1	Cw−	B57	DR7
						c	A33	Cw−	B61	DR2
Family 3										
20,0	24	65	1.1	Grandmother	NIDDM	a	A2	Cw−	B60	DR2
						e	A−	Cw−	B44	DR7
18,0	27	38	2.1	Mother	NIDDM	a	A2	Cw−	B60	DR2
						b	A1	Cw1	B55	DR1
6,9	22	36	2.2	Aunt	Normal	a	A2	Cw−	B60	DR2
						f	A−	Cw−	B44	DR7
5,8	26	45	2.3	Father	Normal	c	A24	Cw−	B35	DR4
						d	A−	Cw−	B58	DR−
13,0	39	15	3.1	Child 1 (F)	NIDDM	b	A1	Cw1	B55	DR1
						d	A24	Cw−	B35	DR4
Family 4										
			1.1	Grandmother	NIDDM*					
20,2	23	45	2.1	Mother	NIDDM	a	A28	Cw−	B52	DR2
						b	A31	Cw−	B51	DR2
7,4	25	48	2.2	Father	Normal	c	A1	Cw−	B60	DR2
						d	A1	Cw−	B60	DR10
18,2	25	29	3.1	Child 1 (F)	NIDDM	a	A28	Cw−	B52	DR2
						c	A1	Cw−	B60	DR2
15,8	19	19	3.2	Child 2 (M)	NIDDM	b	A31	Cw−	B51	DR2
						c	A1	Cw−	B60	DR2
13,6	22	18	3.3	Child 3 (F)	NIDDM	b	A31	Cw−	B51	DR2
						d	A1	Cw−	B60	DR10

M, male, F, female; BMI, body mass index.
*Not tested (died).

to HLA types. Thus far all the studies were confined to Caucasoid patients. In an attempt to determine whether a similar situation pertained in a non-Caucasoid population, we studied a migrant Asian group. In our study, a further antigen HLA-DR was also measured. Similar findings were observed in this group of Indian patients. It thus appears that with respect to HLA status, NIDDM in the young in Indians is in no way different from that which manifests itself in Caucasoids.

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REFERENCES

[1] Jackson, W. P. U.: Epidemiology of diabetes in South Africa. Adv. Metab. Disorders 1978; 9:112–46.

[2] Asmal, A. C., Dayal, B., and Jialal, I.: Non-insulin-dependent diabetes mellitus with early onset in Blacks and Indians. S. Afr. Med. J. 1981; 60:93–96.

[3] Jialal, I., Joubert, S. M., Asmal, A. C., and Jenkins, N.: The insulin and glucose response to an oral glucose load in non-insulin-dependent diabetes in young. S. Afr. Med. J. 1982; 61:351–54.

[4] Fajans, S. S.: Heterogeneity between various familes with non-insulin-dependent diabetes of the MODY type. *In* Genetics of Diabetes Mellitus. Kobberling, J., and Tattersall, R. B., Eds. New York, Academic, 1982:251–60.

[5] Nelson, P. G., and Pyke, D. A.: Genetic diabetes not linked to the HLA locus. Br. Med. J. 1976; 1:196–97.

[6] Faber, O. K., Thomas, I. B., Binker, C., Platz, P., and Svejgaard, A.: HLA antigens in a family with maturity onset type diabetes mellitus. Acta Endocrinol. 1978; 88:329–38.

[7] Platz, P., Jakobsen, B. K., Svejgaard, A., Thomsen, B. S., Jensen, K. B., Henningsen, K., and Lamm, L. V.: No evidence for linkage between HLA and maturity onset type of diabetes in young people. Diabetologia 1982; 23:16–18.

[8] Barbosa, J.: No linkage between HLA and maturity onset hyperglycaemia in the young. Diabetologia 1983; 24:137.

[9] WHO Expert Committee on Diabetes Mellitus. Second Report (Geneva 1980). Tech. Rep. Ser. 646:10–12.

[10] Terasaki, P. I., and McLelland, J. D.: Microdroplet assay of human serum cytotoxins. Nature (Lond.) 1964; 204:998–1000.

[11] Boyum, A.: Separation of leucocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. 1968; 21 (Suppl.):97.

[12] Danilovs, J., Terasak , P. I., Park, M. S., and Ayoub, G.: B lymphocyte isolation by thrombin-nylon wool. *In* Histocompatibility Testing. Los Angeles, UCLA Typing Laboratory, 1980:287–89.

[13] Barbosa, J.: HLA and diabetes mellitus. Lancet 1977; 1:906–907.

[14] Barbosa, J., King, R., Goetz, F. C., Noreen, H., and Yunis, E. J.: HLA and maturity-onset type of hyperglycaemia in the young. Arch. Intern. Med. 1978; 138:90–93.

Multiple Herpetic Whitlows in a Child Performing Self-Monitoring of Blood Glucose

Self-monitoring of blood glucose (SMBG) has become the recommended tool for management of type I diabetes. In our diabetes clinic we have noted no bacterial infections of any significance in the 400 patients using this procedure 2–4 times daily over a period of 4 yr. Ryan et al.[1] described two cases of digital sepsis with osteomyelitis and gangrene eventually requiring amputation in immunocompromised hosts undergoing dialysis or renal transplantation. Knezevic and Mastaslia[2] reported a similar case. This communication concerns a proven case of multiple digital herpes simplex whitlows in a boy performing SMBG on a regular basis for 3 yr.

J.F., a 9-yr-old boy requiring insulin since the age of 20 mo and also mildly asthmatic, developed painful erythematous, indurated, and vesicular lesions on the tips of the middle three fingers of each hand (Figure 1). There was moderate bilateral enlargement of the epitrochlear and axillary lymph nodes, general malaise, anorexia, and low-grade fever. A crusted herpetic lesion was noted on the lower lip and the patient had a history of recurrent labial herpes for 1 yr before this event. On admission the child's blood glucose was 416 mg/dl and he required intensified insulin treatment. There was no ketosis or acidosis.

TABLE 1
Immunologic investigations on patient and mother

Tests	Patient	Mother	Normal range
IgG (mg/dl)	1225	1400	700–1600
IgM (mg/dl)	105	74	36–260
IgA (mg/dl)	295	155	46–490
IgD (mg/dl)	0.6	1.0	0–41
IgE (U/ml)	800	65	0.3–215
C3 (mg/dl)	135	129	88–252
C4 (mg/dl)	25.5	36	13–72
CH5O classic (U/ml)	141	158	90–160
CH5O alternative (U/ml)	17	27	13–30
Rheumatoid factor	Positive	Negative	Negative
Antinuclear antibody	Negative	Negative	Negative
E rosettes (%)	78	81	65–88
OKT3 (%)	67	64	51–87
OKT4 (%)	38	28	15–52
OKT8 (%)	21	14	13–44
B cells (%)	16	12	6–14
PHA response (cpm)	184,233	171,766	>150,000

HLA Class I and II Antigens in South African Indians With NIDDM

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The relationship between the HLA system and non-insulin-dependent diabetes mellitus (NIDDM) in South African Indians, a migrant Indian group, was evaluated by testing HLA-A, -B, and -C antigens in 184 patients and 1444 control subjects and HLA-DR antigens in 104 patients and 330 control subjects. There was a significant increase in the frequency of HLA-Bw61 in patients compared with control subjects (27.7 vs. 18%, $P = .00155$), although the degree of association was not very strong (relative risk 1.7). A similar association has been noted in Fiji Indians, another migrant Indian group. However, no relationship could be established at the DR locus. It is suggested that the relatively high frequency of the Bw61 allele in South African Indians could, in the presence of some environmental factor like obesity, confer increased susceptibility to NIDDM. *Diabetes* **37:796–99, 1988**

South African Indians, like other migrant Indian groups, show a high prevalence of non-insulin-dependent diabetes mellitus (NIDDM) (1). On the basis of studies showing a high concordance rate of the disease in identical twins, there is little doubt that genetic factors play a role in the pathogenesis of the disease, although the precise mechanism remains obscure (2). Previous studies have highlighted the paucity of any relationship between NIDDM and the HLA system of antigens among Caucasians (3–6). Data based on the detection of only HLA class I antigens (HLA-A, -B, and -C) have shown some associations in young Pima Indians, Chinese, certain Pacific population groups, young South African Indians of northern Indian origin, and South African Blacks of Xhosa descent (6–13). HLA-Bw61 has been shown to be associated with the disease in a small study involving Fiji Indians, another migrant Indian group, but no relationship has been established at the HLA-DR locus (9). Apart from this and a small family study showing no association between NIDDM of the young in Indians and HLA class II (HLA-DR) antigens (14), data on any possible association between classic NIDDM and HLA class II antigens in non-Caucasian populations are virtually nonexistent. Our study was therefore undertaken to evaluate the relationship between NIDDM and HLA class I and II antigens in a large group of South African Indians with NIDDM.

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PATIENTS AND METHODS

One hundred eighty-four unrelated subjects with NIDDM, diagnosed and classified on the basis of the revised World Health Organization diagnostic criteria, were selected for the study (15). They were patients attending the Diabetes Clinic of King Edward VIII Hospital, which is a teaching hospital attached to the University of Natal Medical School. The mean age at diagnosis was 48 ± 10 yr (SD) with a range of 35–70 yr, and the mean duration of disease was 10 ± 6 yr with a range of 2–30 yr. None of the patients had ever had ketosis, and their diabetes had been controlled by diet alone or by diet and oral hypoglycemic agents for ≥2 yr. They did not have malnutrition-related diabetes, which is extremely rare in this population group (16). In addition, 1444 healthy control subjects with no history of diabetes mellitus were studied, none being a first- or second-degree relative of other control subjects or of the patients studied. Because the control subjects did not undergo a glucose tolerance test, it is possible that the odd case of asymptomatic diabetes was missed in this group. However, because virtually all HLA studies have similar control data, our control group should be a reasonable basis for comparison.

The sex distribution of the diabetic subjects was 149 women and 35 men and that of the control subjects was 626 women and 818 men. Thus, owing to the relatively small number of diabetic men, it would be difficult to evaluate any

TABLE 1
Frequency of HLA-DR (class II) antigens and genes in South African Indians

Antigen	Control subjects (n = 330)		Patients (n = 104)	
	Antigen frequency (%)	Gene frequency	Antigen frequency (%)	Gene frequency
DR1	5.2	0.0263	8.7	0.0444
DR2	39.7	0.2234	48.1	0.2795
DR3	13.0	0.0672	11.5	0.0592
DR4	20.6	0.0189	24.0	0.1282
DR5	16.1	0.0840	14.4	0.0747
DRw6	14.2	0.0737	18.3	0.0961
DR7	28.2	0.1526	22.1	0.1173
DRw8	2.4	0.0120	7.7	0.0392
DRw9	0.6	0.0030	1.0	0.0050
DRw10	8.8	0.0450	11.5	0.0592
One antigen	50.9	0.2034	32.7	0.0966

sex-dependent differences in HLA distribution among the patients. The reason for the female predominance among the diabetic subjects may lie in women being more likely than men to seek medical attention for the disease. This reason notwithstanding, however, previous epidemiological studies involving this population group have established a higher prevalence rate of NIDDM in women (1,16).

The Indians in Natal are descendants of migrant Indians who emigrated from India between 1860 and 1911. All were born in Natal and represent third, fourth, and fifth generations. They can be divided into two major groups, Dravidians from southern India and Aryans from northern India (17). The Dravidian Indians in Natal are mainly Tamil and Telugu speaking. The Aryan Indians are more conveniently divided by religion into Hindus from the northeast and Muslims from the northwest. The Hindus in Natal are mainly Hindi speaking, although there are a few Gujarati speakers. The Muslims speak mainly Urdu, but there are also Gujarati speakers (17). There has been virtually no intermarriage between the Indians and other ethnic groups in South Africa because of the Group Areas Act (an apartheid legislation).

The control group comprised randomly selected staff and blood donors, many of who had been typed for International Histocompatibility Testing Workshops. Both the patient and control groups had similar socioeconomic backgrounds.

The HLA class I antigens were determined in all patients and control subjects by a two-stage lymphocytotoxicity test (18) with 180 antisera. They consisted of local sera that have been requested for use in International Histocompatibility Testing Workshops, local sera that have been verified with International Workshop sera, and sera that have been exchanged with other laboratories worldwide. Similarly, 120 sera helped to define the class II antigens on B-lymphocyte enriched lymphocyte suspensions prepared with the aid of straws packed with nylon wool (19). The class II antigens were determined in 104 patients and 330 control subjects (Table 1).

The definition of Bw60 with operationally monospecific antisera is clear, but the definition of Bw61 depends on the difference in reaction patterns between the broad antigen B40 and Bw60 (20,21). This means that it is not possible to detect Bw61 in the presence of Bw60, and, therefore, ~3% of the patients with both Bw60 and Bw61 were counted as having Bw60 and a "blank." Thus, the frequency of Bw61 was underestimated by ~3% in the patients and by ~2% in the control subjects. However, the antigen frequencies in Table 2 have not been modified because there are many other cross-reacting groups where antigens may be "hidden," e.g., A10, B5, B15, and further corrections would have been necessary to allow for homozygosity.

Differences in HLA frequencies were tested for signifi-

TABLE 2
Frequency of HLA class I antigens and genes in South African Indians

Antigen	Control subjects (n = 1444)		Patients (n = 184)	
	Antigen frequency (%)	Gene frequency	Antigen frequency (%)	Gene frequency
A1	28.7	0.1556	20.7	0.1094
A2	31.5	0.1723	35.3	0.1956
A3	12.7	0.0656	14.1	0.0731
A11	27.8	0.1502	25.5	0.1368
A23	1.3	0.0065	0	0
A24	28.7	0.1556	35.3	0.1956
A25	1.7	0.0085	1.6	0.0080
A26	6.6	0.0335	4.4	0.0222
A28	12.8	0.0661	11.6	0.0587
A29	1.2	0.0060	1.6	0.0080
A30	3.2	0.0161	2.7	0.0135
A31	3.5	0.0176	8.2*	0.0418
A32	4.4	0.0222	3.3	0.0166
A33	14.3	0.0742	15.2	0.0791
Aw36	0.3	0.0015	0	0
One antigen	21.3	0.0478	20.7	0.0409
B7	13.2	0.0683	16.3	0.0851
B8	6.2	0.0314	10.3	0.0528
B13	7.1	0.0361	6.0	0.0304
B14	0.5	0.0025	0	0
B15	10.1	0.0518	11.4	0.0587
B16	3.5	0.0176	3.8	0.0191
B17	21.3	0.1128	12.0	0.0619
B18	3.8	0.0191	3.8	0.0191
B21	3.4	0.0171	3.8	0.0191
Bw22	4.3	0.0217	3.3	0.0166
B27	2.1	0.0105	3.8	0.0191
B35	20.9	0.1106	20.1	0.1061
B37	5.9	0.0299	3.8	0.0191
Bw41	0.3	0.0015	0	0
Bw47	0.2	0.0010	1.1	0.0055
Bw42	0	0	0	0
B44	12.7	0.0656	12.0	0.0619
B45	0.3	0.0015	0	0
B51	16.7	0.0873	19.6	0.1033
Bw52	13.9	0.0720	12.5	0.0645
Bw53	0.8	0.0040	0.5	0.0025
B51	3.5	0.0176	2.7	0.0135
Bw60	11.5	0.0592	10.9	0.0566
Bw61†	18.0	0.0944	27.7†	0.1497
Bw70	3.3	0.0166	1.6	0.0080
One antigen	16.8	0.0488	13.0	0.0269
Cw1	5.7	0.0290	7.6	0.1087
Cw2	3.8	0.0192	1.6	0.0080
Cw3	11.4	0.0587	10.3	0.0529
Cw4	15.7	0.0818	22.8	0.1214
Cw5	1.4	0.0070	1.6	0.0080

*P = .0022, uncorrected; P = .0954, corrected; relative risk 2.5.
†P = .0016, uncorrected; P = .0689, corrected; relative risk 1.8.

TABLE 3
HLA antigen frequencies in control subjects

Antigen	Antigen frequency in subjects (%)	
	<35 yr old	>35 yr old
A1	28.1	30.1
A2	31.6	31.3
A3	12.7	12.5
A11	27.5	28.8
A23	1.4	1.1
A24	28.8	28.6
A25	1.7	1.6
A26	6.1	8.0
A28	13.4	10.9
A29	1.4	0.3
A30	2.1	6.4
A31	3.7	2.6
A32	4.4	4.5
A33	16.0	9.4
One antigen	20.8	22.7
B7	13.3	12.8
B8	6.0	6.6
B13	6.5	9.0
B14	0.6	0.26
B15	10.9	6.1
B16	3.7	26.7
B17	21.2	22.0
B18	3.2	5.6
B21	3.4	3.5
Bw22	5.5	0.80
B27	2.3	1.33
B35	21.6	18.9
B37	5.7	6.4
Bw41	0.3	0.26
Bw47	0.2	0.26
Bw42	0.0	0.0
B44	12.7	12.5
B45	0.3	0.26
B51	16.5	17.1
Bw52	14.2	12.8
Bw53	0.4	1.8
B51	3.1	4.5
Bw60	10.6	14.2
Bw61	17.7	18.9
Bw70	4.0	1.2
One antigen	16.1	18.7
Cw1	5.6	5.8
Cw2	3.6	4.5
Cw3	11.4	11.5
Cw4	15.6	15.8
Cw5	1.0	2.4
DR1	5.7	
DR2	39.7	40.0
DR3	11.7	26.6
DR4	19.7	30.0
DR5	19.3	16.6
DRw6	10.7	50.0
DR7	26.0	50.0
DRw8	2.3	3.3
DRw9	0.3	3.3
DRw10	8.0	16.6
One antigen	50.0	60.0

cance with the χ^2-test (without Yates' correction), and the probability was corrected by multiplying the P value by the number of comparisons made, i.e., the number of different antigen tests (22). When an antigen was shown to be associated with NIDDM in a population group elsewhere, an uncorrected P value <.01 was considered significant (22,23). Relative risk was calculated according to the formula recommended by Woolf (24).

RESULTS

The frequencies of various HLA antigens in patients and control subjects are shown in Tables 1 and 2. At the A, C, and DR loci there were no significant differences in the frequency of any of the antigens between patients and control subjects. There was a significant increase in the frequency of Bw61 in patients compared with control subjects (27.7 vs. 18%, P = .00167). Despite the fact that the corrected P value fell short of statistical significance (P = .0689), the difference remains significant, due to a prior hypothesis, because the same antigen has been shown to be associated with NIDDM in another migrant Indian group (12,23).

Although increased frequencies of HLA-A31 and -Cw4 were seen in the patients compared with control subjects, the differences were not significant when the P value was corrected.

There were no significant differences in the frequencies of any of the HLA antigens between control subjects <35 yr and those >35 yr (Table 3).

DISCUSSION

Although a relationship between insulin-dependent diabetes mellitus (IDDM) and the HLA system of antigens has been clearly established, their association with NIDDM remains controversial (3,6,25). Caucasians show an inconsistent relationship at the class I locus of genes (6). Pooled data from several independent studies have shown a significant association with HLA-B8, and a study in Scandinavia has established a relationship at the Cw4 locus (6,26). South African Indians, however, show a significant increase in the frequency of HLA-Bw61. This finding is of particular interest in light of a previous study showing an association between the same antigen and NIDDM in Fijian Indians (10), another migrant population group with the same origin as South African Indians, i.e., India. The uncorrected P value in the latter study (P = .01) was much higher than that in this study (P = .0015). In contrast, the relative risk in the Fijian study was higher (4.8), possibly because of the relatively lower frequency of HLA-Bw61 (9%) in the control group and the smaller number of patients (n = 58) and control subjects (n = 47) studied. Young South African Indians with NIDDM have also been found to show a somewhat weak relationship at the Bw61 locus (11).

The frequency of HLA-Bw61 is much higher among Indians originating from the Indian subcontinent compared with Caucasians or Blacks (27). In fact, it is virtually nonexistent among Black population groups (27). Thus, it is tempting to speculate that the high prevalence of diabetes in South African Indians compared with other population groups is due to the increased frequency of this antigen. Yet, Indians from southern India and northern India, in whom the frequencies of Bw61 are as high as 16% and 15%, respectively (27), show much lower prevalence rates of NIDDM (28,29), thereby seemingly negating such a hypothesis. However, it is possible that in the presence of an environmental factor, e.g., obesity, which appears to be a risk factor for diabetes among South African Indians (1), HLA-Bw61 confers in-

creased susceptibility to the disease, at least in a proportion of subjects.

Like IDDM, NIDDM also seems to be characterized by differences in the specific allelic associations among the various ethnic groups. Thus, South African Indians with NIDDM do not show an increased frequency of A2 as seen in Pima Indians (7), of B54 as seen in the Chinese (8), of B22 as seen in Micronesians and Polynesians (9,29), or of Bw62 (B15) as seen in Papuans (New Guinea; 13) In addition, no relationship could be established with HLA-Bw41, which shows a weak association with NIDDM in South African Xhosas (12). At the C locus, there was a weak association with HLA-Cw4 (P .013, uncorrected; P .598, corrected), which shows a strong relationship with NIDDM in Scandinavians (26; P .002, corrected). In regard to the age distribution of the control subjects, only 26% were >35 yr of age, when the mean age of the diabetic subjects was 48 yr. Hence, based on the observation that the prevalence of diabetes increases with age, it is quite possible that further significant associations (e.g., HLA-A31) would have manifested themselves had there been a larger number of older control subjects. Despite these limitations, there appeared to be no significant differences between the HLA distribution of control subjects >35 yr and those <35 yr of age.

In most population groups studied thus far, IDDM shows a stronger association with the class II antigens than with the class I antigens (3,6,25). South African Indians with NIDDM, however, do not show such a tendency, because no relationship could be established with any of the HLA class II antigens. Caucasians with the disease also do not show any consistent relationship involving HLA class II antigens (6). In regard to other population groups, i.e., Melanesians, Polynesians, Papuans, Pima Indians, and Indians from India, published data on the relationship between NIDDM and HLA class II antigens are not available.

REFERENCES

1. Omar MAK, Seedat MA, Dyer RB, Motala AA, Rajput MC, Joubert SM: The prevalence of diabetes mellitus in a large group of South African Indians. *S Afr Med J* 67:923–26, 1985
2. Barnett AH, Eff C, Leslie RDG, Pyke DA: Diabetes in identical twins: a study of 200 pairs. *Diabetologia* 20:87–93, 1981
3. Rotter JI, Rimoin DL: Diabetes mellitus: the search for genetic markers. *Diabetes Care* 2:215–26, 1979
4. Platz P, Jakobsen BK, Thomsen BS, Jensen KB, Henningsen K: No evidence of linkage between HLA and maturity onset type of diabetes in young people. *Diabetologia* 23:16–18, 1982
5. Arnaiz Vellena A, Rodriguez De Cordoba S, Dujorne IL, Regueiro JR, Bootello A, Serrano-Rios M: HLA factors in non insulin dependent diabetes mellitus. *N Engl J Med* 303:1065, 1980
6. Tiwari JL, Terasaki PI: Endocrinology. In *HLA and Disease Associations*. New York, Springer-Verlag, 1985, p. 210–12
7. Williams RC, Knowles WC, Butler WJ, Pettitt DJ, Lisse JR, Bennett PH, Terasaki PI: HLA-A2 and Type II (insulin independent) diabetes mellitus in Pima Indians: an association of allele frequency with age. *Diabetologia* 21:460–63, 1981
8. Zhao T, Chi Z, Waug H, Shen M, Zhou B, Bu K: HLA and diabetes mellitus in China. *Chin Med J* (Engl Ed) 95:609–12, 1982
9. Serjeantson SW, Ryan DP, Zimmet P, Taylor R, Cross R, Charpin M, Gonidec G: HLA antigens in four Pacific populations with non-insulin dependent diabetes mellitus. *Ann Hum Biol* 9:69–84, 1982
10. Serjeantson SW, Ryan DP, Ram P, Zimmet P: HLA and non-insulin dependent diabetes in Fiji Indians. *Med J Aust* 1:462–63, 1981
11. Omar MAK, Hammond MG, Seedat MA, Asmal AC: HLA antigens and non insulin dependent diabetes mellitus in young South African Indians. *S Afr Med J* 67:130–32, 1985
12. Briggs BR, Jackson WPU, DuToit ED, Botha MC: The histocompatibility (HLA) antigen distribution in diabetes in Southern African Blacks (Xhosa). *Diabetes* 29:68–71, 1980
13. Bhatia K, Patel M, Gorogo M: Type 2 (non-insulin dependent) diabetes mellitus and HLA antigens in Papua, New Guinea. *Diabetologia* 27:370–73, 1984
14. Naidoo C, Jialal I, Hammond MG, Omar MAK, Joubert SM: HLA and NIDDM in the young. *Diabetes Care* 9:436–38, 1986
15. WHO Expert Committee: *Diabetes Mellitus*. 2nd report. Geneva, World Health Org., 1980, p. 8–12 (Tech. Rep. Ser. 646)
16. Omar MAK, Asmal AC: Patterns of diabetes mellitus in young South African Blacks and Indians. *Trop Geogr Med* 36:133–38, 1984
17. Mistry SD: Ethnic groups of Indians in South Africa. *S Afr Med J* 39:691, 1965
18. Terasaki PI, McClelland JD: Microdriplet assay of human serum cytoxins. *Nature* (Lond) 204:998, 1964
19. Danilovs JA, Ayoub G, Terasaki PI: B lymphocyte isolation by thrombin-nylon wool. In *Histocompatibility Testing 1980*. Terasaki PI, Ed. Los Angeles, Univ. of California Press, 1980
20. Goldmann SF, Middleton D, Kennedy LJ: Antigen report: HLA-Bw60. In *Histocompatibility Testing 1984*. Albert ED, Baur MP, Mayr WR, Eds. Berlin, Springer-Verlag, 1984, p. 167
21. Goldmann SF, Middleton D, Kennedy LJ: Antigen report: HLA-Bw61. In *Histocompatibility Testing 1984*. Albert ED, Baur MP, Mayr WR, Eds. Berlin, Springer-Verlag, 1984, p. 168
22. Svejgaard A, Jersild L, Staub-Nielsen L, Bodmer WF: HLA antigens and disease: statistical and genetical considerations. *Tissue Antigens* 4:95–105, 1974
23. Tiwari JL, Terasaki PI: *HLA and Disease Associations*. New York, Springer-Verlag, 1985, p. 20
24. Woolf B: On estimating the relation between blood group and disease. *Ann Hum Gen* 19:251–53, 1955
25. Christy M, Green A, Christau B, Kromann H, Nerup J, Platz P, Thomsen M, Ryder LP, Svejgaard A: Studies of the HLA system and insulin-dependent diabetes mellitus. *Diabetes Care* 2:209–14, 1979
26. Groop L, Koskimies S, Pelkonene R, Topaaned EM: Increased frequency of HLA-Cw4 in type II diabetes. *Acta Endocrinol* 104:475–78, 1983
27. Kamura H, Kohara S, Shimizu K, Azaka T: Antigen report. In *Proc Asia-Oceania Histocompatibility Workshop 1986*. Aizawa M, Ed. Hokkaido, Japan, Hokkaido Univ. Press, 1986, p. 117–22
28. Ashok A, Ahujamms, Wasir HS: Prevalence of IGT, diabetes mellitus and cardiovascular risk factors in semi-urban populations of North India using WHO criteria (Abstract). *Diabetes Res Clin Pract* (Suppl. 1):526, 1985
29. Serjeantson SW, Owerbach D, Zimmet P, Nerup J, Thoma K: Genetics of diabetes in Nauru: effects of foreign admixture, HLA antigens and the insulin-gene-linked polymorphism. *Diabetologia* 25:13–15, 1983

p370 Stastny P, Barger BD, Batchelor JR, Bertrams J, Betuel H, Botha MC, Braun WE, Carpenter CB, Darke C, Dawkins RL, Engelfriet CP, Fauchet R, Festenstein H, Gazit E, Gorodezky C, Hammond MG, Hansen JA, Juji T, Mayr WR, Mayer S, McConnachie PR, Oh JH, Perez-Rojans GE, Petranyi GG, Radvany R, Rodey GE, Sasazuki T, Sucia-Foca N, Walford R and Winchester RJ. **Joint Report: Rheumatoid Arthritis**. In: Terasaki PI (ed) *Histocompatibility Testing 1980* p681. UCLA Tissue Typing Laboratory, Los Angeles. 1980

p376 Christiansen FT, Komori K, Dawkins RL, Mehra M, Bashir H, Sekiguchi S, Saito H, Mehra M, Hammond MG, Chandanayingyong D, and Chan SH. **Rheumatoid Arthritis** In: Simons MJ, Tait Bd (eds) *Proceedings of the Second Asia-Oceania Histocompatibility Workshop* p348. Immunopublishing, Toorak, Australia, 1983

p382 Mody GM, Hammond MG and Naidoo PD. **HLA associations with rheumatoid arthritis in African blacks.** *J Rheum* 16:10 1326 1989

p385 Mody GM, Hammond MG, et al. **HLA and rheumatoid arthritis in South African Indians.** (submitted).

RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) patients present with a variety of syndromes. There are marked differences in the severity of the arthritis and in the prominence of other features such as episcleritis, vasculitis, peripheral neuropathy, pulmonary disease, hypersplenism, and nodule formation. A minority of patients with typical adult RA can be distinguished because they do not make rheumatoid factor (RF). In children the disease takes three main forms: (a) systemic onset with fever; (b) polyarticular onset, sometimes associated with rheumatoid factor and nodules; and (c) pauciarticular onset.

In previous studies (1-6) including that of the 7th International Histocompatibility Workshop (7), HLA-DW4 and DR4 were found to be increased in adult RA in Caucasians having erosive arthritis by X-ray and with positive RF tests. This increase was not observed in patients with

Table 1. HLA-DR antigens in unrelated adult RA patients.

Group Studied	Number Subjects	Antigen Frequency (%) DR1	DR2	DR3	DR4	DR5	DRW6	DR7	DRW8
				Caucasians					
Control	662	17.4	24.9	21.0	24.9	19.2	6.3	22.4	6.8
RA	329	20.1	13.4***	16.1	47.4***	7.3***	2.4*	14.3**	3.7
				Japanese					
Control	792	10.7	35.6	1.9	40.7	3.4	9.5	0.6	16.8
RA	104	15.4	24.0*	0.0	62.5***	4.8	8.7	0.0	21.4
				Negroes					
Control	193	10.9	35.2	29.5	9.8	26.4	12.4	19.7	20.2
RA	56	17.9	28.6	16.1	35.7***	10.7*	0.0*	21.4	16.1

* = p <0.05, ** = p <0.01, *** = p <0.001

Table 2. HLA-DR antigens in unrelated adult RA patients of other ethnic groups.

Subjects Studied	Ethnic Group[1]	Number Subjects	Antigen Frequency (%) DR1	DR2	DR3	DR4	DR5	DRW6	DR7	DRW8
					Hungarian					
Control	22	66	9.1	53.1	34.8	10.6	10.6	4.5	9.1	19.7
RA		41	22.0	17.1***	24.4	39.0***	14.6	4.9	12.9	0.0
					Latinoamerican					
Control	M,V	19	10.5	36.8	26.3	5.3	26.3	0.0	42.1	21.1
RA		41	10.0	22.5	22.5	57.5***	15.0	5.0	22.5	10.0
					Jewish					
Control	24	38	2.6	13.2	10.5	31.6	42.1	15.8	31.6	5.3
RA		25	24.0*	20.0	0.0	48.0	32.0	0.0	36.0	--
Control	25	22	13.6	22.7	9.1	22.7	45.5	9.1	22.7	4.5
RA		15	26.7	46.7	20.0	26.7	20.0	6.7	13.3	--
					Asian Indian					
Control	13	10	20.0	50.0	0.0	10.0	0.0	0.0	20.0	10.0
RA		17	0.0	70.6	5.9	23.5	23.5	0.0	23.5	5.9

[1] 22 = Hungarian; M,V = Mexican and Venezuelan; 24 = Ashkenazi; 25 = Non-Ashkenazi; 13 = Asian Indian

juvenile rheumatoid arthritis (JRA) (8).

For the present study patients were classified using the criteria of the American Rheumatism Association for adult RA (9) and those of the JRA criteria subcommittee (10) for children with arthritis. Detailed clinical information was collected on all patients submitted to the study. The final diagnostic classification was performed by computer on the basis of objective criteria and verified by the participating clinicians. Data on RF included information about number of tests on record, time between first and last test, consistency of results, and average titer. Adult patients with negative RF tests were required to have been tested at least twice and to meet criteria for classification as either definite or classical adult RA.

Unrelated Adult RA. As in previous studies small deviations were observed in the frequencies of some HLA-A,B, and C antigens in patients with adult RA. The important differences were in the antigens of the HLA-DR series. No data on HLA-D typing was submitted. In the major population groups (Table 1) the highly significant increase of HLA-DR4 was uniformly present. Several antigens were decreased including DR2, DR5, DRW6, and DR7. Similar changes, with a major increase of DR4, were also observed in the Hungarian population and in Latinoamericans typed in Mexico and Venezuela (Table 2). The remaining three populations in Table 2, did not show an increase in DR4.

There were no significant differences in antigen frequencies when the patients were separated by sex (Table 3) or age of onset of disease (Table 4).

To evaluate the possibility of a relationship between HLA-DR antigens and severity of RA, three approaches were taken. Clinical activity was evaluated on the basis of morning stiffness, joint pain, joint tenderness and joint swelling (11), the most severe functional grade was determined according to Steinbrocker and coworkers (12), and the relative rate of progression was scored by the clinicians as slow, moderate, or rapid. Data obtained by the last method are shown in Table 5. There was no evidence from any of these analyses of a correlation between severity of disease and the HLA-DR antigens in patients with definite or classical adult RA. The absence of correlation between severity of disease and presence of DR4 may depend in part on the selection of a patient population having only definite or classical RA and has been observed previously in such groups (13,14).

The majority of adult RA patients had positive tests for RF. In addition, 46 RF negative Caucasian patients were accepted in the study according to criteria given above. Interestingly, the frequency of DR4 in this group of definite or classical RA without RF was no different from that of controls (Table 6). To further examine the relationship between RF and HLA, the RF positive group was subdivided according to RF titer. Patients with high titer RF had a higher frequency of DR4 than those with low titer (Table 6).

Thus, it appears that seropositive and seronegative RA are separate diseases with different immunogenetic factors. These findings confirm earlier reports by Jaraquemada and coworkers (15) and Dobloug and coworkers (16) who observed correlation between presence of RF and DR4. In view of the data suggesting correlation between DR4 and titer of RF, the possibility of an effect of DR4 on the immune response to autologous IgG should be considered. However, it is known that most normal individuals can make both IgM and IgG RF if properly challenged (17) and DW4 was not increased in patients having RF due to conditions other than RA (18).

Groups of patients having extraarticular manifestations of RA were small, with the exception of those with subcutaneous nodules (Table 7). When the HLA-DR antigens

Table 3. HLA-DR antigens in RA patients in relation to sex.

Subset Studied	Number Subjects	Antigen Frequency (%) DR1	DR2	DR3	DR4	DR5	DRW6	DR7	DRW8
				Caucasians					
Male	105	18	11	19	44	05	05	16	06
Female	189	23	16	12	46	09	02	15	03
				Japanese					
Male	20	15	00*	00	80	10	10	00	30
Female	84	16	30	00	58	04	08	00	19
				Negroes					
Male	6	17	17	33	17	17	00	17	33
Female	49	18	31	14	39	10	00	22	14

* $p < 0.05$

Table 4. HLA-DR antigens in RA patients in relation to age at onset of disease.

Age at Onset	Number Subjects	Antigen Frequency (%) DR1	DR2	DR3	DR4	DR5	DRW6	DR7	DRW8
				Caucasians					
17-39 yrs	92	18	15	13	41	11	02	16	04
>40 yrs	202	22	14	15	47	06	03	15	04
				Japanese					
17-39 yrs	39	05	26	00	64	05	10	00	13
>40 yrs	65	22*	23	00	62	05	08	00	27
				Negroes					
17-39 yrs	23	13	30	17	39	13	00	35	13
>40 yrs	33	21	27	15	33	09	00	12	18

* p <0.05

Table 5. HLA-DR antigens in RA patients in relation to rate of progression of the disease.

Progression Score[1]	Number Subjects	Antigen Frequency (%) DR1	DR2	DR3	DR4	DR5	DRW6	DR7	DRW8
				Caucasians					
1	80	20	19	16	46	11	01	24*	04
2	132	22	13	16	45	08	04	11	05
3	75	20	13	09	45	03	01	13	04
				Japanese					
1	34	27	24	00	59	00	09	00	27
2	48	13	21	00	65	06	13	00	21
3	18	06	28	00	67	11	00	00	17
				Negroes					
1	20	10	10	25*	30	15	00	20	30
2	23	26	39	09	39	09	00	13	09
3	12	17	42	17	33	08	00	33	08

*p <0.05
[1]Progression scores: 1 = slow, 2 = moderate, 3 = rapid

Table 6. HLA-DR antigens in Caucasian RA patients in relation to rheumatoid factor.

Group Studied	RF Status	Number Subjects	Antigen Frequency (%) DR1	DR2	DR3	DR4	DR5	DRW6	DR7	DRW8
Controls	--	662	17	25	21	25	19	06	22	07
RA	Neg	46	24	17	13	24	13	02	26	02
RA	Pos	227	20	13	17	52***	06	03	13	04
RA	Low	76	20	20	18	39	13	05	15	03
RA	Med	117	17	11	09	52	03	03	10	04
RA	High	48	29	13	15	58(*)	04	00	17	08

*** p <0.001 for difference from controls
(*) p <0.05 for difference between low and high titer groups

were compared in each case with the RA population without the extraarticular condition in question, few significant deviations were observed. Two groups of patients with pulmonary disease developing in coal miners were submitted by Darke: patients with pulmonary nodules (Caplan's syndrome) appeared to have an increase in DR3; patients with pulmonary fibrosis had an increase in DR1 (Table 7). Neither of these would be significant if the P values were corrected for the number of antigens tested. Further studies will be of interest.

Family studies in adult RA. Family studies were performed by the following laboratories: Batchelor, Braun, Dawkins, Engelfriet, Hammond, Sasazuki, and Stastny. There were 28 families with at least two members having adult RA and meeting the criteria established for the study.

The frequency of DR4 in these families was quite high, but there was no difference between the affected and the unaffected first degree relatives (Table 8). The families contained 67 sib pairs. In 21 cases the index case and the sib both had RA; in 45 instances the sib was unaffected (Table 9). The distribution of shared haplotypes was different in the two groups. There were only three affected sibs that shared no haplotype with the primary case. One was from a family in which seven sibs had the disease, with two affected haplotypes inherited from a DR4 homozygous mother. The other two were instances of RF positive propositi having sibs who were RF negative (Table 9).

These results appear to confirm the different nature of factor positive and factor negative RA. If the three subjects are not considered then all the affected sib pairs shared at least one haplotype, whereas among the unaffected sibs 22% had no haplotype in common with the index case. This difference between affected and unaffected sibs suggests a major effect of HLA genes on the development of RA.

Unrelated JRA. Data on JRA were submitted by seven laboratories (Table 10). The overall frequencies of HLA-DR antigens in Caucasians with JRA showed an increase in DR5 and DRW8. The frequency of DR4 was not elevated. HLA-DR5 appeared to be highest in the systemic onset group. DRW8 was increased in patients with pauciarticular and polyarticular onset.

Previous results had shown the absence of DW4 and DR4 and the increased frequency of DW/DRW8 among patients with JRA (19). The increased frequency of DW/DR5 has also been recently observed (20,21). Because of the clinical heterogeneity of JRA, large numbers of patients are needed and careful attention must be given to their classification. Further work will be needed to clarify the relationships of the clinical subsets with HLA-D and DR.

REFERENCES

1. Stastny P. Mixed lymphocyte culture typing cells from patients with rheumatoid arthritis. Tissue Antigens 1974, 4:571.
2. Stastny P. Mixed lymphocyte cultures in rheumatoid arthritis. J Clin Invest 1976, 57:1148.
3. Stastny P. Association of the B-cell alloantigen DRW4

Table 8. HLA-DR4 in 28 families with multiple cases of adult rheumatoid arthritis.

Primary Type	Case No.	Relatives Type	Relatives No.	Affected No.	Affected DR4+	Normal No.	Normal DR4+
Parent	8	Child	19	10	6(60%)	9	8(89%)
Child	6	Parent	11	6	5(83%)	5	4(80%)
Sib	22	Sib	66	23	16(70%)	43	29(67%)

Table 9. Inheritance of HLA haplotypes among sibs in 28 families with multiple cases of adult rheumatoid arthritis.

Type of Sibs	Number	Shared Haplotypes 2 N	2 (%)	1 N	1 (%)	0 N	0 (%)
Affected	21	7	(33)	11	(52)	3*	(14)
Normal	45	11	(24)	24	(53)	10	(22)

* Two were rheumatoid factor negative; one was from a family in which 7 sibs had the disease.

Table 7. HLA-DR antigens in Caucasian RA patients in relation to extraarticular manifestations.

Extraarticular Condition	Number Subjects	Antigen Frequency (%) DR1	DR2	DR3	DR4	DR5	DRw6	DR7	DRw8
Serositis	17	41	06	12	53	00	00	00	12
Eye Lesions	23	17	09	22	48	00	09	17	05
Sjögren's	17	35	12	24	35	00	00	12	18
Vasculitis	19	42	16	26	42	00	00	16	05
Subcut. Nod.	117	21	11	15	50	03	03	15	06
Pulm. Nod.	21	24	05	33*	29	00	05	24	05
Pulm. Fibrosis	12	50**	08	17	33	00	00	08	17

* $p < 0.05$, ** $p < 0.01$ for difference between subset with extraarticular manifestation and RA patients without it.

with rheumatoid arthritis. N Engl J Med 1978, 298:869.
4. Panayi GS, Wooley P, Batchelor JR. Genetic basis of rheumatoid diseases: HLA antigens, disease manifestations, and toxic reactions to drugs. Br Med J 1978, 2:1326.
5. Roitt IM, Corbett M, Festenstein H, et al. HLA-DRW4 and prognosis in rheumatoid arthritis. Lancet 1978, 1:990.
6. Thomsen M, Morling N, Snorrason E. HLA-DW4 and rheumatoid arthritis. Tissue Antigens 1979, 13:56.
7. Batchelor JR, Morris PJ, eds, HLA and disease. Rheumatoid arthritis. In *Histocompatibility Testing 1977*, Bodmer WF, et al, eds, Munksgaard, Copenhagen, 1978, 218.
8. Stastny P, Fink CW. Different HLA-D associations in adult and juvenile rheumatoid arthritis. J Clin Invest 1979, 63:124.
9. Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA. 1958 revision of diagnostic criteria for rheumatoid arthritis. Bull Rheum 1958, 9:175.
10. Brewer EJ, Bass J, Baum J, et al. Current proposed revision of JRA criteria. Arthritis Rheum 1977, 20 (Suppl): 196.
11. Srinivascan R, Miller BL, Paulus HE. Long-term chrysotherapy in rheumatoid arthritis. Arthritis Rheum 1979, 22:105.
12. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. JAMA 1949, 140:659.
13. Husby G, Gran JT, Ostensen M, Johannessen A, Thorsby E. HLA-DRW4 and rheumatoid arthritis. Lancet 1979, 1:549.
14. Stastny P. HLA-D in the rheumatic disease. J Reticuloendothelial Soc 1979, 26:601.
15. Jaraquemada D, Pachoula-Papasteriadis C, Festenstein H, et al. HLA-D and DR determinants in rheumatoid arthritis. Transplant Proc 1979, 11:1306.
16. Dobloug JH, Forre O, Thorsby E. HLA-DRW4 and rheumatoid arthritis. Lancet 1979, 1:548.
17. Carson DA, Bayer AS, Eisenberg RA, Lawrence S, Theofilopoulos A. IgG rheumatoid factor in subacute bacterial endocarditis: relationship to IgM rheumatoid factor and circulating immune complexes. Clin Exp Immunol 1978, 31:100.
18. Engleman E, Sponzilli EE, Batey ME, Ramchran S, McDevitt HO. Mixed lymphocyte reaction in healthy women with rheumatoid factor: lack of association with HLA-DW4. Arthritis Rheum 1978, 21:690.
19. Stastny P, Fink CW. Different HLA-D associations in adult and juvenile rheumatoid arthritis. J Clin Invest 1979, 63:124.
20. Suciu-Foca N, Godfrey M, Jacobs J, et al. Increased frequency of DRW5 in pauciarticular JRA. This volume.
21. Fink CW, Stastny P. Results of serologic HLA-DR typing in juvenile arthritis and adult RA. Arthritis Rheum, In press.

Table 10. HLA-DR antigens in Caucasian patients with juvenile arthritis (JA).

LAB	Onset[1]	No.	DR1	DR2	DR3	DR4	DR5	DRW6	DR7	DRW8
					(Number)					
BER	Pauci	3	0	1	3	0	0	0	1	0
	Poly	4	0	0	0	1	1	0	2	0
DAW	Poly	1	0	0	0	1	0	0	1	0
	Syst	1	0	0	1	0	1	0	0	0
ENG	Pauci	5	3	2	1	2	1	0	1	-
	Poly	3	0	0	0	1	1	2	2	-
	Syst	2	0	0	0	2	1	0	0	-
PTR	Pauci	6	0	2	2	0	2	0	2	2
	Poly	2	0	0	0	1	0	0	1	0
	Syst	3	0	2	1	0	1	0	0	1
STA	Pauci	41	10	13	11	3	7	2	3	8
	Poly	16	3	1	4	4	4	0	1	8
	Syst	12	2	7	3	2	4	0	3	1
SUF	Pauci	19	1	4	1	7	7	7	3	3
	Poly	4	1	2	0	1	1	1	2	0
	Syst	3	1	0	0	0	3	0	0	0
					(Percent)					
ALL	Pauci	74	19	30	24	16	23	17	14	18**
	Poly	30	13	10	13	30	23	01	30	27***
	Syst	21	14	43	24	19	48**	00	14	10
Total	--	125	17	27	22	20	27*	10	18	18***

[1] Classification according to clinical form of onset: Pauci - Pauciarticular, Poly = Polyarticular, Syst = Systemic

Rheumatoid Arthritis.

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Background

In the Eighth International Workshop, the increased prevalence of DR4 in rheumatoid arthritis (RA) was confirmed in Caucasoids, Japanese and Negroids. This increase was however not observed in Jews while in Asian Indians the findings were inconclusive (Stastny, 1980). There were no studies of South East Asian ethnic groups. Attempts to relate disease severity, age of onset or sex with DR4 were negative though DR4 was only increased in the "seropositive" rheumatoids. Chromosome 6 markers other than HLA were not studied and there was no attempt to identify a particular haplotype associated with disease.

In this study, the following major questions were considered:

(i) Is DR4 associated with disease in a number of ethnic groups in the South East Asian region when the disease definition is well standardized?
(ii) Does DR4 relate to disease severity, sex or seropositivity?
(iii) Is there another chromosome 6 marker strongly associated with RA and can a unique disease associated haplotype be identified?

In this report, we present our findings in relation to these questions.

Methods

Patients included in this study were required to have symmetrical small joint synovitis (tenderness and swelling) involving at least PIP, MCP, wrist or MTP joints with at least one joint from each side involved, together with radiological erosions typical of RA, at least involving PIP, MCP, wrist or MTP joints, while the exclusions listed in the ARA criteria were not to be present.

In addition, sera were sent to the Perth Laboratory to enable a standardized testing for rheumatoid factor using the Rheumaton (Denver) test. Whenever possible, additional EDTA plasma samples were collected for complement allotyping.

Results and Discussion

The numbers studied in the various ethnic groups are shown in table 1. Insufficient numbers of Chinese were typed though additional cases will be studied. In all groups females were predominant and, as shown in table 1, most were seropositive based on the testing of the available serum sample on the laboratory's submission when this information was available. Seropositivity was considered an important requirement for disease definition and for confirmation in a study involving a number of different centres.

DR4 Definition

In this Workshop, definition of DR4 was complicated by a lack of monospecific sera. Essentially two monspecific sera 634 and 813 were used as key sera. Other longer DR4 sera 631, 633, 636, 640 and 811 (with DR7 and/or DRW9 extras) were also used. These criteria are very similar to those recommended by the DR4 antigen chairman. The reaction patterns of these defining sera were not different between RA patients and controls. Cells with incomplete DR typing data or unsuitable DR serological data (eg apparent presence of triplets or hyper reactive cells) were excluded in the analysis.

HLA Antigen Frequencies

The frequncies of DR antigens in the various racial groups are presented in table 2. A highly significant increase of DR4 is present in Caucasoids (in both laboratory groups

and the combined data) and in Asian Indians from the Vaidya laboratory. There was only a marginal increase in DR4 in those Asian Indians residing in Africa and typed by Dr Hammond. This group may represent a more heterogeneous population derived from different regions of the Indian subcontinent and further radiological and serological studies of disease criteria are needed. Interestingly, Woodrow et al (1981) failed to show an increase in DR4 in Asian Indians residing in England, though information regarding diagnostic criteria was lacking.

In the Japanese, the results are less clear. In one laboratory (SAI) there was an increased frequency of DR4 (81% versus 51% in controls. RR = 4.1 $x^2 = 4.62$). However, in the combined Japanese data the frequency of DR4 in RA was 67.1% (table 2), which though similar to that found in the 8th International Workshop RA study (62.5%, n = 104) was not significantly higher than in controls where the frequency of DR4 seemed unexpectedly high. For example, the frequency of DR4 in the total Japanese disease study controls including laboratories SAI and SEK was 39.1% (n = 371) which is also similar to frequency in the controls of the 8th International Workshop RA study (40.7%, n = 792). Taken together with previous reports (Stastny 1980, Nakai et al, 1981) it is apparent that the frequency of DR4 is increased in Japanese rheumatoids.

In Thais, there was a slight but not statistically significant increase in DR4 (RR 1.8, X^2 0.52). Interestingly there was a similar non significant increase in DR9 (table 2). Given the present frequencies, it would require about four times the numbers of patients and controls to show a statistically significant (at the 5% level) increase in DR4. These data suggest that Thais RA may be exceptional in that they do not show a strong association with DR4 though further cases are required. On the other hand these patients did appear to have rheumatoid arthritis: they had a symmetrical polyarthritis, most were seropositive and x- rays show typical symmetrical erosions in the majority.

As reported in previous studies there was a decrease in the frequency of DR7 in all racial groups except Japanese and a decrease in DR2 in Caucasians, Asian Indians and Japanese. These were mostly not significant and apparently secondary to the increased frequency of DR4.

The only significant deviations of the HLA A B C antigens occurred in Caucasians among whom HLA B15 (28.2% in RA versus 11.3% in controls) and CW3 (45.6% versus 22.9%) were slightly increased. These increases were not significant when corrected for the number of antigens studied and are apparently secondary to the known linkage disequilibrium between these antigens and DR4.

Relationship to Sex and Seropositivity

When available, all cases were tested for rheumatoid factor on the serum samples provided and classified on this basis as seropositive or seronegative. The frequency of DR4 was similar in both groups as shown in table 3. DR4 was not associated with seropositivity in the Thai patients.

Data on the presence of rheumatoid factor at any stage during the clinical course were incomplete and inadequatley standardized. However, data from the Caucasoid and Indian laboratories suggest a relationship between DR4 and high titre rheumatoid factor.

The influence of sex on the prevalence of DR4 is shown in table 4. The numbers of males are small but there is no obvious sex difference.

Complement Markers

Suitable typing has only been undertaken on Caucasoid patients. A rare C4B allele designated C4B3 has been observed in 7 out of 43 patients but none of the Caucasoid controls studied as part of the Second AOHWC (table 5). The phenotypes of these individuals are presented in table 6. All seven individuals share the antigens BFS, C4A3, C2C and DR4, six of the seven having BW62. These results suggest that RA is associated with a rare haplotype (BW62), BFS; C4A3; C4B3; C2C; DR4. Whether similar rare haplotypes can be identified in any other racial groups awaits further study.

Conclusion

The data presented while confirming the strong association of DR4 with RA in some races adds support to the view that DR4 is not associated with RA in all races. Accordingly, the finding of an association with C4B3 and a particular haploype in Caucasoids is of great interest and may allow the identification of high risk haplotypes.

References

1. Nakai Y., Wakasaka A., Aizawa M., Hakura K., Nakai H.,and Ohashi A.: HLA and Rheumatoid Arthritis in the Japanese. Arth. & Rheum. (1981) 24, 722-725
2. Stastny, P.: In Histocompatibility Testing, 1980 - Terasaki P. ed. 681
3. Woodrow, J.C., Nichol, F.E., and Saphiropoulos, G.: DR antigens and rheumatoid arthritis: a study of the populations. Brit. Med. J. 1981 283, 1287-1288.

Table 1.

PARTICIPATING LABORATORIES, PATIENT NUMBERS AND FREQUENCY OF SEROPOSITIVITY

LAB.	ETHNIC GROUP	NUMBER PATIENTS	NUMBER CONTROLS	NUMBER SEROPOSITIVE *‖	NUMBER SEROPOSITIVE AT ANY TIME †‖
BAS	CAUCASIAN	18	18	11/13	12/13
DAW		53 (71)	19 (37)	16/26	23/35
SAI	JAPANESE	21	58	16/20	N/A
SEK		43 (64)	44 (102)		N/A
VAI	ASIAN INDIANS	40	38	20/36	36/40
HAM	S. AFRICAN INDIANS	26	21	N/A	22/25
DCH	THAI	32	50	17/30	N/A
CHA	SINGAPORE-CHINESE	6	-	N/A	N/A

* BY PERTH LAB. TESTING ON SUBMITTED SERUM SAMPLES.

† BY SUBMITTING LABORATORY CODING ON CARD 16.

‖ INCLUDES ONLY THOSE TESTED FOR DR ANTIGENS.

N/A NOT AVAILABLE.

Table 2.

HLA-DR ANTIGEN FREQUENCY IN PATIENTS WITH RA AND CONTROLS

	CAUCASIAN		ASIAN INDIAN		AFRICAN INDIAN		THAI		JAPANESE	
N / Allele	RA 48	CONT. 98	RA 37	CONT. 38	RA 26	CONT. 21	RA 30	CONT. 49	RA 64	CONT. 101
DR1	20.8%	16.3%	8.1%	15.7%	3.8%	14.2%	3.3%	0%	15.6%	11.8%
DR2	16.6	24.4	24.3	39.4	38.4	38.0	40.0	34.6	20.3	33.6
DR3	25.0	25.5	24.3	21.0	7.6	14.2	0	4.0	3.1	0.9
DR4	70.8[1)]	33.6	67.5[2)]	13.1	34.6	28.5	23.3	14.2	67.1	54.4
DR5	4.1	5.1	16.2	18.2	30.7	33.3	3.3	6.1	10.9	8.9
DR7	10.4[3)]	31.6	24.3	36.8	15.3	28.5	6.6	30.6	0	0.9
DRW8	6.2	2.0	0	2.6	3.8	4.7	0	2.0	4.6	17.8
DRW9	2.0	0	5.4	7.8	7.6	4.7	26.6	12.2	28.1	24.7

Disease controls from Labs. BAS, DAW, ROB, TAI were used.

@ DRW6 related antigens were excluded from this table.

1) RR = 4.7 X^2 = 17.9 $p < 5 \times 10^{-5}$

2) RR = 13.7 X^2 = 23.12 $p < 10^{-5}$

3) RR = 0.25 X^2 = 8.7 $p < 5 \times 10^{-3}$

Table 3.

HLA-DR4 IN RA --RELATION TO RHEUMATOID FACTOR STATUS*

	BAS	DAW	VAI	SEK	SAI	DCH	HAM
sero-positive	9/11(82%)	12/16(75%)	13/20(65%)	13/22(59%)	12/16(75%)	2/17(11%)	N/A
sero-negative	1/2 (50%)	7/10(70%)	12/16(75%)	10/17(58%)	4/4 (100%)	5/13(38%)	N/A

*: Based on Perth Lab. testing.

Table 4.

HLA-DR IN RA - RELATION TO SEX

SEX	CAUCASIAN			JAPANESE			ASIAN INDIAN	AFRICAN INDIAN	THAI
	DAW	BAS	TOTAL	SEK	SAI	TOTAL	VAI	HAM	DCH
F	$^{23}/_{31}$ (74%)	$^{5}/_{7}$ (71%)	$^{28}/_{38}$ (73%)	$^{24}/_{36}$ (67.1%)	$^{13}/_{16}$ (81%)	$^{37}/_{52}$ (71%)	$^{20}/_{31}$ (65%)	$^{9}/_{25}$ (36%)	$^{4}/_{23}$ (19%)
M	$^{1}/_{4}$ (25%)	$^{5}/_{6}$ (83%)	$^{6}/_{10}$ (60%)	$^{3}/_{7}$ (43%)	$^{3}/_{4}$ (75%)	$^{6}/_{11}$ (55%)	$^{5}/_{6}$ (83%)		$^{3}/_{8}$ (38%

Table 5

Association of C4B3 with RA in Caucasoids

	C4B3	
	+	−
RA	7	36
NON RA	0	53

$p_{\chi^2} < 0.005$

Table 6

Caucasian RA Patients – C4 B3 Positive

	A	C	B	Bf	C4A	C4B	C2	DR
JAC	2, 11	3, 5	62, 44	S S	3	3	C	4 3
FAU	2, 24	3, 2	62, 14	S S	3	3 1	C	4 1
RID	1, 32	3, 7	62, 8	S S	3	3 1	C	4
GIB	2, 28	3, 5	62, 44	S S	3	3	C	4
CAR	3	3, 4	62, 22	S F	3	3	C	4
MUN	2	3	62, 50	S F	3	3	C	4
JUN	1	6, 9	8, 37	S F	3	3 1	C	4

HLA Associations with Rheumatoid Arthritis In African Blacks

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Abstract. **The HLA-A, B, C and DR antigens were determined in a group of 100 blacks with classical or definite rheumatoid arthritis (RA) in Durban, South Africa. Fifty-six of these patients were also tested for the DQ antigens. There was a significant association of HLA-DR4 with RA (χ^2 = 77.2; $p < 0.0001$). The frequency of DR4 in RA was 44% in comparison with 10% in controls (relative risk 7.4). An unusual finding was a significant increase in the frequency of HLA-B8 in 35% of patients with RA compared to 12.5% of controls ($p < 0.0001$; relative risk 3.8). There was no linkage disequilibrium between DR4 and B8 to explain the latter association. (*J Rheumatol 1989;16:1326–8*)**

Key Indexing Terms:
RHEUMATOID ARTHRITIS BLACKS HLA-DR4 HLA-B8

The HLA-DR4 antigen is associated with rheumatoid arthritis (RA) in Caucasians, American blacks and many other populations[1-4]. However, normal frequencies of DR4 have been reported in Asians in Britain[3] and Jews[5]. American and African blacks with ankylosing spondylitis have a lower prevalence of HLA-B27 than Caucasians[6]. Our survey was undertaken to determine whether the HLA associations with RA in African blacks were similar to Caucasians and American blacks or whether there were genetic differences.

MATERIALS AND METHODS

A group of 100 unrelated blacks with classical or definite RA[7] who attended the rheumatology clinic at the King Edward VIII Hospital in Durban, South Africa were studied. All the patients were of Zulu descent. The mean age of the patients was 43.7 years (range 21 to 66 years) and the female:male ratio was 3.8:1.

The HLA-A,B and C antigens were identified using a 2 stage lymphocytotoxicity test[8] and 180 antisera. The HLA-DR and DQ antigens were defined with 120 antisera on B cell enriched lymphocyte suspensions prepared by the use of straws packed with nylon wool[9]. The HLA-A,B,C and DR antigens were determined in all 100 patients. The DQ antigens were also tested during the course of the study and were determined in 56 patients.

The control group consisted of blood donors and staff who were also of Zulu descent. The HLA-A,B and C antigens were determined in 1985 controls, DR antigens in 513 and DQ antigens in 340 controls.

The difference in frequency of the various antigens between patients and controls were tested for significance by means of the χ^2 test (without Yates' correction). The resulting probabilities were multiplied by the number of HLA specificities tested to determine the corrected value. Relative risk was calculated according to Svejgaard, *et al*[10]. Haplotype frequencies were calculated by the methods of Matthiuz, *et al*[11].

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RESULTS

There was no significant association of RA with any of the HLA-A and C antigens. The frequency of the HLA-B antigens in controls and patients with RA are shown in Table 1. There was a significant increase in the prevalence of HLA-B8 which was noted in 12.5% of the controls and 35% of patients with RA ($p < 0.0001$; relative risk 3.8). There was no linkage disequilibrium between DR4 and B8 to explain the increased frequency of B8.

The results of the DR and DQ antigens are shown in Table 2. There was a significant association of DR4 with RA (χ^2 = 77.2; $p < 0.0001$). There was no significant increase in the frequency of the DQ antigens. The results of some of the haplotype frequencies in patients with RA and controls are shown in Table 3.

DISCUSSION

A significant association between HLA-DR4 and RA, which has been reported in American blacks, Caucasians and other populations, is confirmed in African blacks[1-5]. The prevalence of DR4 in controls and patients with RA is about 30 and 70%, respectively, in Caucasians[12], 7 and 22% in American blacks[4] and 10 and 44% in African blacks. Therefore, although American and African blacks with RA show a significant association with DR4, the frequency of DR4 is lower in both patients with RA and controls in comparison with Caucasians.

The DR3 and DR4 subsets and DQw4 were not tested at the time of this study. There was no increase in the frequency of DQw3 in our patients with RA. The DR4-DQw3 haplotype showed significant linkage disequilibrium in the African black controls as noted in other populations[13]. However, there was no linkage disequilibrium between DR4 and DQw3 in the African blacks with RA. Singal, *et al*[14] have shown

Table 1. *HLA-B antigens in controls and patients with RA*

HLA antigens	Controls (n = 1985)	RA Patients (n = 100)	χ^2	R-R*
	%	%		
B7	22.4	26.00	0.70	1.2
B8	12.5	35.00	41.11	3.8
B13	3.4	3.00	0.04	0.9
B14	5.5	5.00	0.05	0.9
B15	3.4	4.00	0.11	1.2
B16	3.6	1.00	1.90	0.3
B17	37.2	34.00	0.41	0.9
B18	5.5	8.00	1.08	1.5
B21	2.0	3.00	0.52	1.5
B22	0.1	0.00	0.05	0.0
B27	0.3	0.00	0.30	0.0
B35	6.9	10.00	1.39	1.5
B37	0.1	0.00	0.10	0.0
B41	1.9	3.00	0.65	1.6
B42	21.3	14.00	3.03	0.6
B44	15.3	12.00	0.79	0.8
B45	9.2	4.00	3.18	0.4
B47	0.1	0.00	0.10	0.0
B48	0.1	0.00	0.05	0.0
B51	1.3	1.00	0.05	0.8
B52	0	0.00	0	0
B53	1.5	0.00	1.53	0.0
B54	0	0.00	0	0
B60	0.1	1.00	5.36	10.00
B61	0.1	0.00	0.05	0.0
B70	23.5	20.00	0.64	0.8

* R-R: relative risk

Table 2. *HLA-DR and DQ antigens in controls and patients*

HLA-DR Antigens	Controls (n = 513) %	RA Patients (n = 100) %	X^2	R-R
DR1	4.7	4.0	0.09	0.8
DR2	24.2	15.0	4.01	0.6
DR3	35.3	31.0	0.68	0.8
DR4	9.6	44.0	77.17	7.4
DR5	32.2	22.0	4.08	0.6
DRw6	17.9	15.0	0.50	0.8
DR7	15.4	15.0	0.01	1.0
DRw8	3.9	3.0	0.19	0.8
DR9	0.8	0.0	0.78	0.0
DRw10	2.1	6.0	4.61	2.9
HLA-DQ Antigens	(n = 340) %	(n = 56) %		
DQw1	62.7	44.6	6.50	0.5
DQw2	22.4	28.6	1.04	1.4
DQw3	30.0	35.7	0.74	1.3

Table 3. *Estimated haplotype frequencies in controls and patients with RA*

HAPLOTYPE	Frequency/10,000	Delta	Delta/SE*
Patients			
DR4-DQw3	89	31	0.7
DR4-B8	37	−12	−0.4
DR3-B8	49	16	0.6
DR3-Bw42	48	36	2.1**
DR4-B17	110	63	2.2**
Controls			
DR4-DQw3	25	20	2.8**
DR4-B8	2	−1	−0.2
DR3-B8	30	18	2.5**
DR3-Bw42	63	42	4.5***
DR4-B17	16	5	0.7

* SE - standard error.
** $p < 0.05$.
*** $p < 0.01$.

that all DR4 positive patients with RA carried the DQw3.1 subtype in comparison with 19% of healthy DR4 positive controls. We did not study the DQw3 subtypes in our patients and therefore there may still be an association between DR4 and DQw3.1.

An unusual finding in the African blacks with RA was the significant increase in the frequency of the HLA-B8 antigen which is associated with many other autoimmune diseases[15]. The significant linkage disequilibrium between DR4 and B17 in the patients but not in controls may reflect differences in the frequencies of the Class III complement genes which lie between the B and DR loci.

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REFERENCES

1. Stastny P: Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. *N Engl J Med 1978;*298:869-71.
2. Jaraquemada D, Ollier W, Awad J, *et al:* HLA and rheumatoid arthritis: a combined analysis of 440 British subjects. *Ann Rheum Dis 1986;*45:627-36.
3. Woodrow JC, Nichol FE, Zaphiropoulos G: DR antigens and rheumatoid arthritis: a study of two populations. *Br Med J 1981;*283:1287-8.
4. Alarcon GS, Koopman WJ, Acton RT, Barger BO: DR4 antigen distribution in blacks with rheumatoid arthritis. *J Rheumatol 1983;*10:579-83.
5. Schiff B, Mizrachi Y, Orgad S, Yaron M, Gazit E: Association of HLA-Aw31 and HLA-DR1 with adult rheumatoid arthritis. *Ann Rheum Dis 1982;*41:403-4.
6. Chalmers IM: Ankylosing spondylitis in African blacks. *Arthritis Rheum 1980;*23:1366-70.
7. Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA: 1958 revision of the diagnostic criteria for rheumatoid arthritis. *Bull Rheum Dis 1958;*9:175-6.
8. Mittal KK, Mickey MR, Singal DP, Terasaki PI: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation 1968;*6:913-27.

9. Danilov JA, Ayoub G, Terasaki PI: Joint report: B lymphocyte isolation by thrombin-nylon wool. In: Terasaki PI, ed. *Histocompatibility Testing 1980.* Los Angeles: UCLA Tissue Typing Laboratory, 1980:287–8.
10. Svejgaard A, Platz P, Ryder LP, Staub-Nielsen L, Thomsen M: HLA and disease associations — a survey. *Transplant Rev 1975;*22:3-43.
11. Mattiuz PL, Ilde D, Pizazza A, Ceppellini R, Bodmer WF: New approaches to the population genetic and segregation analysis of the HLA system. In: Terasaki PI, ed. *Histocompatibility Testing 1970.* Los Angeles: UCLA Tissue Typing Laboratory, 1970:193.
12. McCusker CT, Singal DP: Molecular relationships between the class II antigens susceptibility to rheumatoid arthritis (editorial). *J Rheumatol 1988;*15:1050-3.
13. Wallin J, Carlsson B, Storm H, *et al:* A DR4-associated DR-DQ haplotype is significantly associated with rheumatoid arthritis. *Arthritis Rheum 1988;*31:72-9.
14. Singal D, D'Souza M, Reid B, *et al:* HLA-DQ beta chain polymorphism in HLA-DR4 haplotypes associated with rheumatoid arthritis. *Lancet 1987;*2:1118-20.
15. Tiwari JL, Terasaki PI: *HLA and Disease Associations.* New York: Springer-Verlag, 1985.

HLA ASSOCIATIONS WITH RHEUMATOID ARTHRITIS AMONG THE VARIOUS MIGRANT INDIAN COMMUNITIES IN SOUTH AFRICA

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SUMMARY

Rheumatoid arthritis (RA) in Indians has been shown to be associated with HLA DR4 in North India and with DR1 in the United Kingdom. We studied a migrant Indian population in South Africa to determine their genetic associations with RA. A group of 123 unrelated RA patients from three communities (Hindi, Muslims and Tamils) were studied. Only the Muslims showed a significant association with DR4 whereas the Hindi and Tamils showed a significant association with DR10. This survey shows that the Indian community is a heterogenous group regarding the HLA association with RA and different associations are noted in the various communities.

INTRODUCTION

The association of HLA DR4 with rheumatoid arthritis (RA) has been documented in Caucasians, American and African Blacks, Japanese and many other communities.

Mehra et al.[1] have shown a strong association with DR4 in a group of 40 North Indian patients with RA in India. The frequency of DR4 was 70% in RA patients compared to 12% in controls. In the United Kingdom, Woodrow et al.[2] studied a group of 35 Indians with RA and found a significant association with DR1 and not DR4; DR1 was detected in 60% of patients with RA compared to 17 % of controls.

Indians from India first arrived in South Africa in 1860 to work in the sugar cane fields in Natal. Presently there are nearly one million Indians in South Africa. The three major groups of Indians are the Hindi and Muslims, who are North Indians of Aryan descent and Tamils, who are South Indians of Dravidian descent. Previous surveys have shown that there are differences in the prevalence of the various Class I antigens in control groups of Muslims, Hindi and Tamils in South Africa.[3,4]

This survey was undertaken to determine whether there were any differences in the genetic associations with RA among the various migrant Indian communities in South Africa when compared to Indians in India and the United Kingdom.

PATIENTS AND METHODS

A group of 123 unrelated Indians with classical or definite RA[5] who were attending the rheumatology clinic at King Edward VIII Hospital were studied. The study population consisted of 53 Tamils, 39 hindi and 31 Muslims. The mean age of the patients was 44.7 years and the female to male ratio was 5.8 to 1. The number of patients and controls in the various communities who were studied for the HLA A, B, C, DR and DQ antigens is shown in Table 1. The control group consisted of 1458 normal adults who were either staff or randomly selected blood donors of Indian descent. HLA Class I antigens were determined in all patients and control subjects by a two-stage microlymphocytotoxicity test (1) with 180 antisera. They consisted of local sera that have been requested for use in International Histocompatibility Testing Workshops, local sera that have been verified with International Workshop sera and sera that have been exchanged with other laboratories worldwide. Similarly, 120 sera were used to define the Class II antigens on B-lymphocyte enriched lymphocyte suspensions prepared with the aid of straws packed with nylon wool (2). The Class II antigens were determined in 446 control subjects except that there were only 319 control subjects that were tested for HLA DQ antigens. Although over 2000 individuals have been tested for HLA DQ locus antigens in our laboratory the majority were Caucasoid or patients with selected diseases. As a consequence, only 319 normal, healthy Indian individuals have been tested for HLA DQ antigens. The difference in the frequency of each antigen in patients and controls was tested for significance by means of the chi-squared test (without Yate's correction). The resulting probabilities were multiplied by the number of specificities tested to determine the corrected value. Relative risk was calculated according to Woolf et al.[8]. Haplotype frequencies were calculated by the method of Mattiuz et al.[9].

RESULTS

There were no significant associations of RA with the HLA C locus antigens in any of the Indian communities. The HLA antigens which showed a significant association in the different communities are summarised in Table 2.

The Tamils showed a significantly increased frequency of HLA A2, B37 and DR10 and although DR2 was also increased, the difference was not statistically significant. The Hindi patients showed a significant increase only in DR10. They also had an increase of B44 and a reduction in the frequency of DR5. The Muslims showed a significant association with B21 and DR4.

DISCUSSION

When the results of the HLA associations with RA in our study are compared with Indians from North India[1] and the United Kingdom[2], we note that a significant association with DR4 was only seen in North India and in our Muslim patients.

In the United Kingdom Woodrow et al.2 found an increased frequency of only DR1 among their Indian patients. At the time of their study only DR1 to DR7 were being tested. Since then DR8 to DR10 have been defined together with many splits and many DR1 antisera contain antibodies to DR10. Thus the increase in DR1 which was seen in The United Kingdom may be related to the increased DR10 which we saw in our Hindi and Tamil patients. A significant association with HLA DR1 and not DR4 has also been reported in Jews[10].

Although American[11] and African Blacks[12] have also shown a significant association of DR4 with RA, the frequency of DR4 was only 22% and 44% in American and African blacks respectively. The frequency of DR4 in Caucasians[13] with RA is about 70%. We have found that although there is a significant association of DR4 or DR10 in our Indian communities, the frequency of these antigens is less than 40%in all the communities. Thus there may be other subsets or epitopes which were not studied in this survey which may show a stronger association with RA.

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Table 1

NUMBER OF PATIENTS AND CONTROLS WHO WERE TESTED FOR THE VARIOUS CLASS 1 AND CLASS 2 ANTIGENS

NUMBER STUDIED	HINDI	MUSLIMS	TAMILS
HLA-ABC antigens			
Patients	39	31	53
Controls	490	176	792
HLA-DR antigens			
Patients	38	30	53
Controls	135	49	262
HLA DQw1-DQw3			
Patients	33	25	43
Controls	100	25	194

Table 2

HLA ANTIGENS WHICH SHOWED A SIGNIFICANT ASSOCIATION IN INDIANS WITH RHEUMATOID ARTHRITIS

Antigen	Controls %	Patients %	R-R*	Chi-square	p Value
TAMILS					
A 2	28	49	2,5	10,6	<0,001
B 37	7	23	3,8	15,8	<0,001
DR10	11	32	3,8	15,6	<0,001
HINDI					
B 44	21	38	2,4	6,7	<0,01
DR10	7	32	5,8	15,6	<0,001
MUSLIMS					
B 21	1	10	18,8	11,5	<0,001
DR4	6	37	8,9	11,9	<0,001

*R-R - relative risk

REFERENCES

1. Mehra NK, Vaidya MC, Taneja V, Agarwal A, Malaviya AN. HLA-DR antigens in rheumatoid arthritis in North India.
   Tissue Antigens 1982; 20 : 300-302.
2. Woodrow JC, Nichol FE, Zaphiropoulos G. DR antigens and rheumatoid arthritis : a study of two populations.
   Br Med J 1981; 293 : 1287-1288.
3. Wadee AA, Du Toit ED. HLA Frequencies in the Indian population of Johannesburg. S Afr Med J 1989; 76 : 331-334.
4. Hammond MG, Appadoo B, Brain P. Subdivision of HLA 5 and comparative studies of HLA polymorphism in South African Indians. Tissue Antigens 1974; 4 : 42.
5. Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA. 1958 revision of the diagnostic criteria for rheumatoid arthritis.
   Bull Rheum Dis 1958; 9 : 175-6.
6. Mittal KK, Mickey MR, Singal DP, Terasaki PI : Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytoxicity test. Transplantation 1968; 6 : 913-27.
7. Danilov JA, Ayoub G, Terasaki PI. Joint report : B lymphocyte isolation by thrombin-nylon wool. In : Terasaki PI ed. Histocompatibility Testing 1980. Los Angeles : UCLA Tissue Typing Laboratory, 1980 : 287-8.

8. Woolf B. On estimating the relation between blood group and diseases. Am J Hum Genet 1955; 19 : 251.

9. Mattiuz PL, Ihde D, Pizazza, Ceppellini R, Bodmer WF : New approaches to the population genetic segregation analysis of the HLA system. In Terasaki PI ed. Histocompatibility Testing 1970. Los Angeles : UCLA Tissue Typing Laboratory, 1970 : 193.

10. Schiff B, Mizrachi Y, Orgad S, Yaron M, Gazit E. Association of HLA-AW31 and HLA-DR1 with adult rheumatoid arthritis. Ann Rheum Dis 1982; 41 : 403-4.

11. Alarcon GS, Koopman WJ, Acton RT, Barger BO. DR4 antigen distribution in blacks with rheumatoid arthritis. J Rheumatol 1983; 10 : 579-83.

12. Mody GM, Hammond MG, Naidoo PD. HLA associations with rheumatoid arthritis in African blacks. J Rheumatol 1989; 16 : 1362-1328.

13. Jaraquemada D, Ollier W, Awad J et al. HLA and rheumatoid arthritis : a combined analysis of 440 British subjects. Ann Rheum Dis 1986; 45 : 627-36.

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Association between histocompatibility type and the ability to make anti-Rh antibodies

The HL-A types of Rh-negative women with and without anti-Rh antibodies were examined. In those who made Rh antibodies, there was an excess of HL-A1, and a deficiency of HL-A2 and W10, approaching the conventional level of significance. There was, however, a highly significant lack, in the antibody-making group, of subjects with only one antigen detected at the first (LA) locus. The capacity to make anti-Rh antibodies and that to make cytotoxic anti-HL-A antibodies were strongly correlated.

1. Introduction

We started this investigation because Jerne [1] had predicted that the ability to make antibodies against certain nonhistocompatibility antigens should be correlated with histocompatibility type, and further, that individuals possessing a wide range of histocompatibility alleles should be able to make a wider variety of antibodies against nonhistocompatibility antigens than individuals with a smaller assortment of histocompatibility alleles. The major histocompatibility system of man (HL-A) is determined by genes at two closely linked loci, called "LA" and "FOUR". It occurred to us, therefore, that a subject who was heterozygous at both these loci, and thus had at least four different HL-A factors, might be expected to make a wider variety of antibodies more readily than a subject who was homozygous at one of the loci (three factors), and particularly than one who was homozygous at both loci (two factors). An earlier attempt by one of us [2] to show this effect with the isoagglutinins of the ABO

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blood group system was inconclusive. We decided, therefore, to examine next the ability to make anti-Rh antibodies in terms of HL-A type. We chose this system because Mollison, Frame and Ross [3] have shown that some Rh negative women make these antibodies readily; whereas others, given the same stimulus by intravenous injection of Rh positive cells, make anti-Rh antibodies with difficulty or not at all. About 50 % of women fall into each of the two groups, and the difference is presumably genetically determined. Such control, of course, need not in this case be of the kind postulated by Jerne: Mollison et al. suggest that it may be connected with Rh genotype, and it might equally well be due to the effect of an immune response gene belonging to neither the HL-A nor the Rh system. The system was attractive to us, however, because it seemed to provide a clear distinction between two classes of individuals in the ability to make a particular antibody, and because we were equipped to test for both the Rh and the HL-A factors.

In this paper, therefore, we compare the HL-A types of two groups of Rh negative women, those who made Rh antibodies and those who did not. We looked both for a specific effect of particular HL-A alleles, and for the effect of homozygosity or heterozygosity at one or both loci, on the ability to make anti-Rh antibodies. As an afterthought, we decided to examine also the frequency with which the two groups of women made antibodies against HL-A antigens, since such antibodies, like those in the Rh system, are frequently made during pregnancy against antigens which the fetus, but not the mother, possesses.

2. Materials and methods

There were 96 women in the group of Rh negative subjects who made antibodies against factors included in the Rh system. Exact data on pregnancies was available for 93 of them, who had had a total of 344 pregnancies, a mean of 3.7 per subject. The control group consisted of 78 Rh negative women whose husbands were all Rh positive; none had had fewer than two pregnancies, and Rh antibodies had been sought in all pregnancies, but never detected. This group had a total of 233 pregnancies, mean 3.0. Since ABO incompatibility between fetus and mother has been thought [4] to influence sensitization to Rh antigens, we obtained where possible the ABO groups of the husbands (those of the women were known). Such data for both partners were available in respect of 63 women in the group making antibodies; 71 % of the couples were compatible, in the sense that the mother could not bear by that husband a child incompatible with herself in terms of ABO. In the control group there were data for 50 couples, of whom 60 % were compatible. This difference between the groups was not significant at the conventional level ($\chi^2 = 1.6$ for 1 d.f., $p = 0.2$). The random population cited for reference in Table 1 consisted of 454 normal unrelated blood donors and staff members of either sex; none of them had been examined because of any disease, or for pregnancy. All the subjects in all three groups were Caucasians of Western European origin.

Rh grouping and antibody detection were done by conventional methods in the laboratories of a large transfusion service. HL-A typing was by the two-stage microcytotoxicity test [5]; 60 sera were used to test for the following antigens: first (LA) locus: HL-A1, 2, 3, 9, 10, 11, 28; W19, 29, 31; second (FOUR) locus: HL-A5, 7, 8, 12, 13, 14, 17, 27; W5, 10, 15, 22.

At the same time, the serum of every woman in the first two groups was examined for cytotoxic anti-HL-A antibodies, using a panel of cells from 12 donors selected to possess all the antigens mentioned above. The data were transferred to punch cards and a computer program was used to calculate phenotype and gene frequencies, with haplotype and delta (gametic association) values from phenotypic data according to Mattiuz et al. [6].

3. Results

Table 1 shows the phenotype frequencies of the HL-A antigens in the two groups and in the random Caucasian population. The proportions of women in the two groups who made cytotoxic HL-A antibodies are also compared. The symbols 'X' and 'Y' are used to indicate subjects in whom only one antigen was detected at the first and at the second locus respectively. The χ^2 values are for comparisons between the frequencies of HL-A antigens in the two groups of Rh negative women, but probabilities obtained from tables of χ^2 have been corrected as described by Walford [7] by multiplying them by the reciprocal of the frequency of the specificity concerned in the general population.

Table 1. HL-A antigen frequencies in Rh negative women with and without Rh antibodies, and in the general population

Antigen	Random Caucasian population (454 subjects) (%)	Rh negative women: With anti-Rh antibodies (96 subjects) No.	(%)	Without anti-Rh antibodies (78 subjects) No.	(%)	χ^2	P <	Corrected p <
HL-A 1	30.6	39	40.7	18	23.1	6.02	0.025	.08
HL-A 2	45.2	38	39.6	46	59.0	6.48	0.025	.06
HL-A 3	29.7	38	39.6	21	26.9	3.08	0.1	.33
HL-A 9	16.3	23	24.0	9	11.5	4.42	0.05	.31
HL-A10′	9.0	1	1.0	3	3.9	1.51		
HL-A10″		6	6.3	4	5.1	0.08		
HL-A11	11.7	13	13.5	9	11.5	0.16		
HL-A28	7.9	8	8.3	6	7.7	0.02		
W19	9.0	3	3.1	3	3.9	0.07		
W29	4.0	3	3.1	2	2.6	0.05		
W31	3.3	3	3.1	3	3.9	0.07		
X (only one antigen detected at first locus)	33.3	17	17.7	32	41.0	11.57	0.001	.003
HL-A 5	9.9	9	9.4	9	11.5	0.22		
HL-A 7	25.1	24	25.0	15	19.2	0.82		
HL-A 8	22.0	32	33.3	14	18.0	5.24	0.025	0.11
HL-A12	29.1	26	27.1	30	38.5	2.55		
HL-A13	6.2	4	4.2	3	3.9	0.01		
HL-A14	5.1	10	10.4	8	10.3	0.00		
HL-A17	8.8	5	5.2	1	1.3	1.99		
HL-A27	8.4	3	3.1	4	5.1	0.45		
W5	15.2	17	17.7	6	7.7	3.76		
W10	15.0	3	3.1	11	14.1	7.01	0.01	.06
W15	10.8	11	11.5	10	12.8	0.08		
W22	3.3	6	6.3	3	3.9	0.51		
Y (only one antigen detected at second locus)	41.1	42	43.8	42	53.9	1.76		
Cytotoxic HL-A antibodies		16/93	17.2	1/78	1.3		0.00028[a]	

a) Probability by Fisher's exact method.

Table 2. Haplotype frequencies with coefficients of gametic association (Δ) and standard errors (S.E.) in Rh negative women with and without anti-Rh antibodies, and in the general population (all figures x 10^3)

	Random Caucasian population			Rh negative women with anti-Rh antibodies			without anti-Rh antibodies		
Haplotype	Freq.	S.E.	Δ	Freq.	S.E.	Δ	Freq.	S.E.	Δ
HL-A 1/8	87	14.9	67.8	120	34.5	78.0	64	39.5	52.9
HL-A 2/12	73	14.4	31.7	48	29.1	17.1	130	44.0	52.2
HL-A 3/7	63	14.0	41.5	78	31.5	48.5	39	37.5	24.3
HL-A 1/17	20	12.3	12.0	20	26.5	13.5	6	34.6	5.6
W 29/HL-A 12	16	12.1	12.9	16	26.2	13.4	13	35.2	10.1
HL-A 2/W 10	30	12.7	10.1	3	24.8	−0.8	10	34.9	−16.3
W 28/HL-A12	9	11.8	2.1	10	25.6	1.9	39	37.5	30.8
HL-A 9/W 10	10	11.9	3.8	4	24.9	1.7	31	36.8	27.0

4. Discussion

We do not know, of course, that all the women in the control group have had the opportunity of making anti-Rh antibodies; some, with husbands heterozygous for Rh, may never have had an Rh positive fetus. It is safe to say, however, that a good proportion of them will have been exposed to Rh antigens.

Of the individual HL-A factors studied, HL-A1, 2 and W10 show differences in frequency between the two groups that approach the conventional level of significance, and a more extensive study might give interesting results. The only highly significant difference obtained, however, is in the proportions from the two groups having only one antigen detected at the first locus. Such subjects are far less common in the group that made antibodies than in the control group. We may safely assume that many of the subjects in whom only one antigen was detected at one locus are in fact homozygous for that antigen and not heterozygous for it and an unknown factor, since we have antisera against all the known factors that occur commonly in Caucasians. At the second locus this effect is less marked, though the figures show the same trend. Such an effect of homozygosity might be expected from Jerne's theory. Our finding, however, that the ability to make Rh antibodies was strongly correlated in this study with the capacity to make antibodies in the HL-A system, would not be expected at all. It probably has quite a different explanation. One possibly is that fetal material, bearing both Rh and HL-A antigens, crosses the placenta readily in some women and not in others; but this will not explain Mollison's nonresponding women, who received their antigens by intravenous injection. Another might be that some pregnant women respond more readily than others to *any* foreign antigens on the fetus, owing to the lessened activity of some mechanism whose function it is to damp down nonspecifically the immune response; such mechanisms have been described by several authors, for example Hill et al. [8].

We do not feel that we ought to try to explain our findings at this stage: if the effects of individual HL-A factors, and particularly of homozygosity at one or both loci, are subsequently confirmed by other studies, we may then profitably consider whether they are a direct effect of HL-A genotype, or of an immune response gene linked to HL-A. Since we have exhausted our material, it will not be possible for us to carry out such studies in the near future; we are therefore publishing this preliminary report in the hope that other laboratories will investigate the subject.

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5. References

1 Jerne, N.K., *Eur. J. Immunol.* 1971. *1*: 1.

2 Brain, P., *Transplantation* 1972. *13*: 530.

3 Mollison, P.L., Frame, M. and Ross, M.E., *Brit. J. Haematol.* 1970. *19*: 257.

4 Levine, P., *J. Hered.* 1943. *34*: 71.

5 Brand, D.L., Ray, J.G., Hare, D.B., Kayhoe, D.E. and McClelland, J.D., in Terasaki, P.I. (Ed.) *Histocompatibility Testing*, Munksgaard, Copenhagen 1970, p. 357.

6 Mattiuz, P.L., Ihde, D., Piazza, A., Ceppellini, R. and Bodmer, W.F., in Terasaki, P.I. (Ed.) *Histocompatibility Testing*, Munksgaard, Copenhagen 1970, p. 193.

7 Walford, R.L., Zeller, E., Combs, L. and Konrad, P., *Transplant. Proc.* 1971. *3*: 1297.

8 Hill, C.A.St., Finn, R. and Denye, V., *Brit. Med. J.* 1973. *3*: 513.

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Human leucocyte antigen status in African women with eclampsia

NICHOLAS JOHNSON, JACK MOODLEY, MICHAEL G. HAMMOND

Summary. Investigation of the HLA system in 53 African eclamptic or imminently eclamptic women showed that they were significantly more likely to be heterozygous at the B locus than were normal controls. This did not apply to the A or D related loci.

The aetiology of eclampsia remains unknown, but suggestions that it may be an immunogenetic disorder date back to the beginning of the century (Beer & Need 1985). Redman *et al.* (1978) reported that women who had only one detectable surface antigen determined by the HLA-B locus were at an increased risk of developing pre-eclampsia in pregnancy. This work has yet to be confirmed. To examine this association, we studied the HLA system in women who developed eclampsia and compared it with that found in the normal population.

Patients and methods

The study included 53 black African women from Zululand admitted to the labour ward of King Edward VIII hospital who had systolic blood pressures greater than 160 mmHg and diastolic blood pressures greater than 115 mmHg, gross oedema and at least 2+ proteinuria by standard turbinometric methods in a catheter specimen of urine. Forty-two patients had already suffered at least one seizure before admission and the remaining 11 all complained of headache, nausea and visual disturbances. All were either irritable or had intellectual clouding and an independent observer described those who had not had a seizure as being hyper-reflexic with clonus before therapy. Patients with a history of neuropathology, diabetes, hypertension, renal disease, recurrent miscarriage or a recent blood transfusion were all excluded. Patients who were still proteinuric or hypertensive (>140/90 mmHg) 12 days after delivery were also excluded. Three patients with eclampsia did not know their parents, but the remainder denied that they could be the product of consanguineous marriage or matings.

The control group for the A and B locus of the HLA system consisted of 1416 blood donors of the same tribe and resident within the same hospital catchment area and 412 of them also acted as controls for the DR locus.

The HLA, A, B and DR antigens were determined by a two-stage lymphocytotoxicity test (Terasaki & McClelland 1964). Patients with only one antigen per locus were considered to be homozygous at that locus. Frequency differences between the eclamptic patients and the controls were tested for significance with the χ^2-test. The formula given by Haldane (1956) was used to combine data from the available published series with the data presented. Relative risk was defined as the number of times more often the disease occurred in women positive for that antigen than in those negative for that antigen (Woolf 1955).

Results

Clinical details of the subject group are recorded in Table 1. Women with eclampsia or imminent

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Table 1. Clinical details of patients with eclampsia

Variable	Number
No. of patients	53
No. with previous viable pregnancy	26 (49%)
Nulliparous	27 (51%)
Preterm labour (< 35 weeks gestation)	26 (49%)
Age (years)	
< 20	16
20–24	21
25–30	11
> 30	5
Maternal deaths	1*

*Intracranial haemorrhage.

eclampsia were less likely to have only one detectable antigen at the B locus than were the normal population, the difference was statistically significant ($P < 0{\cdot}01$, $\chi^2 = 7{\cdot}4$). The relative risk in patients in whom both antigens were detected at the B locus is thus increased to 2·3. This does not apply to the A locus or at the DR locus (Table 2). No specific A, B or DR antigen occurred more commonly in eclamptic patients.

Discussion

Two antigen types are inherited, one from each parent. If only one antigen can be detected, then it can be inferred that the individual has either inherited the same antigen from each parent, thus making her homozygous at that locus, or that she has an antigen yet to be discovered. As it is believed that over 98% of the B locus antigens are known to us, the finding of a single B locus antigen is presumed to be synonymous with homozygosity. Pregnant Zulu women suffering from eclampsia or imminent eclampsia are less likely to have only one detectable human lymphocyte antigen at the B locus than are the normal, healthy population from the same tribe and district. Our eclamptics are more likely to be heterozygous at the B locus. It is perhaps significant that women born of a consanguineous relationship, and thus relatively homozygous, have some protection from developing eclampsia in pregnancy (Stevenson *et al.* 1976). However, heterozygosity in eclamptics does not occur at the A or DR locus, this observation is unlikely to be related.

Our data contradict the findings of Redman *et al.* (1978). They studied 80 Oxfordshire women suffering from pre-eclampsia and computed a *P* value of 0·025 supporting an association between pre-eclampsia and homozygosity at the B locus. Simon *et al.* (1980) also reported that French pre-eclamptic patients were relatively homozygous, but they only recruited 26 patients (six were homozygous) and their control group was limited to 16 men, none of whom was homozygous. However, Persitz *et al.* (1983) investigated 40 women in Israel, and Scott *et al.* (1976) studied 46 women from Iowa with eclampsia and pre-eclampsia and both studies failed to show such a relation. It is difficult to understand why English pre-eclamptic patients should tend towards homozygosity at the A and more particularly at the B locus yet Africans with eclampsia are more likely to be heterozygous. An immunogenetic explanation seems unlikely. It is true, however, that some disorders are associated with an HLA type only in certain races, e.g. HLA-B54 is associated with juvenile diabetes mellitus in Japanese, but not in Caucasians. No condition yet described is associated with such marked polarization as demonstrated here. If the explanation of such conflicting results is not within the different racial study groups then it may be with the disease. We have presumed that pre-eclamptics become eclamptics and therefore the two groups are comparable. However, patients presenting with acute eclampsia may have a different genotype compared with those presenting with proteinuric hypertension in pregnancy. Finally, it must be noted that 30% of our control population are

Table 2. Frequency of human leucocyte antigen (HLA) homozygosity in patients with eclampsia and in normal controls

HLA locus	Eclamptic patients		Normal controls	
	n	(%)	*n*	(%)
A	19/53	(35·8)	374/1416	(26·4)
B	7/53	(13·2)	435/1416	(30·7)*
DR	22/53	(41·5)	223/412	(54·1)

Significance of difference between the two groups. * $P < 0{\cdot}01$, $\chi^2 = 7{\cdot}4$.

homozygous. This remarkably high figure may be a reflection of African tribal society and the immobility of its members due to political and economic constraints. Such nuclear communities are not a feature of Oxfordshire, Iowa or France.

The history of HLA associations with certain diseases has been a major breakthrough in our understanding of the genetics of many diseases. The exciting work by Redman *et al.* (1978) associating pre-eclampsia with homozygosity at the B locus promoted many ideas and strengthened the immunogenetic interest in the subject. It is not clear why in African women we found a significant association between heterozygosity and eclampsia, just the opposite of what we expected. If all the available literature is gathered it conflicts and when it is summated no trend emerges. Eclampsia is either independent of HLA status or the association is so complicated it defies present comprehension.

References

Beer, A. E. & Need, J. A. (1985) Immunological aspects of pre-eclampsia/eclampsia. *Birth Defects* **21 (5),** 131-154.

Haldane, J. B. S. (1956) The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* **20,** 309-311.

Persitz, E., Oksenberg, J., Amar, A., Margalioth, E. J., Cohen, O. & Brautbar, C. (1983) Histocompatibility antigens, mixed lymphocyte reactivity and severe pre-eclampsia in Israel. *Gynecol Obstet Invest* **16,** 283-291.

Redman, C. W. G., Bodmer, J. G., Bodmer, W. F., Beilin, L. J. & Bonnar, J. (1978) HLA antigens in severe pre-eclampsia. *Lancet* **ii,** 397-399.

Scott, J. R., Beer, A. E. & Stansty, P. (1976) Immunogenetic factors in preeclampsia and eclampsia; erythrocyte, histocompatibility and Y-dependent antigens. *JAMA* **235,** 402-404.

Simon, P., Fauchet, R., Menault, M. *et al.* (1980) HLA A, B and DR antigens in pre-eclampsia: preliminary results. *Kidney Int* **17,** 705-706.

Stevenson, A. C., Say, R., Ustaoglu, S. & Durmus, A. (1976) Aspects of pre-eclamptic toxaemia of pregnancy, consanguinity and twinning in Ankara. *J Med Genet* **13,** 1-8.

Terasaki, P. I. & McClelland, J. D. (1964) Microdroplet assay of human serum cytotoxins. *Nature (Lond)* **204,** 998-1000.

Woolf, B. (1955) On estimating the relation between blood group and disease. *Ann Hum Genet* **19,** 251-253.

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HLA STATUS OF THE FETUS BORN TO AFRICAN WOMEN WITH ECLAMPSIA

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ABSTRACT

The HLA status of 37 babies who were born to African mothers suffering from eclampsia was determined. The B35 antigen was more prevalent in babies born to eclamptic mothers than one would expect ($p=0 \cdot 01$). The number of shared antigens between mother and baby at the A and B locus was similar to that of a normal population.

INTRODUCTION

There is no published evidence to support the hypothesis that eclampsia has an association with any maternal human leucocyte antigen (HLA). Combining the literature [2,12-14,16-19] a total of 330 pre-eclamptic or eclamptic mothers have been studied and no HLA association with the disease has emerged. Two hundred and seventeen fathers have

been studied and they are indistinguishable from the normal population. However there are no studies of babies born to eclamptic mothers and there are only two papers reporting on the infant delivered to pre-eclamptic mothers. Scott et al [18] measured the HLA status at the A locus in 10 babies born to pre-eclamptic mothers and no trend emerged. Kilpatrick et al [14] studied 41 cases of mild and severe pre-eclampsia and suggested an association with the fetal DR4 antigen.

Cooper et al [8] recently examined the inheritability of eclampsia and concluded that the fetal genotype influences the susceptibility to the disease. He presumes that these genes are the human leucocyte antigen (HLA) genes or are linked to the HLA system and this prompted us to analyse our data regarding the HLA status of African babies born to eclamptic mothers.

PATIENTS AND METHODS

Thirty seven black African females of Zulu origin who presented with a diagnosis of eclampsia or severe pre-eclampsia were studied over a one year period. Only mothers with all the classic features of the disease were included (systolic blood pressure greater than 160 mm of mercury, a diastolic pressure greater than 115 mm of mercury, gross oedema and at least 2 pluses of proteinuria measured on

a catheter specimen of urine with Ames sticks). Thirty mothers had already suffered at least one seizure before admission, the remaining seven all had irritability or intellectual clouding and were hyperreflexic with clonus. Mothers with previous hypertension (booking blood pressure $>^{140}/_{80}$) or a past history of renal disease or those who had not fully recovered by the seventh post-partum day to an aproteinuric normotensive state were excluded. As far as possible, samples were collected consecutively but 11 cases were lost, either because insufficient blood was obtained (n=4) or because of administrative reasons (mothers delivering late on Friday night or on Saturday; n=7).

The HLA, A, B and DR antigens were determined by a 2 stage lymphocytotoxicity test [21] on 20 ml of cord blood obtained at the time of delivery. In 3 cases of stillbirth, cord blood was insufficient and blood was taken by cardiac puncture from the stillborn infant. We were unable to measure the paternal HLA antigens.

The prevalence of the A and B locus on the HLA system in the normal population was determined by analysing 1416 blood donors of the same tribe and resident within the same hospital catchment area. Four hundred and twelve of these also acted as controls for the DR locus. If only 1 antigen per locus could be detected, patients were considered to be homozygous at that locus.

Statistical analysis was performed by Chi squared testing. As this the first study of its kind, we had no preconceived hypothesis to test and therefore multiple comparisons were performed and the p value was corrected by the Bonferoni inequality method [10].

RESULTS

The frequency of the HLA antigens in the normal population and in the fetus born to eclamptic mothers is shown in table I. The B35 and the B14 antigen were more prevalent in babies born to sufferers from eclampsia than one would expect (B35 frequency = 22% compared with 6.7% in the controls - Chi squared = 12.2, p corrected for multiple comparisons of the mean = 0·01; B14 frequency = 16% compared with 7.5% in the control population - Chi squared = 7.1; p = 0·2 when corrected for multiple comparisons of the mean). The B8 antigen frequency was lower in babies born to eclamptics but this probably occures by chance (Chi squared = 5.6, p corrected for multiple comparisons of the mean = 0·5). When compared with a control group babies born to eclamptic mothers showed no variation in the number of antigens at any locus and no specific A or D related antigen recurred more commonly. The sample includes 3 neonatal deaths and 3 fresh stillbirths.

DISCUSSION

The evidence that there is an immunogenetic aetiology to eclampsia is overwhelming [6,15]. However there is also some evidence that there is a direct genetic component to the aetiology of the disease. Eclampsia has a familial trait [3,4,7], is commoner if the subject's parents are from different racial stock [1] and there is some protection from the disease if the mother is the product of a consanguineous mating [20].

This genetic link has been investigated by examining the maternal histocompatability complex. Jenkins et al [12] suggested that pre-eclampsia may be more common if the mother and father possess the same HLA antigens and Redman et al [17] did suggest an excess of homozygosity at the B locus but this has not been confirmed [13].

Examination of family trees involving cases of eclampsia suggest that the inherited susceptibility of the disease may be linked to the fetus rather than the mother [8].

Our finding that the frequency of B35 antigen is significantly increased in the fetus born to an eclamptic mother (22% compared with 5.7%) adds further support to the suggestion that the disease may be influenced by the genetics of the conceptus. As Coovadia et al [9] has demonstrated that

TABLE I Distribution of human lymphocyte antigens

LOCUS A ANTIGEN	FETUS n = 37	CONTROL 1416
1	2.7%	6.4%
2	24%	21%
3	13%	13%
11	0%	0.1%
23	22%	18%
24	5.4%	4.9%
25	16%	14%
26	22%	10%
28	16%	21%
29	14%	17%
30	22%	37%
31	2.7%	6.0%
32	2.7%	2.3%
33	2.7%	2.2%
homozygous	35%	26%

LOCUS B ANTIGEN	FETUS n =37	CONTROL 1416
5	0%	1%
7	14%	20%
8	0%	13%
13	8.1%	4%
14	16%	6%
15	2.7%	4%
16	0%	3%
17	30%	39%
18	14%	5%
21	0%	2%
22	0%	0.07%
27	0%	0.3%
35	22%	6.7%
37	0%	0.07%
40	0%	0.6%
41	0%	1.5%
42	14%	24%
44	19%	15%
45	5.4%	8.6%
47	0%	0.1%
53	0%	1.6%
70	24%	14%
homozygous	32%	30%

TABLE I (continued)

LOCUS D ANTIGEN	FETUS n=20	CONTROL n=412
1	10%	5%
2	35%	24%
3	35%	36%
4	10%	10%
5	35%	35%
6	15%	15%
7	20%	15%
8	10	2.9%
9	0%	0.7%
10	0%	2.2%
homozygous	30%	5%

neonates of Zulu decent are indistinguishable from the normal blood donating members of the same society, our study and control groups are likely to be representative of the fetus born to eclamptic and non eclamptic mothers respectively. Therefore our finding invites speculation on a possible mechanism that would explain the disease association.

Because of linkage disequilibrium (HLA genes associated with other specific genes) any association between any HLA antigen and a disease means that either (i) the allele responsible for the expression for the HLA antigen is directly involved in the pathogenesis of eclampsia, or (ii) the HLA gene is associated with a second gene which is responsible for determining eclampsia susceptibility.

If the HLA gene was pathogenic it would have to act as either a receptor for a noxious or infective agent, act as a

carrier for a carrier-hapten complex or thirdly, act as a antigen that is familiar to the mother thus inducing an autoimmune response [5]. This explanation seems unlikely for the following reasons;

1) The trophoblast that is exposed to the mother is free of transplant antigens.

2) If pre-eclampsia depended upon a single fatal gene expressed on the fetal side the influence of parity would be difficult to explain.

3) It would be difficult to understand why exposing the mother to a blood transfusion or to her partners leucocytes decreases the incidence of the disease rather than increasing it [11]. Thus our observed association is more likely to be due to the involvement of genes closely linked with the HLA complex. In other words the HLA genes are probably not involved with the causation of the disease but they are neutral markers of it. As the immune response genes and the HLA genes are spatially intimately related on the short arm of chromosome 6 and a functional link is known to exist in animals [22] it is plausible that there is an immune response gene associated with both the B35 antigen and eclampsia susceptibility.

The presence of the disease in only a small fraction of those carrying the antigen may be explained by the following.

1) The association is due to a second as yet undiscovered allele located at a different but closely linked locus and

this gene occurs with a different frequency than the B35 antigen.

2) Eclampsia is influenced by environmental factors and the fetal genotype cannot be expected to influence more that the susceptibility to the disease.

3) Other genetic factors not linked to the B35 antigen may contribute to the disease susceptibility.

4) Eclampsia is almost certainly a disease with a multifactorial aetiology and therefore the observed association may only be apparent in a subset of the population.

Unfortunately our current knowledge of immunology and eclampsia permits little more than speculation. However it is possible that the fetal genotype may possess the genetic make up to influence pathology in the mother.

REFERENCES

1. Alderman B W, Sperling R S, Daling J R. An epidemiological study of the immunogenetic aetiology of Pre-eclampsia. Br Med J 292:372-374 1986

2. Anichkova, S I, Vasilieva Z F, Grigorieva V V. Study of compatibility in married couples according to the HLA antigen system in normal and complicated by late toxaemia pregnancy. Anush Ginekol 8; 20-23 1981

3. Anonymous Genetic control of pre-eclampsia. (Editorial) Lancet (i): 634-635 1980

4. Anonymous. Genetics of pre-eclampsia. (Editorial) Lancet (ii): 778 1988

5. Batchelor J R and McMichael A J, Progress in understanding HLA. Br Med Bull 43 (1); 156-183 1987

6. Beer A E and Need J A. Immunological aspects of pre-eclampsia/eclampsia. Birth Defects. 21(5), 131-154, 1985

7. Chesley L C and Cooper D W. Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. Br J Obstet Gynaecol 93:898-908 1986

8. Cooper D W, Hill J A, Chesley L C Bryans, C I. Genetics control of susceptibility to eclampsia and miscarriage. Br J Obstet Gynaecol 95:644-653 1988

9. Coovadia H M, Wesley A, Hammond M G, Kiepiela P, Measles, histocompatibility leucocyte polymorphism and natural selection in humans. J Infect Dis 144 (2): 142-147 1981

10. Dunn O J. Multiple comparisons amongst means. Am J Stat Assoc 56:52-64 1961

11. Feeney J, Tovey L L, Scott J, Influence of previous blood transfusion on the incidence of pre-eclampsia. Lancet (i) 874-875 1977

12. Jenkins D M, Need J A, Scott J S, Morris H, Pepper M. Human leucocyte antigens and mixed lymphocyte reaction in severe pre-eclampsia. B Med J 1: 592-544 1978

13. Johnson N, Moodley J, Hammond M G. Human leucocyte antigens status in
African women with eclampsia. Br J Obstet Gynaecol 95; 877-879 1988

14. Kilpatrick D C, Liston W A, Jazwinska, E C, Smart G E. Histocompatibility studies in pre-eclampsia. Tissue Antigens 29; 232-236 1987.

15. Kitzmiller J L. Immunological approaches to the subject of pre-eclampsia. Clin Obstet Gynecol 30 717-735 1977

16. Persitz E, Oksenberg J, Amar A, Margalioth E J, Cohen O, Brautbar C. Histocompatibility antigens, mixed lymphocyte reactivity and severe pre-eclampsia in Israel. Gynecol Obstet Invest 16:283-291 1983

17. Redman C W G, Bodmer J G, Boomer W F, Beilin L J, Bonnar J. HLA antigens in severe pre-eclampsia. Lancet (ii) 397-399 1978

18. Scott J R, Beer A E, Stastny P. Immunogenetic factors in pre-eclampsia and eclampsia; Erythrocyte, histocompatibility and Y dependent antigens. JAMA 235(4):402-404 1976

19. Simon P, Fauchet R, Menault M, Bombail D, Le Fiblec B, Panguy E et al HLA A, B, and DR antigens in pre-eclampsia: Preliminary results. Kidney Int 17: 705-706 1980

20. Stevenson A C, Ustuoplus S, Durmas Z, Aspects of pre-eclamptic toxaemia of pregnancy, consanguinity twinning in Ankara. J Med Genet 13; 1-8 1976

21. Terasaki P I and McClelland J D. Microdroplet assay of human serum cytotoxins. Nature (Lond), 204;998-1000 1964

22. Tiwari J L and Terasaki P I. HLA and disease associations. Pub Springer-Verlag, New York. (1985) p30.

HLA AND SELECTIVE MATING

Michael G Hammond[1]

ABSTRACT

The selection of a mate by female semi-wild mice is influenced by the major histocompatibility complex (MHC)[1]. The role of the MHC in human mate selection is investigated by analysing the distribution of human leucocyte antigens (HLA) in couples. The frequency of sharing of HLA antigens showed no significant differences from that expected by chance but there are significant differences in the frequencies of some of the HLA B locus antigens in males selected by females with specific HLA antigens.

ABBREVIATIONS

MHC major histocompatibility complex
BSR basal sharing rate

INTRODUCTION

Potts et al.[1] showed that female semi-wild mice selected males that were disparate for MHC. The lack of homozygous progeny can be explained if the female avoids mating with males that possess the same MHC antigens. Does this selection process occur in humans?

METHODS

The data base I chose for analysis consisted of 837 couples who were typed for HLA to determine paternity of their offspring. There were a further 27 males from paternity investigations involving two men. All subjects were Caucasian.

The number of couples who shared HLA antigens at the A, B or C locus was counted. The probability of two random people sharing HLA antigens was calculated using the formulae of Koyama et al.[2] and defined as the basal sharing rate (BSR). The BSR was calculated using the gene frequencies in males, females and in the combined frequencies. The BSR was also calculated using the gene frequencies in 643 blood donors as a control population. The significance of the differences were determined with the chi-

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squared test. Couples where the man was shown not to be the father were then removed from the data base and the calculations repeated.

Another data base was built up consisting of couples drawn from family studies performed for transplantation. Ninety one couples were analysed in the same way. The results are shown in Table 1.

RESULTS AND DISCUSSION

If selection by females is on the basis of avoiding males who possess the same MHC antigens, then the degree of sharing would be lower than that expected by chance. There were no significant differences in the sharing rate when comparing all possible pairwise combinations at each locus using the chi-square test. No significant differences were observed in couples where there was no exclusion. The sharing rate in family couples was slightly higher but not significantly so. If selection based on HLA was present, then two men selected by the same woman would be expected to have some HLA antigens in common. The sharing rate of 27 male pairs in this category was not significantly different.

However, this does not rule out the possibility that the MHC genes are involved in mate selection in human populations. Perhaps the possession of specific antigens forms the basis of selection? This was investigated by frequency analysis of the males chosen by females possessing specific HLA alleles. Those antigens which were significantly increased ($p<0.01$) in frequency in males are shown in Figure 1. If the increase was highly significant ($p<0.001$), the frequency is shown in a box. The matrix has 44 x 44 entries so that about 20 entries would be expected to be increased by chance and about two entries with $p<0.001$ but there are 38 frequencies that are significantly increased and ten of these have a probability less than 0.001. This indicates that at least some of the HLA antigens may be involved in mate selection.

Most of the increases are in the lower right quadrant of the matrix so that the HLA B locus antigens appear to be more influential. Linkage disequilibrium may account for significant increases at adjoining loci; e.g. the significant increase in the frequency of HLA B27 probably accounts for the increased frequency of Cw2. There are three entries on the diagonal. Do females with HLA A2, HLA B37 and HLA B51 prefer males with the same antigen? This contradicts the overall finding that there was no increase in shared antigens and may be due to chance. An analysis of frequencies that were significantly decreased produced a matrix (Figure 2) with only ten entries which is lower than would be expected. None of the decreases were significant at $p<0.001$.

These findings suggest that in humans, females do not appear to select males on the basis of avoiding males with the same antigens as themselves or by avoiding males with a specific antigen but rather on the basis of selecting males with specific antigens, dependent on their own antigenic phenotype. The HLA B locus appears to be more important than HLA A or HLA C but another independent survey is needed to determine which of the more than 30 antigens at this locus are important for mate selection. It may also be that, as in the many disease associations that have been reported, the Class II antigens (HLA DR, DQ, DP) have a more important role.

References

1 Potts, W. K., Manning, C. J. & Wakeland, E. K. *Nature* **352**, 619-621 (1991).

2 Koyama, M., Saji, F., Takahashi, M., Samejima, Y., Kameds, T., Kimura, T. & Tanizawa, O. *Tissue Antigens* **37**, 211-217 (1991).

Legends

Figure 1 Matrix showing percentage frequencies of HLA antigens that are significantly increased ($p < 0.01$) in males selected by females with the HLA antigen shown at the top of the matrix. Frequencies in boxes are significantly increased with $p < 0.001$. Significance was determined by the chi-squared test.

Figure 2 Matrix showing percentage frequencies of HLA antigens that are significantly decreased ($p < 0.01$) in males selected by females with the HLA antigen shown at the top of the matrix. There were no frequencies significantly decreased with $p < 0.001$. Significance was determined by the chi-squared test.

Table 1 Observed and expected sharing of HLA antigens in percent.

	HLA LOCUS		
	A	B	C
Number of antigens tested	14	23	7
Observed in 864 couples	46.5	21.9	11.9
Observed in 653 couples with no exclusion	45.8	20.7	12.1
Observed in 27 male pairs	37.0	25.9	7.4
Observed in 91 families	47.3	30.8	6.6
BSR 864 males	43.2	22.8	10.9
BSR 837 females	43.6	25.3	10.9
BSR 1701 both	43.3	23.9	10.8
BSR 643 controls	40.3	27.1	10.5

*BSR Basal Sharing Rate calculated by the method of Koyama et al.[2]

F E M A L E A N T I G E N

CONTROL %	MALE	A1	A3	A11	A2	A28	A23	A24	A25	A26	A29	A30	A31	A32	A33	Cw1	Cw2	Cw3	Cw4	Cw5	Cw6	Cw7	B7	B22	B27	B8	B14	B16	B18	B37	B44	B45	B21	B51	B52	B35	B62	B63	B70	B57	B58	B60	B61	B41	B13	MALE	CONT %
25.97	A1																																													A1	25.97
27.68	A3																																													A3	27.68
11.35	A11																																													A11	11.35
44.95	A2																																													A2	44.95
10.11	A28																																													A28	10.11
3.27	A23																																													A23	3.27
19.44	A24																																													A24	19.44
2.95	A25																																													A25	2.95
6.84	A26																																													A26	6.84
7.93	A29																																													A29	7.93
3.89	A30																																													A30	3.89
3.42	A31																																													A31	3.42
4.67	A32																																													A32	4.67
2.95	A33																																													A33	2.95
5.29	Cw1																																													Cw1	5.29
7.62	Cw2																																													Cw2	7.62
20.22	Cw3																																													Cw3	20.22
17.11	Cw4																		8																	5		7										Cw4	17.11
8.86	Cw5																						3								3																	Cw5	8.86
4.04	Cw6				1																																									Cw6	4.04
13.53	Cw7																																													Cw7	13.53
29.39	B7																									18																				B7	29.39
3.42	B22																																													B22	3.42
7.47	B27																																													B27	7.47
21.93	B8																											7																		B8	21.93
8.40	B14			1																																										B14	8.40
6.84	B16																																													B16	6.84
6.69	B18																																													B18	6.69
2.18	B37																																													B37	2.18
25.66	B44																																													B44	25.66
2.33	B45																																													B45	2.33
2.95	B21																																													B21	2.95
6.69	B51																																													B51	6.69
2.64	B52																																													B52	2.64
16.17	B35																																	5												B35	16.17
10.73	B62																																													B62	10.73
2.33	B63																																													B63	2.33
0.93	B70																																													B70	0.93
4.82	B57																																													B57	4.82
4.04	B58																																													B58	4.04
7.93	B60																																													B60	7.93
2.95	B61																																													B61	2.95
0.78	B41																																													B41	0.78
4.35	B13																																													B13	4.35

F E M A L E A N T I G E N

CONTROL %	MALE	A1	A3	A11	A2	A28	A23	A24	A25	A26	A29	A30	A31	A32	A33
25.97	A1											43			
27.68	A3														
11.35	A11														
44.95	A2				53										
10.11	A28														
3.27	A23														
19.44	A24														
2.95	A25														
6.84	A26														
7.93	A29														
3.89	A30														
3.42	A31														
4.67	A32														
2.95	A33														
5.29	Cw1														
7.62	Cw2								24						
20.22	Cw3										36				
17.11	Cw4														
8.86	Cw5														
4.04	Cw6														
13.53	Cw7														
29.39	B7														
3.42	B22														
7.47	B27								**31**						
21.93	B8														
8.40	B14														
6.84	B16														
6.69	B18								21						
2.18	B37														
25.66	B44														
2.33	B45														
2.95	B21														
6.69	B51			14		15				16					
2.64	B52						11								
16.17	B35														
10.73	B62														
2.33	B63														
0.93	B70														
4.82	B57														
4.04	B58														
7.93	B60														
2.95	B61														
0.78	B41											8	7		
4.35	B13	9				10									

CONTROL %	MALE	Cw1	Cw2	Cw3	Cw4	Cw5	Cw6	Cw7
25.97	A1							
27.68	A3							
11.35	A11							
44.95	A2							
10.11	A28							
3.27	A23							
19.44	A24							
2.95	A25							
6.84	A26	19						
7.93	A29							
3.89	A30							
3.42	A31							
4.67	A32							
2.95	A33							
5.29	Cw1						**30**	
7.62	Cw2							
20.22	Cw3							
17.11	Cw4							
8.86	Cw5							
4.04	Cw6							
13.53	Cw7							
29.39	B7							
3.42	B22							
7.47	B27							
21.93	B8							
8.40	B14							
6.84	B16							
6.69	B18							
2.18	B37							
25.66	B44							
2.33	B45							
2.95	B21							
6.69	B51			13				
2.64	B52							
16.17	B35							
10.73	B62	**30**						
2.33	B63							
0.93	B70		6					
4.82	B57							
4.04	B58							
7.93	B60							
2.95	B61							
0.78	B41						**20**	
4.35	B13							

MALE	B7	B22	B27	B8	B14	B16	B18	B37	B44	B45	B21	B51	B52	B35	B62	B63	B70	B57	B58	B60	B61	B41	B13	MALE	CONT %
A1																								A1	25.97
A3																								A3	27.68
A11																								A11	11.35
A2																								A2	44.95
A28																								A28	10.11
A23																								A23	3.27
A24																								A24	19.44
A25																								A25	2.95
A26																								A26	6.84
A29																								A29	7.93
A30																								A30	3.89
A31																								A31	3.42
A32						13																		A32	4.67
A33																								A33	2.95
Cw1																								Cw1	5.29
Cw2					17																			Cw2	7.62
Cw3																								Cw3	20.22
Cw4																						44		Cw4	17.11
Cw5																								Cw5	8.86
Cw6																								Cw6	4.04
Cw7																								Cw7	13.53
B7											57													B7	29.39
B22																								B22	3.42
B27																								B27	7.47
B8																								B8	21.93
B14																								B14	8.40
B16																								B16	6.84
B18																								B18	6.69
B37								**22**																B37	2.18
B44																			45					B44	25.66
B45																								B45	2.33
B21			**11**																					B21	2.95
B51												**19**												B51	6.69
B52																								B52	2.64
B35																								B35	16.17
B62			21		22																			B62	10.73
B63																								B63	2.33
B70								11																B70	0.93
B57																								B57	4.82
B58																								B58	4.04
B60											24			16	16									B60	7.93
B61																								B61	2.95
B41											**10**						**13**							B41	0.78
B13													**27**					13						B13	4.35

HLA AND OTHER DISEASES

p465 Maharaj B and Hammond MG. **HLA-A, B, DR and DQ Antigens in Black patients with idiopathic dilated cardiomyopathy.** *Amer J Cardiology* 65; 1402. 1990

p467 O'Farrell N and Hammond MG. **HLA antigens in Donovanosis (Granuloma Inguinale).** *Genitourin Med*; 67:400-402 1991

p470 Bhigjee AI, Bill PLA, Hammond MG and Windsor IM. **HLA Profile and HTLV-I Associated Myelopathy (HAM/TSP) in Natal, South Africa.** *J Neurology, Neurosurgery and Psychiatry*; 55:329-330 1992

p472 Hammond MG. **Paternity calculations in court.** (submitted to *Forensic Science International*).

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SHORT COMMUNICATION

HLA and Duodenal Ulcer in South African Indians

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Clearly established genetic factors associated with duodenal ulcer are blood group O and non-secretor status. In a search for further genetic factors Rotter et al. (1979) determined the HLA antigens of 77 patients with duodenal ulcer and found a significant increase in the frequency of HLA–B5 in the 54 Caucasian patients. We therefore decided to test Indians with duodenal ulcer because the frequency of B5 in the Indian population is relatively high (34%) and a survey of duodenal ulcer in Indians by Robbs & Moshal (1979) has shown that Durban may be regarded as an area of high prevalence and that this disease is a major problem in the Indian population.

A total of 180 antisera were used in a two-stage microlymphocytotoxicity test to determine the HLA antigens of 94 Indians with duodenal ulcer (confirmed by endoscopy).

The antigen B5 IND was assigned to those B5 cells that are not Bw51 or Bw52 and probably includes the recently described antigen Bu (Laundy et al. 1978).

The antigen frequencies are listed in Table 1. The distribution of antigens at the A and B loci conformed to Hardy-Weinberg equilibrium. In contrast to the findings of Rotter et al. (1979), the frequency of B5 was decreased and the frequency of Bw51 was small enough to give an uncorrected P value of less than 0.01. The frequency of Bw52 was approximately the same in the patients and the controls.

There was an increased frequency of B40.2 (uncorrected $P < 0.01$) and a decrease in the frequency of B40.1. None of these differences retained their significance after correcting for the number of antigens tested. The splitting of B5 and B40 into subdivisions still poses problems which may be solved in the future by better sera and International Workshops.

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Table 1
Percentage frequency of HLA antigens in Indians with duodenal ulcer (DU)

HLA	Control 632	DU 94	HLA	Control 632	DU 94
A1	27.9	30.9	B7	12.5	14.9
A2	31.3	29.8	B8	5.9	4.3
A3	14.6	11.7	B13	6.8	2.1
A11	27.5	31.9	B14	0.3	1.1
Aw23	0.6	0	B15	10.9	11.7
Aw24	27.1	31.9	B16	2.2	3.2
A25	1.9	2.1	B17	21.2	22.3
A26	6.3	5.3	B18	3.0	1.1
A28	14.4	5.3	Bw21	1.7	2.1
A29	0.8	2.1	Bw22	2.5	4.3
Aw30	4.0	3.2	B27	2.5	7.4
Aw31	3.5	2.1	Bw35	20.6	19.1
Aw32	2.5	0	B37	4.1	7.4
Aw33	7.4	12.8	B40	29.1	35.1
1 Antigen	30.2	30.9	B40.1	13.4	6.4
			B40.2	15.7	28.7*
			Bw42	0	0
			Bw44	12.0	10.6
			Bw45	0.2	1.1
			B5	34.2	25.5
			Bw51	22.5	10.6*
			Bw52	8.4	10.6
			Bw53	1.9	1.1
			B5 IND	3.3	4.3
			1 Antigen	28.3	25.5

* P (uncorrected) < 0.01.

Details of ethnic subgroup and phenotype of each patient have been submitted to the HLA and Disease Registry.

References

Laundy, G. J., Entwistle, C. C. & Hassenkamp, K. (1978) Bu – A new antigen at the HLA–B locus. *Tissue Antigens* **11**, 121.

Robbs, J. V. & Moshal, M. G. (1979) Duodenal ulceration in Indians and Blacks in Durban. *S. Afr. Med. J.* 55, 39–42.

Rotter, J. I., Rimoin, D. L. Gursky, J. M. Terasaki, P. I. & Sturdevant, R. A. L. (1979) HLA B5 associated with duodenal ulcer. *Gastroenterology* 73, 438–440.

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MEASLES, HISTOCOMPATIBILITY LEUKOCYTE ANTIGEN POLYMORPHISM, AND NATURAL SELECTION IN HUMANS

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Measles, Histocompatibility Leukocyte Antigen Polymorphism, and Natural Selection in Humans

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Profound lymphocytopenia (<2,000 lymphocytes/mm³) occurring within two days of rash in 69 South African black children with measles predicted either death or progression to chronic lung disease in 51 (77%) of 66 children who were followed for at least six weeks. Lymphocytopenia was significantly associated with the presence of histocompatibility leukocyte antigen (HLA) AW32 ($P = 0.01$), with a relative risk of 5.5. There was a trend toward an association between the presence of particular antigens in the HLA complex and the various indices of humoral and cellular immunity studied. These findings are discussed in terms of variation in the clinical spectrum of the disease and in relation to the evolution of HLA polymorphism.

The consequences of infection with measles virus are recovery, chronicity, or death. This clinical spectrum is determined by the severity of immunoparesis at the onset of illness [1]. One component of this immunoparesis is the specific antibody response, which, if impaired, leads to severe disease if cell-mediated immunity is also impaired [2]. The ability of the human host to produce antibody to measles virus is controlled by genes linked to the histocompatibility leukocyte antigen (HLA) complex [3–5], but there is little evidence to suggest that clinical outcome is under a similar influence [5]. In both experimental animals [6, 7] and in humans [8–10], genes linked to the major histocompatibility complex control immune responsiveness and can therefore modify the course and outcome of infectious illnesses [11–13].

It is believed that the extreme polymorphism of the HLA system has arisen during evolution through the process of natural selection [14]. Infectious diseases, which have taken a massive toll of human life throughout history, may have exerted selective pressure on antigens of the HLA complex. Several investigators have studied the association between particular antigens of the HLA complex and infections [12, 15], but few [10, 16] have linked the HLA complex with the clinical features or immune responses of a disease that has been and still is a major human killer. Even in the absence of protein-calorie malnutrition, measles can be a severe disease in children in developing countries [17]. We report a study of the HLA system in relation to both clinical severity and immune responsiveness in children with measles.

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Subjects and Methods

HLA frequencies were determined in 69 South African black children with severe measles.

Nutritional status. The nutritional status of the patients studied was satisfactory. All patients were between the 10th and 75th Harvard percentiles for weight [18], with serum albumin levels of >30 g/liter and without any of the clinical features of protein-calorie malnutrition.

Age and sex distribution. The median age of the 33 female children was 12 months (range, 25 months); that of the 36 male children was about 16 months (range, 54 months).

Definition of severe measles. Severe measles was defined by the presence of a count in peripheral blood of <2,000 lymphocytes/mm³ within two days of the appearance of rash. This degree and timing of lymphocytopenia have been shown to be indicative of subsequent death or progression to chronic chest disease in 77% of patients [17].

Outcome of measles. The clinical outcome was assessed six weeks after the onset of rash, when patients were classified as having recovered,

died, or developed chronic chest disease. Recovery or chronicity was determined by the presence or absence of pneumonia, respectively, detected radiologically [17]. Bronchopulmonary changes were graded on a total of 11 points; an abnormal score was ≥4. Children with an abnormal score were classified as having chronic chest disease, and those with a score of <4 were classified as recovered. Chronic chest disease or death was a poor outcome, and recovery was a good outcome.

Control subjects. Because the attack rate for measles is almost 100%, the frequencies of particular HLA types among healthy adult black Africans, many of whom were studied for international workshops [19, 20], were used as the normal distribution. The African population of Durban, South Africa, consists mainly of Zulus, and the patients and control subjects were of pure descent. The possibility that a comparison of HLA frequencies in infants with HLA frequencies in adults may not be valid was investigated by comparing frequencies in normal infants (never infected with measles virus). Cord blood specimens from 51 black neonates and sera from 32 black infants younger than five years of age who were subjects of paternity disputes were tested for HLA types.

Immunologic tests. The results of immunologic tests (table 1) performed within 48 hr of the onset of measles rash were used for correlations with HLA typing. The criteria are based on previous observations of lymphocyte subpopulations [17] and are arbitrary cutoff points for titers of CF and HAI antibodies, inhibition index (see below), and levels of C3. Immune functions were categorized as good or poor.

HLA typing. The patients were typed for HLA-A, -B, and -C specificities using 180 antisera in a two-stage lymphocytotoxicity test [21]. Lymphocytes were isolated on a Ficoll-Hypaque density gradient. Differences in HLA frequencies were tested for significance with a χ^2 test (without Yates's correction), and the resulting probabilities were corrected by multiplying by the number of antigens tested.

Lymphocyte subpopulations. Mononuclear cells were obtained from defibrinated peripheral blood that had been passed through columns of Ficoll-Hypaque. Lymphocyte subpopulations were counted in a single preparation by means of sheep erythrocytes and by an immunofluorescence method for detecting immunoglobulins [22]. Peripheral lymphocytes were classified as rosetting cells (T), fluorescing cells (B), cells with no markers (null), and those with both markers.

Table 1. Criteria used for categorizing immune functions in African children with severe measles within 48 hr of the onset of rash.

Function	Response	
	Good	Poor
CF antibody titer	≥1:8	<1:8
HAI antibody titer	>1:8	≤1:8
Inhibition index*	Positive	Negative
T cells (cells/mm³)	≥1,268	<1,268
B cells (cells/mm³)	≥556	<556
Null cells (cells/mm³)†	≥116	<116
C3 (mg/100 ml)	≥70	<70

NOTE. The criteria are based on previous observations of lymphocyte subpopulations [17] and are arbitrary cutoff points for the other functions.

* See Subjects and Methods.

† Cells lacking surface markers of B or T cells.

Antibodies to measles virus. Titers of CF antibody to measles virus were measured in sera by a microtiter method using specific antigen. Titers of measles-specific antibody were also measured by the HAI test with antigen from Behringwerke (Marburg, Federal Republic of Germany).

Inhibition of leukocyte migration. Leukocytes obtained by dextran sedimentation of whole blood that had been treated with heparin were incubated for 24 hr in agarose petri dishes in the presence or absence of measles virus CF antigen. The degree of migration was measured by projection. The percentage migration inhibition was calculated as follows: (the extent of migration with antigen/the extent of migration without antigen) × 100. The percentage inhibition index was calculated as: 100% − the percentage migration inhibition.

C3. Levels of C3 in plasma were measured by radial immunodiffusion.

Results

The clinical outcome at six weeks could be assessed in 66 of the 69 children with severe measles. Five children died, the illness progressed to chronic chest disease in 46 children, and 15 children recovered.

There were no significant differences in the frequencies of HLA types among the 51 neonates, the 32 subjects of paternity disputes, and the 1,081

Table 2. Percentage frequency of individual antigens of the histocompatibility leukocyte antigen (HLA) complex in South African black children with severe measles and in control subjects.

HLA type	Controls (n = 1,132)	Patients (n = 69)
A1	6.4	11.6
A2	21.3	10.1
A3	13.1	7.2
A11	0.1	1.4
AW23	18.4	14.5
AW24	3.8	4.3
A25	15.3	20.3
A26	8.8	11.6
A28	21.1	26.1
A29	16.2	20.3
AW30	37.5	26.1
AW31	9.3	7.2
AW32	2.0*	10.1*
AW33	2.7†	4.3
One antigen‡	24.0	24.6
B7	18.2	21.7
B8	14.1	10.1
B13	4.4	1.4
B14	5.7	1.4
B15	4.9	1.4
BW16	2.4	4.3
B17	38.7	44.9
B18	4.9	5.8
BW21	1.1	1.4
BW22	0	0
B27	0.3	0
BW35	6.3	7.2
B37	0	0
BW41	2.1†	0
BW42	24.7	15.9
BW44	15.7	15.9
BW45	7.6	8.7
BW46	0	0
BW51	1.8	4.3
BW52	0	0
BW53	3.4	1.4
BW60	1.0	2.9
BW61	0	0
One antigen‡	42.7	50.7

NOTE. Severe measles was defined by the presence of a count in peripheral blood of <2,000 lymphocytes/mm³ within two days of the onset of rash. The control group comprised 1,081 randomly chosen adults and 51 neonates.

* $P < 0.016$ (corrected for the number of antigens tested).

† Of 146 controls.

‡ Only one antigen detected at the A or B locus.

randomly chosen adults. The AW32 antigen was found in two (3.9%) neonates and one (3.1%) of the infants younger than five years of age—three (3.6%) of the combined group. Nineteen (1.8%) of the 1,081 randomly chosen adults possessed the AW32 antigen. We therefore combined as the control group the randomly chosen adults and the neonates and excluded the infants tested in paternity disputes; the frequencies of HLA types in the control group were compared with those in the patients with severe measles.

There was a significant excess of HLA-AW32 in the group of 69 children with severe measles (<2,000 lymphocytes/mm³) as compared with the control group (corrected $P = 0.016$) (table 2). The relative risk of developing lymphocytopenia in individuals possessing HLA-AW32 was 5.5. None of the other HLA types examined showed significant variations between patients with measles and the control group when corrections were made for the number of antigens tested.

The distribution of HLA types in children with a good clinical outcome from severe measles was similar to that in those with a poor outcome, and neither clinical subgroup had a significantly different distribution of HLA types from that detected in normal persons. HLA-AW32 (as would be expected from its deviation from a normal distribution in the control group) was increased in comparison to the control group in both clinical subgroups.

No other individual HLA types and the parameters of immunity studied were significantly associated (table 3). However, there was a trend toward the presence of HLA-A1 in good responders in tests of humoral and cellular immunity—CF and HAI antibodies; T, B, and null cells; and inhibition index—and toward the presence of HLA-AW32 in good responders for T, B, and null cells. There was a similar trend toward the absence of HLA-BW42 (a common HLA type in blacks) among good responders for CF and HAI antibodies and T and B cells. A25 and A29 antigens were associated with a poor response for some components of the immune response (table 3).

The presence of particular HLA-C types did not correlate with lymphocytopenia, clinical outcome, or immune responses.

Patients with a good T-cell response also had a good B-cell ($\chi^2 = 24.8$; uncorrected $P < 0.0001$) and good null-cell ($\chi^2 = 6.4$; uncorrected $P < 0.02$) response. Those with a good null-cell response also had a good B-cell response ($\chi^2 = 6.8$; uncorrected $P < 0.01$).

Discussion

Measles, like most other infections, causes minor

Table 3. Percentage frequency of nine antigens of the histocompatibility leukocyte antigen complex in 69 South African black children with severe measles.

Immune function, response (*n*)	A1	A25	A29	AW30	AW32	B7	B18	BW42	BW51	Only one antigen at the B locus
CF antibodies										
Good (17)	17.6	17.6	17.6	29.4	5.9	17.6	1.8	5.9	0	70.6
Poor (37)	8.1	18.9	21.6	24.3	10.8	27.0	5.4	16.2	5.4	37.8
HAI antibodies										
Good (11)	27.3	9.1	9.1	36.4	9.1	36.4	9.1	0	0	54.5
Poor (24)	8.3	33.3	33.3	25.0	8.3	20.8	4.2	25.0	0	50.0
Inhibition index*										
Good (20)	25.0	25.0	15.0	35.0	5.0	30.0	0	15.0	5.0	55.0
Poor (15)	0	33.3	40.0	20.0	13.3	20.0	6.7	13.3	13.3	33.3
T cells										
Good (19)	21.1	5.3	5.3	31.6	21.1	21.1	5.8	5.3	10.5	57.9
Poor (37)	10.8	29.7	32.4	21.6	8.1	27.0	2.7	13.5	2.7	45.9
B cells										
Good (11)	27.3	0	0	36.4	27.3	18.2	9.1	0	0	63.6
Poor (42)	11.9	26.2	26.2	21.4	9.5	28.6	7.1	9.5	7.1	45.2
Null cells†										
Good (16)	25.0	18.8	6.3	18.8	25.0	18.8	6.3	12.5	6.3	50.0
Poor (35)	11.4	20.0	28.6	28.6	8.6	25.7	8.6	8.6	5.7	51.4
C3										
Good (22)	13.6	18.2	13.6	18.2	13.6	31.8	9.1	22.7	4.5	40.9
Poor (40)	12.5	22.5	25.0	35.0	10.0	15.0	5.0	10.0	5.0	55.0

NOTE. Severe measles was defined by the presence of a count in peripheral blood of <2,000 lymphocytes/mm³ within two days of the onset of rash. See table 1 for definitions of good and poor immune responses.

* See Subjects and Methods.

† Cells lacking surface markers of B or T cells.

effects in the vast majority of children in the developed world. Among poorer nations, the adverse effects of protein-calorie malnutrition in children with measles result in high morbidity and mortality. However, even when protein-calorie malnutrition has been carefully excluded, measles remains a severe disease in a significant minority of hospitalized African children [17]. The proportion of this minority of children with severe measles can vary between communities, and the probable reason for this variation may be a genetic predisposition in some individuals to the development of severe disease. We have shown in the present report that this genetic tendency indeed may be the case.

Severe lymphocytopenia during exanthem in measles has been unequivocally demonstrated to be a reliable index of severity [1, 17]. More than three-quarters of children with counts of <2,000 lymphocytes/mm³ at the onset of measles subsequently die or develop prolonged chest disease. This degree of lymphocytopenia, however, is detected in only a small proportion (9%) of all African children with measles.[1] The present study has shown that the development of severe lymphocytopenia in African children with measles is linked to the presence of HLA-AW32. The antibody response in humans with measles has also been reported to be under the control of genes linked to the HLA system [3–5]. Kreth et al. have demonstrated that there is a major histocompatibility complex–restricted killing of target cells infected with measles virus by cytotoxic T cells [23], but this observation was not confirmed by Perrin et al. [24]. There is suggestive, although not conclusive, evidence in measles-inexperienced populations vaccinated against measles virus to indicate that the febrile response may be influenced by HLA genes [5]. Taken together, these findings lead to the conclusion that HLA-linked genes determine immune responsiveness and may influence clinical outcome in measles. The results of

[1] H. M. Coovadia, "Host Allergic Response in Children with Measles Infection," M.D. thesis, University of Natal, Congella, Durban, South Africa, 1977, p. 85.

the current study reinforce the concept that within a community there is a group that is genetically susceptible to the development of severe measles. The degree of susceptibility might vary between populations, depending on the frequency of the HLA-linked susceptibility. The variation may be the effect of dissimilar historical and evolutionary pressures exerted on different populations. Because the linkage disequilibrium pattern for alleles at HLA loci differs among populations, susceptibility to severe measles may be detected with antigens other than HLA-AW32 in other populations.

Although we used profound lymphocytopenia as a marker of severe clinical measles, 23% of these children subsequently recovered. Associations with particular HLA types were not detected in the subgroup who recovered or in those who did not in comparisons between the subgroups and with the control group. The numbers in the subgroups were, however, small, so that factors other than lymphocytopenia could also have influenced the outcome. These factors may be under separate genetic control. The trend towards linkage between the presence of particular HLA types and the various parameters of immunity studied (table 3) supports this suggestion. Paradoxically, the presence of HLA-AW32, which is associated with severe lymphocytopenia, showed a trend toward good responses for T, B, and null cells (table 3). However, the tendency toward an association between the presence of HLA-AW32 and poor responses for CF antibodies and inhibition index and the failure to detect a correlation between clinical outcome and particular HLA types suggest that severe measles is only indicated by lymphocytopenia (and therefore the presence of HLA-AW32); the outcome is the result of a more complex interplay of immune reactions [25] which the present study has not elucidated.

An important application of associating the presence of HLA types with particular diseases has been the classification of diseases into categories according to the degree of association with one or another locus of the HLA complex. Two examples are the association of ankylosing spondylitis with B-locus antigens and of autoimmune diseases with D- and DR-locus antigens [26]. Tests for detecting D- and DR-locus antigens were unavailable for this study. However, the association of occurrence of severe measles with an A-locus gene is unusual. Two diseases that have shown an association with A-locus antigens are idiopathic hemachromatosis with HLA-A3 and pemphigus with HLA-A10. An explanation for this association may be found in Zinkernagel's hypothesis that clinical outcome is dependent on both host immune responsiveness and virus cytopathogenicity [27]. Accentuated host responsiveness results in autoimmune diseases, whereas low responsiveness predisposes to damage by acute virus infections. It is therefore not unexpected that susceptibility to the damaging effects of measles virus relates to a locus different from that associated with autoimmune diseases. The immune response to measles has been shown to be frequently associated with A-locus genes [4, 5]. The finding of an association between severe measles and a particular HLA type suggests that during previous measles epidemics of catastrophic proportions, individuals without that HLA type or other linked types at this locus would have been favored for survival. This hypothesis would be an example of linkage disequilibrium in which HLA-AW32 occurs in combination with another susceptibility antigen more frequently than would be expected.

Although the relative risk for persons possessing HLA-AW32 of developing severe measles (5.5) is considerably less than that for persons possessing HLA-B27 of developing ankylosing spondylitis and for those possessing D-locus antigens of developing autoimmune diseases [28], it is similar to calculated risks for HLA types and other infectious diseases (for example, tuberculosis and leprosy) [15]. These relative values are in accordance with the idea that associations with particular HLA types are more likely to be detected in autoimmune diseases, which have little effect on the survival of the species, rather than with infectious diseases, which can have major effects [27].

The comparison of HLA frequencies between children with measles and adult control subjects is not inappropriate. The HLA distributions among neonates, infants, and adults of the ethnic group studied were similar, and the distribution of HLA types in the patients conformed to Hardy-Weinberg equilibrium (χ^2_{91} for heterogeneity at the A locus = 89.25, $P = 0.5$; χ^2_{120} for heterogeneity at the B locus = 93.4, $P > 0.95$).

The mechanisms of genetic control of lymphocytopenia in measles are not known. They could, however, involve some of the explanations reviewed by Svejgaard et al. [29]—in particular, those relating to immune response genes, molecular

mimicry, and virus receptor function. HLA-AW32 may in fact serve this last mentioned function.

We have demonstrated an HLA-linked genetic control (which is probably polygenic) of severe measles, discussed the findings in terms of variation in clinical spectrum of the illness among individuals and between communities, and noted the implications for evolution of HLA polymorphism.

References

1. Coovadia, H. M., Wesley, A., Brain, P., Henderson, L. G., Hallett, A. F., Vos, G. H. Immunoparesis and outcome in measles. Lancet 1:619-621, 1977.
2. Mitus, A., Enders, J. F., Craig, J. M., Holloway, A. Persistence of measles virus and depression of antibody formation in patients with giant-cell pneumonia after measles. N. Engl. J. Med. 261:882-889, 1959.
3. Haverkorn, M. J., Hofman, B., Masurel, N., van Rood, J. J. HL-A linked genetic control of immune response in man. Transplantation Reviews 22:120-124, 1975.
4. Jersild, C., Ammitzboll, T., Clausen, J., Fog, T. Association between HL-A antigens and measles antibody in multiple sclerosis [letter]. Lancet 1:151-152, 1973.
5. Black, F. L., Pinheiro, F. de P., Hierholzer, W. J., Lee, R. V. Epidemiology of infectious disease: the example of measles. *In* Health and disease in tribal societies. Elsevier, Amsterdam, 1977, p. 115-135.
6. Benacerraf, B., McDevitt, H. O. Histocompatibility-linked immune response genes. Science 175:273-279, 1972.
7. Dorf, M. E., Balner, H., Benacerraf, B. Mapping of the immune response genes in the major histocompatibility complex of the rhesus monkey. J. Exp. Med. 142:673-693, 1975.
8. Marsh, D. G., Bias, W. B., Hsu, S. H. Association of the HL-A7 cross-reacting group with a specific reaginic antibody response in allergic man. Science 179:691-693, 1973.
9. Greenberg, L. J., Gray, E. D., Yunis, E. J. Association of HL-A 5 and immune responsiveness in vitro to streptococcal antigens. J. Exp. Med. 141:935-943, 1975.
10. Spencer, M. J., Cherry, J. D., Terasaki, P. I. HL-A antigens and antibody response after influenza A vaccination: decreased response associated with HL-A type W16. N. Engl. J. Med. 297:13-16, 1976.
11. McDevitt, H. O., Oldstone, M. B. A., Pincus, T. Histocompatibility-linked genetic control of specific immune responses to viral infection. Transplantation Reviews 19:209-225, 1974.
12. de Vries, R. R. P., Fat, R. F. M. L. A., Nijenhuis, L. E., van Rood, J. J. HLA-linked genetic control of host response to *Mycobacterium leprae*. Lancet 2:1328-1330, 1976.
13. Morris, P. J., Pietsch, M. C. A possible association between paralytic poliomyelitis and multiple sclerosis [letter]. Lancet 2:847-848, 1973.
14. Bodmer, W. F. Evolutionary significance of the HL-A system. Nature 237:139-145, 183, 1972.
15. Ryder, L. P., Andesson, E., Svejgaard, A. [ed.]. HLA and disease registry: 3rd report. Munksgaard, Copenhagen 1979, p. 18-19.
16. de Vries, R. R. P., Kreeftenberg, H. G., Loggen, H. G., van Rood, J. J. In vitro immune responsiveness to vaccinia virus and HLA. N. Engl. J. Med. 297:692-696, 1977.
17. Coovadia, H. M., Wesley, A., Brain, P. Immunological events in acute measles influencing outcome. Arch. Dis. Child. 53:861-867, 1978.
18. Vaughan, V. C. Growth and development. *In* W. E. Nelson, V. C. Vaughan, and R. J. McKay [ed.]. Textbook of Pediatrics. 9th ed. Saunders, Philadelphia, 1969, p. 39-54.
19. Hammond, M. G., Appadoo, B., Brain, P. HL-A antigens in Bantu and Indians. *In* F. Kissmeyer-Nielson [ed.]. Histocompatibility testing. Munksgaard, Copenhagen, 1975, p. 173-178.
20. Hammond, M. G., Appadoo, B., Brain, P. HLA in non-Caucasian populations. *In* W. F. Bodmer [ed.]. Histocompatibility testing. Munksgaard, Copenhagen, 1977, p. 407-408.
21. Terasaki, P. I., McClelland, J. D. Microdroplet assay of human serum cytotoxins [letter]. Nature 204:998-1000, 1964.
22. Brain, P., Cox, J., Duursma, J., Pudifin, D. J. T and B lymphocytes in three population groups. Clin. Exp. Immunol. 23:248-251, 1976.
23. Kreth, H. W., ter Meulen, V., Eckert, G. Demonstration of HLA restricted killer cells in patients with acute measles. Med. Microbiol. Immunol. (Berl.) 165:203-214, 1979.
24. Perrin, L. H., Tishon, A., Oldstone, M. B. A. Immunologic injury in measles virus infection. III. Presence and characterization of human cytotoxic lymphocytes. J. Immunol. 118:282-290, 1977.
25. Coovadia, H. M. Recent advances in the understanding of measles. South African Journal of Hospital Medicine 6:143-148, 1980.
26. Dausset, J. Clinical implications (nosology, diagnosis, prognosis, and therapy). *In* J. Dausset and A. Svejgaard [ed.]. HLA and disease. Munksgaard, Copenhagen, 1977, p. 296-310.
27. Zinkernagel, R. M. Association of disease susceptibility to major histocompatibility antigens. Transplant. Proc. 11:624-627, 1979.
28. Thomsen, M., Morling, N., Platz, P., Ryder, L. P., Svejgaard, A. HLA and disease. Transplant. Proc. 11:633-637, 1979.
29. Svejgaard, A., Platz, P., Ryder, L. P., Neilsen, L. S., Thomsen, M. HL-A and disease associations—a survey. Transplantation Reviews 22:3-43, 1975.

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HLA antigens in black South African children with rheumatic heart disease

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SUMMARY The high incidence of rheumatic heart disease (RHD) in black South African children has been attributed mainly to poor socio-economic status and over-crowding. In order to elucidate whether other factors, in particular genetic, were responsible, the HLA-status of 61 black children with rheumatic heart disease was compared with that of 1165 normal controls. Overall, no differences were found, except a higher incidence of HLA–B25 and BW51 in the group with rheumatic heart disease, when the difference was not of statistical significance. Moreover, when the patients were considered in two groups, viz. (a) a "surgical" group which required cardiac surgery and (b) a "non-surgical" group in which cardiac failure was absent or could be easily controlled by medical therapy, the difference between the two groups was also not of statistical significance, though there was a higher incidence of HLA-A10 (which includes HLA-A25 and A26) in the "non-surgical" group.

These data appear to agree with the results of other studies which found no significant association between HLA-status and RHD.

Introduction

It is well recognized that the incidence of rheumatic heart disease (RHD) in South African blacks is high. In 1972 the Soweto survey showed a prevalence rate of 6·9 per 1000 black children between the ages of two and 18 years (1). Chesler *et al.* working at Baragwanath Hospital, Johannesburg, found an incidence of acute rheumatic fever of the order of 10·6 cases per 1000 paediatric admissions over a period of three years, of whom 87% had carditis (2). In a recent joint survey of hospital admissions for rheumatic fever carried out in Cape Town, Durban and Johannesburg (3), carditis was present in 70% of black children presenting with rheumatic fever for the first time.

It has also been noted by various authors that rheumatic heart disease is much more severe in the black child, and tends to occur at a relatively younger age (4). In the Baragwanath study (2), 13·4% were below five years of age. Most workers attribute the high incidence of rheumatic heart disease in developing countries to overcrowding and poor socio-economic status: the truth of this is undisputed. However, some reports (5, 6) indicate that there may be a familial susceptibility to the acquisition of group A-haemolytic streptococci and of rheumatic fever. Stevenson and Cheeseman (7), in a study of 462 families with 2038 children, found that inheritance was an important factor in rheumatic fever but that it did not follow a Mendelian pattern. Others have found a higher incidence of ABO non-secretors in rheumatic subjects than among healthy school children (8), although the numbers were too small to warrant definite conclusions. A lower frequency of blood group O has also been reported in rheumatic children (8–10). Recently, attention has been focused on a possible association between acute rheumatic fever and/or rheumatic heart disease on the one hand

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and histocompatibility antigens (HLA-antigens) on the other (11–17) (Table I). Since these antigens are genetically inherited, a strong association between a particular HLA-antigen and a specific disease might favour predisposition to that disease in individuals possessing such an antigen. It will be observed from Table I that such an association has not been consistently reported by previous workers.

Because of the high incidence of rheumatic heart disease in black South Africans and of the tendency for many of them to develop severe or advanced cardiac lesions early in life, it was thought that HLA-testing in this particular group of individuals might shed some light on the subject.

Definitions

Materials and methods

From 24 April 1979 to 1 May 1980, 61 children, aged five to eleven, were admitted to the general paediatric wards of King Edward VIII Hospital, Durban, with rheumatic heart disease. Fifty-three had active carditis according to the Modified Jones Criteria (18). In one carditis was thought to be probably active, in two there was complicating infective endocarditis and in the remaining five, without active carditis, there were established mitral valvular lesions due to well-documented previous attacks of rheumatic carditis. None of the children were related. Children with acute rheumatic fever without carditis were not included in the study. In only one case was there a family history of "heart disease". In 54 there was no family history of either rheumatic fever or rheumatic heart disease, and in six no family history could be obtained. Thirteen of the 53 children with active carditis were seen during their first attack of carditis, and in a further eight the attack was probably the first. In 23, an active carditis was superimposed upon chronic rheumatic heart disease, in three the attack was thought to be probably a recurrence, and in six we were unable to ascertain whether their carditis was caused by an initial attack or a recurrence (Table II). Thus, at the time of investigation at least 28 children (probably 31) had established rheumatic heart disease with valvular lesions. Informed consent for investigation was obtained from the parents, and, in some cases where the child was old enough, from the patient.

HLA-Typing

The patients were typed for HLA A-B-C antigens using 180 antisera in a two stage lymphocytotoxicity test (19). Lymphocytes were isolated on a Ficoll-Hypaque density gradient (20). Typing was for the following antigens: HLA-A1, 2, 3, 11, 25, 26, 28, 29; AW23, AW24, AW30, AW31, AW32 and AW33; HLA-B7, 8, 13, 14, 15, 17, 18, 27, 37; BW16, BW21, BW22, BW35, BW41, BW42, BW44, BW45, BW46, BW51, BW52, BW53, BW60 and BW61; and CW1, CW2, CW3, CW4 and CW5 (a total of 42 antigens).

The HLA antigen frequencies in 61 patients were compared with those in a healthy control population consisting of randomly chosen blood donors and staff, many of whom were typed for International Workshops (21, 22). All the patients and controls were typed in the laboratories of the Natal Institute of Immunology using standardized National Institute of Health technique (23). The Negro population of Durban consists mainly of Zulus and the patients and controls studied by us were of pure descent. Comparisons of HLA frequencies in children and adults show no significant differences (24). The same applies to sex (25, 26) and to place of residence, i.e. whether rural or urban (27).

Statistical methods

HLA antigen frequencies in patients and controls were compared using the Chi square (χ^2) test. Yates' correction was used when expected values were less than four. The resulting probabilities were corrected (for multiple testing) by multiplying by the number of comparisons made, i.e. the number of different antigens tested. 5% was taken as the level of significance.

Results

Clinical data:

(i) *Age of children:* Figure 1 shows the age distribution of the 61 children; the peak at seven to nine years of age is in keeping with the findings of Nadas and Fyler (28). There was, however, an appreciable proportion of cases in the five to six year age group.

(ii) *Sex:* There were 34 males and 27 females, giving a male-to-female ratio of 1·3:1.

(iii) *Urban vs. rural place of residence:* Thirty-two (52·5%) children came from rural areas and 29 (47·5%) from urban homes. The relatively large percentage of children from rural areas can probably be explained by the fact that King Edward VIII Hospital is a referral hospital serving the whole of Natal and KwaZulu and that many cases are referred from outlying peripheral hospitals in the rural areas: overcrowding in rural huts is probably an additional factor.

Table I *HLA studies in patients with rheumatic fever or rheumatic heart disease, acquired valvular disease and cardiomyopathy*

Authors	Race of patients	No. of patients	Disease	No. of HLA antigens tested	Findings
Falk J. A. *et al.* (11)	Caucasian (8 non-Caucasian)	76	*Rheumatic fever and/or Rheumatic heart disease	17	↓A3
Caughey D. E. *et al.* (12)	(a) Caucasian	50	*Rheumatic fever ± R.H.D.		↓A28 ↑BW17
	(b) Maori	50	*Rheumatic fever ± R.H.D.	18	↑A3 ↑B8; ↓A10 ↑BW35
Leirisalo M. *et al.* (13)	Caucasian	109	Rheumatic fever (38% had Carditis)	24	No association
			R.H.D.		
Joysey V. C. *et al.* (14)	Caucasian	94	R.H.D.	21	↑BW 15 when compared to 1 group of controls but not when compared to 2 other control groups
Ward C. *et al.* (15)	Caucasian	58	Acquired valvular disease	27	
			(i) No history of rheumatic fever		↑AW30/31, ↑A29
			(ii) With history of rheumatic fever		No association
Murray G. C. *et al.* (16)	Caucasian (Mexican-American)	49	Rheumatic fever with *arthritis* Carditis in only 18% cases	32	No association
Matsumori A. *et al.* (17)	Japanese	20	Rheumatic heart disease		No association
		30	Cardiomyopathy	22	? some role in *familial* cases

*Percentage with carditis not mentioned.

Table II *Summary of cases admitted with active carditis*

Active carditis: first attack	13
Active carditis: probable first attack	8
Active carditis superimposed on chronic RHD	23
Active carditis in patients with probable chronic RHD	3
Active carditis (uncertainty re first or repeat attack)	6
Total number of cases with active carditis	53

(iv) *Other major manifestations of rheumatic fever:* Of the 53 children with active carditis, only seven had associated polyarthritis, five rheumatic nodules, and three Sydenham's chorea either in the past or on subsequent admissions to hospital. No child had erythema marginatum. Sixteen children had joint pains only, without clinical evidence of arthritis, and four others gave a history of joint pains in the past.

(v) *Cardiac lesions:* The valvular lesions are summarised in Table III. It will be noted that mixed mitral valve disease accounted for 60% of the cases. Mitral stenosis, either as an isolated lesion or as the dominant lesion in mixed mitral valve disease, was

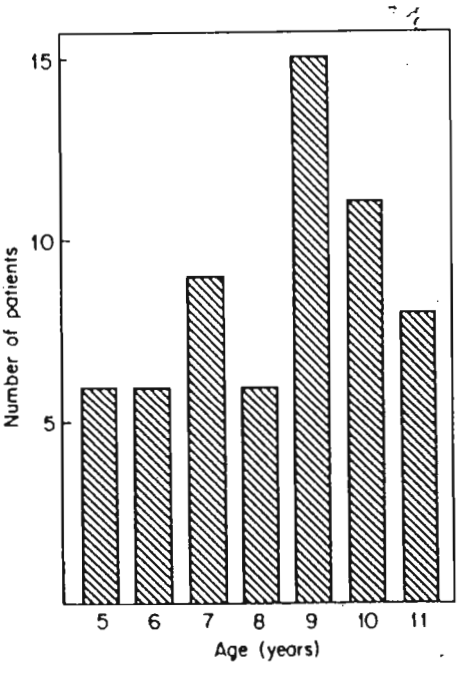

Figure 1 Age incidence of black children with rheumatic heart disease (King Edward VIII Hospital, 24 April 1979 to 1 May 1980).

present in 16% of the patients. This figure is much higher than that quoted by others (29). Pericarditis, as manifested by a friction rub, was present in eight (14·7%) of the 61 children.

(vi) *Cardiac failure:* 51 (83·6%) of the 61 children were admitted in cardiac failure. Of these, nine had left ventricular failure, the remainder biventricular failure.

In 21 of the 51 patients with cardiac failure, active carditis was responsible; these patients later recovered when a negative C-reactive protein and return of a previously elevated ESR to normal indicated cessation of active carditis. In seven additional children on admission failure was precipitated by exertion: but subsequently when haemodynamic problems developed failure became persistent. In the remaining 23 children (of whom 13 had active carditis superimposed upon chronic rheumatic heart disease), failure was considered to be the result of haemodynamic disturbance caused by the valvular lesions. Cardiomegaly, as evidenced by a cardio-thoracic ratio of more than 0·50 on an antero-posterior chest radiograph, was present in 56 (91·8%) of the children. The vast majority of these, on admission (38 cases, or 62% of the total) had a cardio-thoracic ratio greater than 0·60.

Table III *Valvular lesions*

Mixed mitral (Dominant mitral incompetence)	31
Mixed mitral (Dominant mitral stenosis)	6
Pure mitral incompetence	4
Pure mitral stenosis	4
Combined mitral and aortic incompetence	15
Aortic incompetence alone	1
Total	61

HLA-Studies

(i) *Frequency:* Table IV shows the percentage frequency of HLA A-B-C antigens in black South African children with rheumatic heart disease, compared with 1165 controls. A higher frequency of HLA-A25, A10 and BW51 was found in children with rheumatic heart disease, but the differences were not statistically significant (after correction).

(ii) *Presence of only a single antigen at either the A or B locus:* The number of children with only one detectable antigen at the A or B locus was compared with the control population (Table V). Again, no significant differences were shown, indicating that homozygosity for a particular HLA-antigen does not

Table IV *Percentage frequency of HLA antigens in South African negro children with rheumatic heart disease and/or rheumatic carditis*

HLA	Control 1165	RHD/ Carditis 61	HLA	Control 1165	Carditis 61
A1	6·4	9·8	B7	18·2	21·3
A2	21·2	16·4	B8	13·7	11·5
A3	13·2	11·5	B13	4·5	0
A11	0·1	0	B14	5·8	4·9
AW23	18·5	19·7	B15	5·1	1·6
AW24	3·9	3·3	BW16	2·5	3·3
A25	15·2	24·6[1]	B17	38·8	41·0
A26	8·7	11·5	B18	4·6	8·2
A10	23·9	36·1[2]			
A28	20·9	18·0	BW21	1·0	1·6
A29	16·3	8·2	BW22	0	0
AW30	37·7	37·7	B27	0·3	1·6
AW31	9·5	8·2	BW35	6·2	4·9
AW32	2·1	0	B37	0	0
AW33*	2·7	1·6	BW41*	1·1	1·6
			BW42	24·6	23·0
Only 1 A antigen detected	26·1	29·5	BW44	15·8	14·8
			BW45	7·7	6·6
			BW46	0	0
CW1	1·2	0	BW51	1·7	6·6[3]
CW2	14·6	21·3	BW52	0	0
CW3	8·8	6·6	BW53	1·3	3·3
CW4*	18·2	18·0	BW60	1·6	1·6
CW5*	1·7	3·3	BW61	0	0
			Only 1 B antigen detected	45·8	42·6

RHD = Rheumatic heart disease.
* Number of controls = 165.
[1] $\chi^2 = 3·9$ $P < 0·05$ (uncorrected).
[2] $\chi^2 = 4·7$ $P < 0·05$ (uncorrected).
[3] $\chi^2 = 4·8$ $P < 0·05$ (uncorrected).

Table V *HLA and rheumatic heart disease/carditis patient with only a single detectable antigen at the A or B locus*

	Controls $n = 1165$ %	RHD/Carditis $n = 61$ %
One HLA-A Antigen	26·1	29·6
One HLA-B Antigen	45·8	42·6

(No significant difference).

appear to be a factor in the predisposition t rheumatic heart disease.

(ii) *HLA-status in relation to severity of valvula lesions:* The higher incidence of HLA-A25 in th patients with rheumatic heart disease, though nc statistically significant, was further analysed accord

Table VI *HLA-A25 and A26 in rheumatic heart disease (percentage frequency)*

HLA-Antigen	Normal controls $n = 1165$ %	RHD "surgical" $n = 19$ %	RHD/carditis "non-surgical" $n = 42$ %
A25	15·2	10·5	31·0*
A26	8·7	5·3	14·3
A10	23·9	15·8	45·3†

Comparison with controls: * $\chi^2 = 7{\cdot}7$ $P < 0{\cdot}01$ (uncorrected).
† $\chi^2 = 10{\cdot}2$ $P < 0{\cdot}005$ (uncorrected).

ing to the severity of the cardiac lesions. The children were subdivided into two groups, viz.

(a) A "surgical" group consisting of 19 children who required cardiac surgery due to severe haemodynamic problems. In 13 of them surgery was carried out after the acute carditis had subsided, the cause for the failure being residual valve disease. Three died before surgery could be performed; and the remaining three, though booked for surgery did not turn up.

(b) A "non-surgical" group which included all children without any haemodynamic problems and not in cardiac failure, as well as those who were in failure but were adequately controlled by medical means alone.

As shown in Table VI, though there was a much higher incidence of HLA-A25 and A26 (together known as HLA-A10) (30) in the "non-surgical" than in the "surgical" group, the difference was not statistically significant.

Discussion

A number of workers (11–17) have tried to show possible associations between HLA-status on the one hand and rheumatic fever and/or rheumatic heart disease on the other. As Table I shows, the results have been conflicting. This could be due to one or more of the following (16):

(a) Variation in patient selection: some workers grouped acute rheumatic fever (with or without carditis) and rheumatic heart disease together; others studied patients with acute rheumatic fever only. One study included heart disease *presumed* to be due to rheumatic fever. We included only children with heart disease due either to well-documented rheumatic fever in the past or to active rheumatic carditis. Children with acute rheumatic fever but without carditis were excluded.

(b) Possible lack of racial homogeneity: it is known that certain HLA-associations are stronger in some races than in others, notably the association between HLA-B27 and ankylosing spondylitis, in which the frequency of this antigen in whites with this disease is 94% and in blacks only 48%. All our patients were black.

(c) The use of too few specific antisera in identifying the HLA-antigens, as well as the presence of cross-reacting antibodies: these can result in errors in detection of HLA-antigens and in an inability to identify the total HLA-antigen complement on the cell surface. We used 180 antisera in this study.

Our results do not show any definite associations between rheumatic heart disease and HLA-status, though we found a higher incidence of HLA-A25 and BW51 in our patients with rheumatic heart disease and carditis. Moreover, we found that the incidence of HLA-A10 (which includes A25 and A26) (30), was higher, albeit not significantly, in the patients whose lesions were classified as "non-surgical" than in those with gross haemodynamic problems due to valvular lesions severe enough to warrant surgery. Thus on the figures obtained, it appears that there is no definite association between HLA-status and RHD, with or without active carditis, although there seems to be a trend towards a high incidence of HLA-A25 in that condition. A larger series might clarify the significance of this observation.

The incidence of only one detectable antigen at either A or B locus in patients with RHD was no different from that in the control population (Table V). Homozygosity for any particular HLA-antigen, therefore, is not likely to be a factor in the increased predisposition to RHD in the black South African child. This finding is at variance with that of Falk *et al.* (11) who found that the number of antigens detected on lymphocytes from rheumatic patients was significantly lower than that found on cells from non-rheumatic individuals, the majority of their patients, however, were of Caucasian origin.

Finally, the propensity for the black African child to develop RHD at an early age, with a high incidence of severe valvular lesions, is confirmed in this study. Overcrowding and poverty, the root causes of this malady, appear far more important than genetic

factors, although the latter cannot be excluded altogether as HLA-antigens at the D locus, and some at the C locus, were not tested for in this study.

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References

(1) McLaren MJ, Hawkins DM, Koornhof HJ *et al.* Epidemiology of rheumatic heart disease in black children of Soweto, Johannesburg. Br Med J 1975; 3: 474–7.
(2) Chesler E, Levin S, du Plessis L, Freiman I, Rigers M, Joffe N. The pattern of rheumatic heart disease in the urbanised Bantu of Johannesburg. S Afr Med J 1966; 40: 899–904.
(3) Winship WS. Personal communication, 1978.
(4) Schrire V. The racial incidence of heart disease at Groote Schuur Hospital, Cape Town. Part II. Hypertension and valvular disease of the heart. Am Heart J 1958; 56: 742–60.
(5) Wilson MG, Schweitzer MD, Lubschez R. The familial epidemiology of rheumatic fever: genetic and epidemiological studies. I. Genetic Studies. J Pediatr 1943; 22: 468–92.
(6) Wilson MG, Schweitzer M. Pattern of hereditary susceptibility in rheumatic fever. Circulation 1954; 10: 699–704.
(7) Stevenson AC, Cheeseman EA. Heredity and rheumatic fever: a study of 462 families ascertained by an affected child and 51 families ascertained by an affected mother. Ann Eugen London 1953; 17: 177–210.
(8) Clark CA, McConnell RB, Sheppard PM. ABO blood groups and secretor character in rheumatic carditis. Br Med J 1960; 1: 21–3.
(9) Glynn LE, Holborrow EJ. Relation between blood groups, secretor status and susceptibility to rheumatic fever. Arthritis Rheum 1961; 4: 203–7.
(10) Buckwalter JA, Neifeh GS, Auer JE. Rheumatic fever and the blood groups. Br Med J 1962; 2: 1023–7.
(11) Falk JA, Fleischman JL, Zabriskie JB, Falk RE. A study of HLA antigen phenotype in rheumatic fever and rheumatic heart disease patients. Tissue Antigens. 1973; 3: 173–8.
(12) Caughey DE, Douglas R, Wilson W, Hassal IB. HLA-antigens in Europeans and Maoris with rheumatic fever and rheumatic heart disease. J Rheumatol 1975; 2: 319–22.
(13) Leirisalo M, Laitinen O, Tiilikainen A. HLA phenotypes in patients with rheumatic fever, rheumatic heart diseases and Yersinia arthritis. J Rheumatol (Suppl 3) 1977; 4: 78–83.
(14) Joysey VC, Roger JH, Ashworth F. *et al.* Parallel studies of HLA antigens in patients with rheumatic heart disease and schleritis: comparisons with three control populations. J Rheumatol (Suppl 3) 1977; 4: 84–88.
(15) Ward C, Gelstorpe K, Doughty RW, Hardisty CA. HLA antigens and acquired valvular heart diseases. Tissue Antigens. 1976; 7: 227–31.
(16) Murray GC, Montiel MM, Persellin RH. A study of HLA antigens in adults with acute rheumatic fever. Arthritis Rheum. 1978; 21: 652–6.
(17) Matsumori A, Hirose K, Wakabayashi A. *et al.* HLA in hypertrophic cardiomyopathy and rheumatic heart disease. Japanese Circulation J. 1979; 43: 445–9.
(18) *Ad Hoc* Committee of the Council on Rheumatic Fever and Congenital Heart Disease: Jones criteria (revised) for guidance in the diagnosis of rheumatic fever. Circulation 1965; 32: 644.
(19) Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. Nature 1964; 204: 998.
(20) Boyum A. Separation of leucocytes from blood and bone marrow. Scand J Clin Lab Invest 1968; 21: 97.
(21) Hammond MG, Appadoo G, Brain P. HLA antigens in Bantu and Indians. In: Kissmeyer-Nielsen F. (ed) Histocompatibility Testing. Copenhagen: Munksgaard, 1975; 173–8.
(22) Hammond MG, Appadoo G, Brain P. HLA in non-Caucasian populations. In: Bodmer WF. (ed) Histocompatibility Testing. Copenhagen: Munksgaard, 1977; 407–8.
(23) Brand DL, Ray JG, Hare DB, Kayhoe DE, McClelland JD. Preliminary trials towards standardisation of leucocyte typing. In: Terasaki PI (ed) Histocompatibility Testing. Copenhagen: Munksgaard, 1970; 357.
(24) Coovadia HM, Wesley A, Hammond MG, Kiepiela P. Measles, histocompatibility leucocyte polymorphism, and natural selection. J Infect Dis 1981; 144: 142.
(25) Hammond MG. Unpublished data.
(26) Hammond MG, Appadoo G, Brain P. HLA and cancer in South African Negroes. Tissue Antigens 1977; 9: 1.
(27) Hammond MG. Personal communication.
(28) Nadas AS, Fyler DC. Paediatric Cardiology 3rd ed. Philadelphia: W. B. Saunders Company. 1972; Chapter 7.
(29) Aryanpur I, Naxarian I, Siassi B. Chapter 34: Rheumatic Heart Disease in developing countries. In: Moss AJ, Adams FH and Emmnouilides GC. (eds) Heart Disease in Infants, Children and Adolescents 2nd ed. Baltimore: Williams and Wilkins Company. 1977.
(30) World Health Organisation: I.U.I.S. Terminology Committee. Nomenclature for factors of the HLA system. In: Bodmer (ed) Histocompatibility Testing. Copenhagen: Munksgaard, 1977.

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Male transmission of the gene for isolated gonadotropin-releasing hormone deficiency

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Three black women, daughters of the same father but three unrelated mothers, presented with isolated gonadotropin deficiency (IGD). Clinically, the patients had no midline defects and intact smell and taste senses. Biochemically, the essential feature was very low unstimulated and stimulated follicle-stimulating hormone and luteinizing hormone levels, even after priming with gonadotropin-releasing hormone over a 5-day period. Growth hormone response to insulin-induced hypoglycemia was somewhat blunted, but prolactin, cortisol, and thyroid-stimulating hormone responses were quite normal. All three patients had the 46,XX karyotype; clinical or biochemical aberrations could not be demonstated in any of the remaining family members. The disorder was, apparently, transmitted by the deceased father, who manifestly did not have an IGD deficiency nor any of the midline stigmata associated with IGD. The mode of inheritance seems most likely to be autosomal dominant with variable penetrance. Fertil Steril 43:225, 1985

The syndrome of isolated gonadotropin deficiency (IGD), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) has been well characterized in terms of the clinical and endocrine presentation.[1, 2] It is now generally recognized that the syndrome is the result of a congenital deficiency of hypothalamic gonadotropin-releasing hormone (GnRH). Kallman et al.[3] first drew attention to an occurrence of the syndrome in three kindreds. Subsequently, reports suggested that the disorder was transmitted by female carriers to male offspring.[4, 5]

In this article, a black family is reported in which a father had three apparently affected daughters by three unrelated mothers.

Three half sisters presented to the gynecologic endocrine clinic within 12 months with delayed puberty and primary amenorrhea. They had the same father, but each had a different unrelated mother (Fig. 1).

CASE REPORTS

CASE 1 (II.6)

N. N. first presented to the gynecologic clinic at the age of 18 with primary amenorrhea and un-

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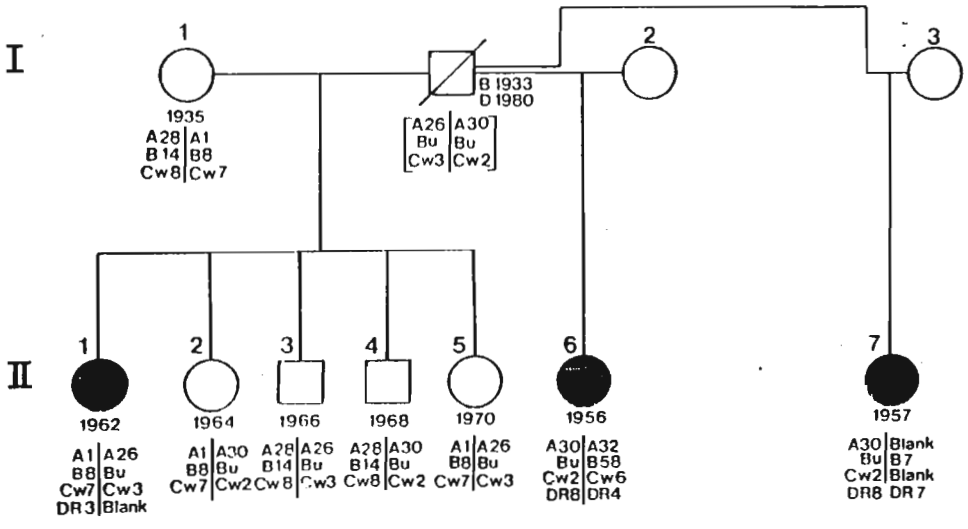

Figure 1
HLA status of family members studied. The probable HLA status of the deceased father was based on those of the children begotten by mother I.1.

derwent laparoscopy; an infantile uterus and normal ovaries and fallopian tubes were found. She was seen again at the age of 28, when she was noted to be eunuchoid (arm span greater than height by 8 cm). There was scanty pubic and axillary hair, and breast development was retarded.

CASE 2 (II.1)

G. N. presented for the first time at the age of 20 with amenorrhea, no breast development, and scanty pubic and axillary hair and was also eunuchoid.

CASE 3 (II.7)

P. N. was 25 years old when she was seen at the clinic with features similar to those of patients 1 and 2.

There were no midline facial defects or anosmia in any patient. X-rays of the skull and ophthalmologic examination were normal in all three patients.

Unstimulated LH and FSH levels were less than the reference range in all three patients (reference range, LH, 3.5 to 30 mIU/ml; FSH, 3 to 16 mIU/ml). Plasma estradiol was low in all three patients (reference range, 30 to 80 pg/ml).

There was no withdrawal bleeding after medication with medroxyprogesterone acetate (Provera, The Upjohn Company, Kalamazoo, MI), 15 mg/day for 5 days, but the patients did bleed on Ovral (0.05 mg ethinyl estradiol, 0.5 mg norgestrel; Wyeth, Isando, RSA) withdrawal after 21 days of medication.

GnRH, 100 μg, thyrotropin-releasing hormone (TRH, 200 μg), and insulin tolerance tests (insulin, 0.1 to 0.15 U/kg body weight) were performed in all three patients. In addition, GnRH (100 μg) was given subcutaneously for 5 days, and the intravenous stimulation was repeated at the end of the period of priming. The GnRH test was also performed on all available nonaffected members of the family. Unfortunately, two of the mothers (I.2 and I.3) were not available to be studied, and the father had died in 1980 of an unknown cause. Human leukocyte antigen (HLA) typing was performed as published previously.[6]

RESULTS

The genetic relationships and HLA status of each patient are shown in Figure 1. Each patient allegedly had the same father but a different mother (I.1, 2, 3). The presumed HLA status of the father was determined by study of the family members II.1 to 5. It is obvious from Figure 1 that the disorder is not linked to HLA type, and the disorder appeared to be transferred from father to daughters.

The results of the GnRH test in patients and relatives are shown in Tables 1 and 2. Although two of the affected patients demonstrated an increased response of LH to stimulation after priming, these values did not reach the reference range concentrations for unaffected patients in the follicular phase of the cycle. Thyroid function was normal in the three patients, and dynamic testing of the hypothalamic-pituitary axis showed

Table 1. *FSH and LH (U/l) Before and After Injection of GnRH (100 μg)*

Patient[a]	FSH					LH				
	−15 min	0 min	15 min	30 min	60 min	−15 min	0 min	15 min	30 min	60 min
N. N.										
Pre	2.0	2.4	4.4	5.4	5.7	< 3	< 3	3.5	4.5	3.8
Post	2.0	2.2	1.4	2.0	1.3	< 3	< 3	14.0	17.0	9.6
G. N.										
Pre	< 1.6	< 1.6	3.2	6.1	8.1	< 3	< 3	4.1	3.2	< 3
Post	2.1	< 1.6	3.5	5.9	7.1	< 3	< 3	7.6	12.0	12.8
P. M.										
Pre	< 1.6	< 1.6	< 1.6	< 1.6	< 1.6	< 3	< 3	< 3	< 3	< 3
Post	< 1.6	< 1.6	< 1.6	< 1.6	< 1.6	< 3	< 3	< 3	< 3	< 3

[a]Pre, test done before priming; Post, test done after priming with 100 μg GnRH daily for 5 days.

normal responses to insulin-induced hypoglycemia and TRH stimulation. Serum and urine osmolality levels indicated that the posterior pituitary function was normal.

Prestimulation gonadotropin concentrations and levels after GnRH stimulation in other members of the family are shown in Table 2. Mother (I.1) is postmenopausal, and one of her daughters (II.2) was pregnant. Patient II.5, although 12 years old and prepubertal, had a normal adult pattern increase of FSH and LH.

All family members had a karyotype appropriate to the phenotypic sex.

DISCUSSION

In the present study, the probable HLA status of the deceased father was based on those of the children begotten by the mother I.1 in Figure 1. The HLA typing of the two daughters born to mothers I.2 and I.3, respectively, is consistent with the haplotype assigned to the presumed father. Objective evidence supporting the paternity claim of patients II.6 and II.7, therefore, exists. If this is correct, this family is probably unique inasmuch as it demonstrates that the disorder can be transmitted by a male to his daughters without manifestly expressing the disorder himself. As far as could be ascertained, the father did not have other associated features of IGD, such as midline defects. It was not possible to establish whether subtle features, such as anosmia or hyposmia, were present in the deceased father.

Other studies[5, 7] have shown more than one member of the same family affected by IGD, and in the majority of recorded instances female to male transmission appeared more likely. In the two kindreds described by Santen and Paulsen,[5] male to male transmission of anosmia was clearly demonstrated, but they did not unequivocally show transmission of IGD. In both these families, male to male transmission, therefore, clearly excludes an X-linked condition.

In the present study the mode of inheritance seems most likely to be autosomal dominant with variable penetrance. This is supported by the absence of the syndrome in patients II.2 and II.5, coupled with the disorder being milder in the father and of varying severity in the three affected daughters. Autosomal recessive inheritance is

Table 2. *FSH and LH (U/l) in Members of the Family Shown in Figure 1*[a]

Case	FSH					LH				
	−15 min	0 min	15 min	30 min	60 min	−15 min	0 min	15 min	30 min	60 min
I.1 (J. N., 47 yrs)	153.2	73.1	187.0	157.7	223.0	110.1	107.3	> 200	> 200	> 200
II.2 (G. N., 20 yrs)	Pregnant									
II.3 (G. N., 17 yrs)	8.2	6.4	10.0	12.0	12.4	7.1	12.2	—	40.0	39.0
II.4 (G. N., 15 yrs)	6.0	4.4	6.1	7.1	8.6	< 7.0	5.5	40.8	42.6	32.4
II.5 (C. N., 12 yrs)	17.5	15.0	30.0	26.3	25.6	12.0	16.2	96.1	66.0	59.0

[a]GnRH (100 μg) was injected intravenously at time 0.

most unlikely, because the three mothers are unrelated. There is no evidence of HLA linkage of IGD in this study.

The present study, therefore, confirms the heterogeneity of the syndrome of IGD, in that the youngest affected patient (II.1) was more severely affected than the other two, both in terms of clinical features and the response to GnRH before and after priming of the pituitary gland. However, none of the patients showed any of the associated features of anosmia, cleft palate, and hare lip; the present family may, indeed, have a condition unrelated to that of some of the patients reported in other series.

REFERENCES

1. Boyar RM, Finkelstein JW, Witkin M, Kapen S, Weitzman E, Hellman L: Studies of endocrine function in "isolated" gonadotropin deficiency. Metabolism 36:64, 1973
2. Spitz IM, Diamant Y, Rosen E, Bell J, Ben David M, Polishuk W, Rabinowitz D: Isolated gonadotropin deficiency. N Engl J Med 290:10, 1974
3. Kallman FJ, Schoenfeld WA, Barrera SE: The genetic aspects of primary eunuchoidism. Am J Ment Defic 48:204, 1944
4. Le Marquand HS: Congenital hypogonadotropic hypogonadism in five members of a family. Proc R Soc Med 47:442, 1954
5. Santen RJ, Paulsen CA: Hypogonadotropic hypogonadism: clinical study of mode of inheritance. J Clin Endocrinol Metab 36:47, 1973
6. Hammond MG, Asmal AC, Omar MAK: HLA typing and insulin-dependent diabetes in South African Negroes. Diabetologia 19:101, 1980
7. Sparkes RS, Simpson RW, Paulsen CA: Familial hypogonadotropic hypogonadism with anosmia. Arch Intern Med 121:534, 1968

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Associations between HLA Antigens and Nephrotic Syndrome in African and Indian Children in South Africa

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Key Words. Nephrotic syndrome · HLA associations · Children, Indian, African

Abstract. The nephrotic syndrome (NS) reported from Southern Africa is distinguished by unusual characteristics in African children and typical features among Indian children. A genetic basis for these differences is explored in 44 African and 33 Indian children with NS in this paper. HLA associations were detected in the 20 Indian children with minimal change NS (MCNS) and 12 African children with membranous NS. Previous studies of HLA antigens, which have all been performed on Caucasian children with MCNS or steroid-responsive NS (SRNS), have detected associations with HLAB and DR locus genes. In this report HLA Bw44, which is part of HLA B12, was found to be significantly more frequent in Indian children with MCNS or SRNS than in controls (45 and 12%, respectively, $p < 0.04$; relative risk 5.8). In contrast, African children with membranous nephropathy had a significantly increased frequency of HLA Bw21 (15% in patients and 1% in controls, $p < 0.04$; relative risk 22.1). HB_sAg was positive in 9 of 11 patients tested in the latter group. We conclude that the interaction between heredity and environmental factors is central to the pathogenesis of membranous nephropathy and similar considerations may be important in the development of MCNS.

Introduction

The spectrum of nephrotic syndrome (NS) in Durban, South Africa, offers a study in contrasts between the expected pattern of the disease as seen in children in most parts of the world and that peculiar to African children in the non-malarious zones of Africa [1].

The former is illustrated by Indian children, the majority of whom have typical minimal change nephrosis (MCNS). In comparison, African children have 'obvious' glomerular lesions, of which one of the commonest types is membranous nephropathy. The aetiology is unknown in the majority of these children except for a likely causal relationship between HB_sAg and membranous NS [2–4]. Significant associations have been detected between specific HLA antigens and MCNS [5], steroid-responsive NS (SRNS) [6–8], with [8, 9], and without [5, 7, 9], atopy and focal glomerulosclerosis [10]. We therefore investigated a possible genetic predisposition to the development of discrete categories of NS in Indian and African children in South Africa.

Patients and Methods

HLA frequencies were determined in a total of 77 nephrotic children, of whom 33 were Indian and 44 African. The nephrotic syndrome was defined according to 3 criteria: hypo-albuminaemia (<3 g/l), gross proteinuria (>2 g/m²/24 h or 3 g/l on random samples) and severe oedema. Patients were routinely investigated for most of the known causes of the NS including the detection of HB_sAg by radio-immuno-assay. Steroid responsiveness was equated with MCNS in Indian children. This is in accordance with the practice adopted by other workers [6, 7] and was supported by the excellent outcome on follow-up (more than 5 years in all children).

HLA Typing

The patients were typed for HLA A, B and C specificities using 180 antisera in a two-stage lymphocytotoxicity test [11]. Lymphocytes were isolated on Ficoll-Hypaque density gradient. Differences in HLA frequencies were tested for significance with a χ^2-test (without Yates correction), and the resulting probabilities were corrected by multiplying by the number of antigens tested. The relative risk was calculated according to the method of *Svejgaard* et al. [12].

Controls

There were 952 African and 856 Indian normal adult controls.

Table I. Percentage frequency of individual antigens tested in the HLA complex in Indian minimal change nephrotic children and adult controls

HLA type	Controls (n = 952)	Patients (n = 20)
A1	28	30
A2	32	40
A3	14	10
A11	27	15
Aw23	1	0
Aw24	29	15
A25	2	0
A26	7	0
A28	13	20
A29	1	0
Aw30	3	5
Aw31	3	0
Aw32	3	5
Aw33	10	25
One antigen	28	35
B7	13	15
B8	6	0
B13	7	10
B14	0.2	0
B15	10	30
Bw16	2	0
B17	21	20
B18	4	0
Bw21	2	5
Bw22	3	0
B27	2	0
Bw35	20	20
B37	5	5
Bw41/7/8	0.2	0
Bw44 (12)	12	45*
Bw45 (12)	0.2	0
Bw51	19	10
Bw52	12	10
Bw53	1	0
Bw60	16	5
Bw61	15	20
Y	25	5

*Significant difference: corrected $p < 0.04$.

Table II. Percentage frequency of individual antigens tested in the HLA complex in African membranous nephrotic children and adult controls

HLA type	Controls (n = 856)	Patients (n = 13)
A1	6	8
A2	20	31
A3	13	0
A11	0.2	0
Aw23	18	33
Aw24	5	0
A25	15	17
A26	9	8
A28	20	23
A29	18	23
Aw30	39	38
Aw31	8	8
Aw32	2	0
Aw33	1	0
One antigen	25	33
B7	19	4
B8	13	8
B13	4	0
B14	5	15
B15	5	8
Bw16	3	0
B17	39	31
B18	4	8
Bw21	1	15*
B27	0.4	0
Bw35	6	8
B37	0	0
Bw41/7/8	1	0
Bw42	25	0
Bw44 (12)	16	23
Bw45 (12)	8	0
Bw51	1	0
Bw52	0	0
Bw53	1	0
?40	1	0
Bu	8	23
Y	37	15

*Significant difference: corrected $p < 0.04$.

The HLA distribution among neonates, infants and adults of the African subjects have been shown to be similar and conform to Hardy-Weinberg equilibrium [13].

Results

Of the 33 Indian and 44 African children, significant HLA associations were detected in 20 Indian children with MCNS and 12 African children with membranous nephropathy. Only these results will be presented. Fourteen of the Indian MCNS and 11 of the African membranous NS cases were males. The mean age of the Indian children was 4.9 years and Africans 8 years. All the African and 13 Indian children had renal biopsies and 7 of the Indian children were steroid sensitive. Nine of the 11 African patients with membranous nephropathy were positive for HB_sAg.

The percentage frequency of individual antigens tested in the HLA complex in Indian MCNS and African membranous NS and their controls are shown in tables I

Table III. Significant HLA associations

Race	HLA	Histological type	Corrected p	Relative risk
Africans	Bw21	membranous	<0.04	22.1
Indians	Bw44	minimal change	<0.04	5.8

Table IV. Studies of significant HLA associations with nephrotic syndrome: with special reference to relative risk

NS	HLA antigens	RR	Reference
SRNS	B12 (atopic)	–	*Thomson* et al. [9]
SRNS	A1, B8,(non-atopic)	–	*Thomson* et a. [9]
MCNS	B8	2.81	*Noss* et al. [5]
SRNS	B8	3.5	*O'Regan* et al. [7]
SRNS	DR7 (atopic)	4.4	*de Mouzon-Cambon* et al. [8]
MCNS	B13	4.65	*Noss* et al. [5]
MCNS	Bw44 (12)	5.8	present study
SRNS	DRw7	5.9	*Alfiler* et al. [6]
MEM	Bw21	22.1	present study

SRNS = Steroid-responsive nephrotic syndrome; MCNS = minimal change nephrotic syndrome; MEM = membranous; RR = relative risk.

and II, respectively. The frequency of HLA Bw44 was increased in Indian MCNS compared to controls (45 vs. 12%, respectively, $p<0.04$; relative risk 5.8). The frequency of Bw21 was increased in African children with membranous nephropathy compared to healthy controls (15 vs. 1%, respectively, $p<0.04$; relative risk 22.1). There were no significant association between HLA antigen frequencies and the other histological groups among either Indians and Africans. Number of patients in most of these sub-groups are, however, small. Results are summarised in table III. Table IV compares the relative risk of developing NS (MCNS or SRNS) with particular HLA frequencies documented in other reports. The current study reveals a relative risk for the development of MCNS in the Indian child, which is close to the highest reported, and an exceedingly high risk for developing membranous nephropathy in the African child.

Discussion

Previous studies of HLA associations and NS [5–9, 14] have all been performed on Caucasian children with MCNS or SRNS. Most of these studies report associations between NS and HLA B and DR locus genes, while some of these show that the relationships are more pronounced in the presence of atopy and occasionally correlate with response to therapy.

In different studies HLA B12, HLA B8 and HLA DR7 have been significantly associated with SRNS. HLA B12 and HLA DR7 have been associated with atopy in patients with this disease and a significant relationship was also detected between HLA B12 and a shortened remission after cyclophosphamide therapy.

In different races, different antigens are found in linkage disequilibrium. HLA B8 is in linkage disequilibrium with HLA DR3 [15] and HLA B12 is in linkage disequilibrium with HLA DR7 in Caucasian populations [8]. The Indian population also shows linkage disequilibrium between these same antigens while in the African population different antigens are found in linkage disequilibrium [16].

We should perhaps note that the HLA B12 antigen can be split into two parts, Bw44 and Bw45. The frequency of Bw45 in the Caucasian and Indian populations is so low that HLA Bw44 and HLA B12 can be regarded as synonymous. In the African population, however, the frequency of Bw45 is about 8% and HLA Bw44 is about 16% [16].

Alfiler et al. [6] found that the increased frequency of HLA DR7 was not accompanied by that of HLA B12. In the present study the frequency of HLA Bw44 was significantly increased in Indian children with SRNS or MCNS (45% in patients vs. 12% in controls; $p<0.04$). The relative risk was 5.8. This means that the Indian child with HLA Bw44 is 5.8 times more susceptible to the development of MCNS as compared to an Indian child without HLA Bw44. This finding supports previous studies in Caucasian children [8, 9] in which an association with HLA B12 has been detected. Taken together, these results suggest that HLA DR7 (which was not tested in the current study) is probably the most important antigen in these relationships.

In contrast, the African children with membranous nephropathy had a significantly increased frequency of HLA Bw21 (15% in patients vs. 1% in controls; $p<0.04$). The relative risk in this group was 22.1. Therefore the African child carrying HLA Bw21 has a 22-fold chance over a child without HLA Bw21 of developing membranous nephropathy. Caucasian adults with membranous nephropathy have been shown to have an increased frequency of DR3 [17]. There is good evidence to suggest that even within the same racial group, adult membranous nephropathy is dissimilar to the childhood form of the disease [17]. Therefore, our findings in African patients

cannot be compared with studies in these adults. In a previous publication, HB_sAg had been causally linked with membranous nephropathy in African patients [2]. The HB_sAg has recently been regarded as an important cause of membranous nephropathy [3, 4]. Some of these patients are included in this study: of 11 membranous patients tested, 9 were positive for HB_sAg. The number of patients who were HB_sAg negative was too few to allow meaningful comparison in HLA frequencies between the two groups. The incidence of HB_sAg in Black adult males is 8.7%, females 3.9%, males 5–10 years 20% and females 5–10 years 21% [18] whereas in Indian technologists the incidence in the males was 0.85% and females 0.39% [19]. Figures are not available for Indian children. There is some evidence to suggest that there is a genetic predisposition to the development of HB_sAg infection [20].

In brief, the association of Indian MCNS with Bw44 reinforces our earlier observation [1] that Indian children resemble other Caucasian children in nearly all respects for this disease. Further, the results obtained in African patients imply that there is a genetic susceptibility among children to the development of membranous nephropathy in response to HB_sAg infection. These conclusions suggest that both heredity and environment may be important in the pathogenesis of membranous nephropathy. It will be important to note whether the use of HB_sAg vaccines will reduce complications, including membranous nephropathy, induced by this virus.

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References

1 Coovadia, H.M.; Adhikari, M.; Morel-Maroger, L.: Clinicopathological features of the nephrotic syndrome in South African children. Q. Jl Med. *48:* 77–91 (1979).
2 Adhikari, M.; Coovadia, H.M.; Crystal, V.: Extramembranous nephropathy in Black South African children. Ann. trop. Paed. *3:* 17–24 (1983).
3 Slusarczyk, J.; Michalak, T.; Nazarewicz-de Mezer, T.; Krawczyński, K.; Nowosławski, A.: Epimembranous glomerulonephritis associated with hepatitis B core antigen immune complexes. Am. J. Path. *98:* 29–39 (1980).
4 Nagy, J.; Bajtai, G.; Brasch, H.; et al.: The role of hepatitis B surface antigen in the pathogenesis of glomerulopathies. Clin. Nephrol. *12:* 109–116 (1979).
5 Noss, G.; Bachmann, H.J.; Olbing, H.: Association of minimal change nephrotic syndrome (MCNS) with HLA-B8 and B13. Clin. Nephrol. *15:* 172–174 (1981).
6 Alfiler, C.A.; Roy, L.P.; Doran, T.; Sheldon, A.; Bashir, H.: HLA DRw7 steroid responsive nephrotic syndrome of childhood. Clin. Nephrol. *14:* 71–74 (1980).
7 O'Regan, D.; O'Callaghan, U.; Dundon, S.; Reen, D.J.: HLA antigens and steroid responsive nephrotic syndrome of childhood. Tissue Antigens *16:* 47–51 (1980).
8 Mouzon-Cambon, A. de; Bouissou, F.; Dutau, G.; Barthe, P.H.; Parr, M.T.; Sevin, A.; Ohayon, E.: HLA-DR7 in children with idiopathic nephrotic syndrome. Correlation with atopy. Tissue Antigens *17:* 518–524 (1981).
9 Thomson, P.D.; Stokes, C.R.; Barratt, T.M.; Turner, M.W.; Soothill, J.F.: HLA antigens and atopic features in steroid responsive nephrotic syndrome of childhood. Lancet *ii:* 765–768 (1976).
10 Lenhard, V.; Dippell, J.; Müller-Wiefel, D.E.; Schröder, D.; Seidle, S.; Schärer, K.: HLA antigens in children with idiopathic nephrotic syndrome. Proc. Eur. Dial. Transplant. Ass. *17:* 673–677 (1980).
11 Terasaki, P.I.; McClelland, J.D.: Microdroplet assay of human serum cytotoxins. Nature, Lond. *204:* 992–1000 (1964).
12 Svejgaard, A.; Platz, P.; Ryder, L.P.; Staub-Nielson, L.; Thomsen, M.: HLA and disease associations. Transplant. Rev. *22:* 3–43 (1975).
13 Coovadia, H.M.; Wesley, A.G.; Hammond, M.; Kiepiela, P.: Measles, histocompatibility leukocyte antigen polymorphism and natural selection in humans. J. infect. Dis. *144:* 142–147 (1981).
14 Trompeter, R.S.; Barratt, T.M.; Kay, R.; Turner, M.W.; Soothill, J.F.: HLA atopy, and cyclophosphamide in steroid responsive childhood nephrotic syndrome. Kidney int. *17:* 113–117 (1980).
15 Bodmer, W.F.; Bodmer, J.G.: Evolution and function of the HLA system. Br. med. Bull. *34:* 309–316 (1978).
16 Terasaki, P.I.: Histocompatibility testing 1980, p. 955 (UCLA Tissue Typing Laboratory, Los Angeles 1980).
17 Mallick, N.P.; Short, C.D.; Manos, J.: Clinical membranous nephropathy (Editorial). Nephron *34:* 209–219 (1983).
18 Vos, G.H.; Rose, E.F.; Marimuthu, T.: Hepatitis B antigen and antibodies in rural and urban Southern African Blacks. S. Afr. med. J. *57:* 868–870 (1980).
19 Marimuthu, T.: Type B viral hepatitis in medical technologists. S.Afr. J. Med. Lab. Tech. *26:* 29–31 (1980).
20 Hillis, W.D.; Hillis, A.; Bias, W.B.; Walker, W.G.: Associations of hepatitis B surface antigenemia with HLA locus B specificities. New. Engl. J. Med. *296:* 1310–1314 (1977).

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JOINT REPORT : HLA AND DISEASE : SLE

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Introduction

Systemic lupus erythematosus(SLE) is an autoimmune connective tissue disease that has shown associations with HLA antigens. Tiwari and Terasaki reviewed an overall increase in B8 and an association with DR2 and DR3 in Caucasians (1). A report by Hashimoto (2) showed a positive association with DR2 and another by Kameda (3) a negative association with DR4 in Japanese but these results were not confirmed yet. In this report, patients with SLE from various ethnic groups in the Asian-Oceania region were tested for HLA using the same set of typing sera to find out whether HLA was associated with SLE or not.

Materials and Methods

The HLA types of 46 Japanese, 26 Northern Chinese, 82 Southern Chinese and 23 Northern Indian patients were determined. In addition 15 Australian Caucasoids, 8 African Black, one Sichuan Chinese and one Southern Indian were determined during the 3rd AOH Workshop. The diagnosis of SLE was made according to the reviced criteria of the American Rheumatism Association (4). The frequency of each antigen in the patient groups was compared with that of normal controls in the corresponding ethnic group. Calculation were done only in Japanese, Northern Chinese, Southern Chinese and Northern Indian, because of the small number of patients in other ethnic groups. A subgroup of Japanese patients with nephritis (lupus nephritis ;LN) was also analysed. HLA Class III antigens were determined in the Japanese patients by Dr. Serjeantson and compared with those of Japanese normal controls. The statistical significance of the difference in frequency of each HLA antigen between patiens and controls was determined by chi square calculation and P value was corrected (Pc) by multiplying by the number of tested antigens. Relative risk (RR), etiologic fraction (EF) and preventive fraction (PF) were calculated according to Svejgaard et al (5).

Results and Discussions

HLA antigens positively or negatively associated with SLE with a P value less than 0.05 are listed in the following Tables. In Table 1, HLA antigens associated with total SLE as well as lupus nephritis in Japanese are listed. The frequency of A24 was decreased and Bw6 was increased in SLE patients and A11, A31 and Bw54 were increased in the

subgroup with nephritis but the only difference that was still significant after correction was the decreased frequency of DQW3. The frequency of DR2 was higher in SLE than in controls (46% vs 35%) in Japanese without significance. As shown in Table 2, the frequency of C4A3 and C4B0 was lower in the patient group while C4A0 was increased as has been reported in Caucasian patients (6). In Northern Chinese statistically higher occurrence of HLA-B15 and DR2 among patients with SLE was observed as shown in Table 3. CW1 and DQW1 were also observed more frequently in SLE than controls, probably due to linkage disequilibrium. DR2 was also significantly more frequent in SLE in Southern Chinese. The tendency of higher occurence of B15 was observed, too, but it was not significant after correction (Table 4). The higher frequency of BW4 in Southern Chinese was different from results of the other ethnic groups, in which rather BW6 was increased in frequency. Amongst the Indian patients, SLE was associated rather strongly with DR4 and B37, (Table 5). Since B37 is rather infrequent in Northern Indians the EF of this antigen was rather low. Almost 100% occurence of BW6 was observed in patients with SLE including the 15 Australian Caucasians and 8 African Blacks, except Southern Chinese patients as mentioned above.

Conclusion

HLA-DR2 was primarily associated with SLE in the Chinese and probably in the Japanese as well. DR4 was the primary antigen associated with SLE patients from Northern India. The association of C4A0, a class III antigen, with SLE in Japanese as well as in Caucasians is suggestive of a common aetiology.

References

(1) Tiwari JL and Terasaki PI : HLA and disease association. p363 Springer-Verlag, 1985.

(2) Hashimoto H, Tsuda H, Matsumoto T et al : HLA antigens associated with systemic lupus erythematosus in Japan. J Rheumatol, 12:919,1985.

(3) Kameda S, Naito S, Tanaka K et al : HLA antigens of patients with systemic lupus erythematosus in Japan. Tissue Antigens, 20:221,1982.

(4) Tan EM, Cohen AS, Fries JF et al : The 1982 revised criteria for the classification of systemic lupus erytematosus. Arthritis Rheum, 25:1271,1982.

(5) Svejgaard A, Platz P and Ryder LP : HLA and disease 1982-A survey. Immunological Rev, 70:193,1983.

(6) Reveill JD, Arnett FC et al : Null Alleles of the Fourth Component of Complement and HLA Haplotypes in Familial Systemic Lupus Erythematosus. Immunogenetics. 1985;21;299-311

Table 1 HLA Antigens associated with SLE(Japanese)

HLA	Disease	Freq.(%) in Pat.	Freq.(%) in Cont.	RR	EF	PF	P	Pc
A11	SLE	26	17	1.7	0.11	-	NS	NS
	LN	35		2.7	0.22	-	0.05	NS
A31	SLE	20	13	1.7	0.07	-	NS	NS
	LN	29		2.9	0.19	-	0.05	NS
BW54	SLE	24	19	1.9	0.11	-	NS	NS
	LN	35		3.3	0.24	-	0.025	NS
BW6	SLE	100	89		-	-	0.025	NS
	LN	100			-	-	NS	NS
A24	SLE	48	68	0.43	-	0.34	0.01	NS
	LN	47		0.42	-	0.39	NS	NS
DQW3	SLE	24	55	0.26	-	0.40	0.0002	0.005
	LN	18		0.17	-	0.46	0.003	0.05

SLE : SLE TOTAL N=46
LN : Lupus nephritis N=17
Control N=404

Table 2 HLA Class III Antigens associated with SLE(Japanese)

Class III	Freq.(%) in Pat.	Freq.(%) in Cont.	RR	EF	PF	P	Pc
C4A3	67	88	0.3	-	0.63	0.02	NS
C4A0	27	8	4.3	0.2	-	0.02	NS
C4B0	2	12	0.1	-	0.01	0.04	NS

Pat. : Patients with SLE N=55
Cont. : Normal Control N=50

Table 3 HLA Antigens associated with SLE(Chinese North)

HLA	Freq.(%) in Pat.	Freq.(%) in Cont.	RR	EF	PF	P	Pc
B15	50	20	4.0	0.37	-	0.0004	0.02
CW1	46	23	2.7	0.29	-	0.02	NS
DR2	69	33	4.4	0.53	-	0.0003	0.004
DQW1	84	59	3.7	0.61	-	0.02	NS

SLE N=26 Controls N=405-431

Table 4 HLA Antigens associated with SLE(Chinese South)

HLA	Freq.(%) in Pat.	Freq.(%) in Cont.	RR	EF	PF	P	Pc
A10	0	7	0	-	-	0.009	NS
B15	33	21	1.8	0.15	-	0.02	NS
B16	16	7	2.3	0.09	-	0.02	NS
BW4	64	39	2.8	0.41	-	0.0003	0.01
BW6	98	82	12.3	0.90	-	0.002	NS
DR2	52	30	2.4	0.30	-	0.002	0.03
DR4	13	26	0.4	-	0.15	0.03	NS
DR5	15	27	0.4	-	0.15	0.04	NS
DQW1	67	48	2.2	0.37	-	0.006	NS
DQW2	11	4	3.0	0.07	-	0.02	NS

SLE N=61-82 Control N=313-356

Table 5 HLA Antigens Associated with SLE(Indian North)

HLA	Freq.(%) in Pat.	Freq.(%) in Cont.	RR	EF	PF	P	Pc
A23	8.7	0.8	11.5	0.07	-	0.02	NS
B37	17	0.8	25.6	0.16	-	0.0001	0.006
CW4	43	21	2.8	0.28	-	0.02	NS
DR4	47	7	11.5	0.43	-	0.00001	0.0001
DR7	0	24	0	-		0.008	NS

SLE N=23 Control N=123

THYROID DISEASE : JOINT REPORT

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Introduction

Thyrotoxic Graves' disease is a relatively common disorder in many ethnic groups and its association with the HLA system has been studied in some detail. Associations with B8 and DR3 have been shown consistently in Caucasians, and DR3 is particularly associated with relapse of the disease following withdrawal of therapy (see review in 11). In Japanese the disease is associated with B35[4,6], especially in patients with disease onset below the age of 30 years[8] but there is no clear association with HLA-DR antigens. Similarly in Chinese, there is a strong association with HLA Bw46[1,5] particularly in patients with early-onset disease, but there is no clear association with HLA-DR antigens. Also in Chinese there is evidence for an association with B5 in patients with disease onset above age 35 years[5]. Thyrotoxic periodic paralysis is a common complication of Graves' disease in Chinese and the association with HLA-Bw46 is particularly strong in patients with this complication[5].

Hashimoto's thyroiditis is less common than Graves' disease in all ethnic groups, and rather less information is available on HLA associations with this disease. In Caucasians there is a weak association with B8[3] and a significant association with DR3[7]. In Japanese there is an association with B35[8] but there is no clear association with HLA-DR antigens. In Chinese there is some evidence for an association of Hashimoto's thyroiditis with Bw46 and B5[12].

Aims of the study

1. Graves' Disease

HLA associations with Graves' disease are fairly clear in Caucasians but there is a need in non-Caucasians in the Region to:-

a) determine whether HLA associations exist in previously unstudied populations
b) clarify previously reported HLA-DR associations
c) confirm HLA associations with early and late onset disease
d) determine whether HLA associations exist with relapse following withdrawal of therapy.

2. Hashimoto's thyroiditis

The major aims were to clarify and confirm previously reported HLA-B associations in non-Caucasians and to determine whether HLA-DR associations exist in these ethnic groups.

Materials and Methods

Contributors were invited to HLA type as many patients as practicable with Graves' disease and/or Hashimoto's thyroiditis using the 3AOH serum set and to include an adequate number of normal controls. Since the major aim was to confirm or clarify previous findings, contributors were requested to use patients who had not been previously included in published surveys.

Contributors were requested to complete a brief questionnaire on each patient giving details of disease category, clinical features, age at onset, associated diseases, and history of relapse if therapy had been withdrawn.

Results

Table 1 provides a summary of the patient categories submitted by each laboratory. Unfortunately, some of the questionnaire data were not available at the time of preparation of this report and complete analyses were not always possible.

Graves' disease

Table 2 shows a comparison of antigen frequencies in controls and patients with Graves' disease for antigens shown to be of interest in previous studies. There was a slight increase in the frequencies of Bw46 and DRw9 in Northern Chinese patients but not at a statistically significant level. In Southern Chinese patients, however, there was a highly significant increase in the frequencies of DRw9 and Bw46 conferring relative risks of 2.6 and 2.2 respectively. In Thai patients there was an extremely significant excess of Bw46 (relative risk 4.0). In all ethnic groups studied B5 was shown to have a negative association with Graves' disease but in no case was this statistically significant.

In view of the small numbers of patients of other ethnic groups only speculative suggestions may be made as to possible HLA associations with Graves' disease in these ethnic groups. Of the 11 African Black patients 45.4% had A23 compared with 20.9% of controls, 54.5% had Bw58 compared with 39.2% of controls, and DR3 had the same frequencies in patients and controls as Bw58. Of the 8 Southern Indian patients 62.5% had A11 compared with 24.6% of controls and 62.5% had B35 compared with 26.6% of controls. In Northern Indian patients 6 of the 7 (86%) had DR2 compared with 45% of controls.

Age at onset data was not available for the Southern Chinese patients with Graves' disease although it is reported elsewhere in this volume that the frequency of Bw46 was significantly higher in patients with disease onset below age 30 years[2].

Table 3 shows the age at onset for Thai and Thai Chinese patients with Graves' disease. In both Thai and Thai Chinese patients it may be seen that Bw46 had an increased frequency irrespective of age at onset whereas the increased frequency of DRw9 appeared to relate particularly to early-onset disease.

There was insufficient data available to compare the HLA associations in patients with and without periodic paralysis or relapse following withdrawal of therapy.

Additional information on patients with Graves' disease included in this study is given in references 2 and 10.

Hashimoto's thyroiditis

Table 4 shows the frequencies of B5, Bw46 and DRw9 in patients with Hashimoto's thyroiditis. In Southern Chinese both Bw46 and DRw9 were strongly associated with the disease, conferring relative risks of 3.1 and 2.6 respectively, whereas in Sichuan Chinese only Bw46 had a statistically significant association.

There were no statistically significant associations between Hashimoto's thyroiditis and Bw46 or DRw9 in either Thai or Thai Chinese.

There was no evidence for an association with B5 in any of the ethnic groups studied.

Additional information on patients with Hashimoto's thyroiditis studied as part of this Workshop is given in references 9 and 10.

Conclusions

Graves' disease

Bw46 has a statistically significant association with Graves' disease in Southern Chinese and Thais but not in Thai Chinese or Northern Chinese. DRw9 is strongly associated with Graves' disease in Southern Chinese but not in the other ethnic groups studied.

Hashimoto's thyroiditis

Bw46 is strongly associated with Hashimoto's thyroiditis in Southern Chinese and Sichuan Chinese but not in Thai or Thai Chinese. DRw9 is strongly associated with this disease in Southern Chinese but not the other ethnic groups studied.

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References

1. Chan, S.H., Yeo, P.P.B., Lui, K.F., Wee, G.B., Woo, K.T., Lim, P. & Cheah, J.S. (1978) HLA and thyrotoxicosis (Graves' disease) in Chinese. *Tissue Antigens*, 12, 109-114.

2. Chan, S.H., Yeo, P.P.B., Tan, S.H., Wee, G.B., Lui, K.F. & Cheah, J.S. (1986) Bw46 and DRw9 associations in Singaporean Chinese Graves' disease. Third Asia-Oceania Histocompatibility Workshop Conference. Abstract 119.

3. Farid, N.R., Barnard, J.M. & Marshall, W.H. (1976) The association of HLA with autoimmune thyroid disease in Newfoundland. The influence of HLA homozygosity in Graves' disease. *Tissue Antigens*, 8, 181-189.

4. Grumet, F.C., Payne, R.O., Konishi, J., Mori, T. & Kriss, J.P. (1975) HL-A antigens in Japanese patients with Graves' disease. *Tissue Antigens*, 6, 347-352.

5. Hawkins, B.R., Ma, J.T.C., Lam, K.S.L., Wang, C.C.L. & Yeung, R.T.T. (1985) Association of HLA antigens with thyrotoxic Graves' disease and periodic paralysis in Hong Kong Chinese. *Clin. Endocrinol.*, 23, 245-252.

6. Kawa, A., Nakamura, S., Nakazawa, M., Sakaguchi, S., Kawabata, T., Maeda, Y. & Kanehisa, T. (1977) HLA-Bw35 and B5 in Japanese patients with Graves' disease. *Acta Endocrinol.*, 86, 754-757.

7. Moens, H. & Farid, N.R. (1978) Hashimoto's thyroiditis is associated with HLA-DRw3. *N. Engl. J. Med.*, 299, 133-134.

8. Nakao, Y., Kishihara, M., Baba, Y., Kuma, K., Fukunishi, T. & Imura, H. (1978) HLA antigens in autoimmune thyroid diseases. *Arch. Int. Med.*, 138, 567-570.

9. Pei, J., Yang, Zh., Zhu, D.Y., Hou, Zh. Ch., Sun, S.X., Mi, X.Y., Wang, L.H. & Han, L.Y. (1986) HLA and Hashimoto's thyroiditis in Sichuan Chinese. Third Asia-Oceania Histocompatibility Workshop Conference. Abstract 77.

10. Sridama, V., Charoenwongse, P., Kangwanshiratada, O. & Nopporpunth, V. (1986) HLA association and autoimmune thyroid diseases in Thai population. Third Asia-Oceania Histocompatibility Workshop Conference. Abstract 78.

11. Tiwari, J.L. & Terasaki, P.I. (1985) HLA and disease associations. New York: Springer Verlag pp 214-220.

12. Yeo, P.P.B., Chan, S.H., Lui, K.F., Wee, G.B., Cheah, J.S. & Lim, P. (1983) HLA and Chinese patients with thyroid diseases. In *Current Problems in Thyroid Research. Proceedings of the Second Asia and Oceania Thyroid Association Meeting, Tokyo, August 19-22, 1982* (eds. N. Ui, K. Torizuka, S. Nagataki & M. Miyai), pp. 325-328. Excerpta Medica, Amsterdam.

Table 1

Summary of data submitted to 3AOH thyroid study

Ethnic origin	Lab Code	Number of patients studied: Graves' disease	Hashimoto's thyroiditis
Northern Chinese	SYP	45	-
	YGY	26	-
Southern Chinese	CSH	62	-
	HAW	-	48
Sichuan Chinese	PEJ	-	59
Thai Chinese	CHA	36	18
Thai	CHA	55	27
African Blacks	HAM	11	-
Southern Indian	HAM	8	-
Northern Indian	HAM	7	-

Table 2

Distribution of selected HLA antigens in patients with Graves' disease

Ethnic origin	Antigen	Patients obs	%	Controls obs	%	RR*	χ^2
Northern Chinese		(n=71)		(n=430)			
	B5	13	18.3	92	21.4	0.8	0.19
	Bw46	15	21.1	55	12.8	1.8	2.86
	DRw9	25	35.2	105	25.3	1.7	3.15
Southern Chinese		(n=62)		(n=407)			
	B5	5	8.1	65	16.0	0.5	2.1
	Bw46	28	45.2	112	27.6	2.2	7.18
	DRw9	32	51.6	120	32.5	2.6	11.04
Thai Chinese		(n= 36)		(n=86)			
	B5	2	5.5	7	8.1	0.6	0.01
	Bw46	13	36.1	17	19.8	2.3	2.83
	DRw9	11	30.5	22	25.6	1.3	0.12
Thai		(n= 55)		(n=138)			
	B5	2	3.6	17	12.3	0.3	2.43
	Bw46	25	45.5	24	17.4	4.0	14.9
	DRw9	17	30.9	25	18.1	2.0	3.07

* RR = Relative risk

Table 3

Distribution of selected HLA antigens in patients with Graves' disease of early and late onset

Ethnic origin	Antigen	Early onset (<30 years) obs	%	Late onset (>30 years) obs	%	Controls obs	%
Thai Chinese		(n=19)		(n=17)		(n=86)	
	B5	0	0	2	11.8	7	8.1
	Bw46	7	36.8	6	35.3	17	19.8
	DRw9	7	36.8	4	23.5	22	25.6
Thai		(n=30)		(n=25)		(n=138)	
	B5	1	3.3	1	4.0	17	12.3
	Bw46	16	53.3	9	36.0	24	17.4
	DRw9	12	40.0	5	20.0	25	18.1

Table 4

Distribution of selected HLA antigens in patients with Hashimoto's thyroiditis

Ethnic origin	Antigen	Patients obs	%	Controls obs	%	RR*	χ^2
Southern Chinese		(n=48)		(n=407)			
	B5	4	8.3	65	16.0	0.4	1.40
	Bw46	26	54.2	112	27.6	3.1	13.19
	DRw9	25	52.1	120	32.5	2.6	9.09
Sichuan Chinese		(n=59)		(n=145)			
	B5	9	15.3	18	12.4	1.3	0.09
	Bw46	30	50.9	40	27.6	2.7	9.06
	DRw9	28	47.5	57	39.3	1.4	0.8
Thai Chinese		(n=18)		(n=86)			
	B5	4	22.2	7	8.1	3.2	1.81
	Bw46	7	38.9	17	19.8	2.6	2.08
	DRw9	8	44.4	22	25.6	2.3	1.74
Thai		(n=27)		(n=138)			
	B5	3	11.1	17	12.3	0.9	0.02
	Bw46	9	33.3	24	17.4	2.4	2.66
	DRw9	7	25.9	25	18.1	1.6	0.45

* RR = Relative risk

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Histocompatibility antigens in Indian patients with myocardial infarction

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The frequency of HLA-A, B, C and DR tissue antigens in 103 Indian men aged 40 years or under who had experienced a myocardial infarction was compared with the frequency in 760 healthy Indian controls. No significant differences in antigen frequencies were found. The findings in this study provide no support for either a genetic or an immunological basis for myocardial infarction in young Indian men.

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Hypertension, hyperlipidaemia, smoking, diabetes mellitus are well known risk factors for the development of myocardial infarction (Kannel & McGee 1979). The frequent occurrence of coronary events in family members is well established and suggests that genetic factors may contribute to the development of coronary artery disease (Epstein 1964). The mechanism by which heredity exerts an influence on the incidence of coronary artery disease has been questioned for many years. It is uncertain whether the familial aggregation of coronary heart disease is mediated by familial clustering of risk factors or by some unknown mechanism (Schweitzer et al. 1962, McKwick 1959). The studies investigating the relationship of histocompability (HLA) antigens to the presence of coronary artery disease have not indicated a clear association (Scott et al. 1976, Logan et al. 1977). Significant correlations have been demonstrated between the frequency of the antigen HLA-B8 and A1-B8 and the death rate from coronary artery disease (Mathews 1975). Other investigators have been unable to confirm this association (Scott et al. 1976, Logan et al. 1977).

We therefore undertook this study in which the frequencies of HLA tissue antigens in young Indian males with myocardial infarction was compared with those in a healthy control group.

Material and methods

One hundred and three Indian male patients with myocardial infarction between the ages of 24–40 years (mean 36 yrs) were selected for HLA typing. The criteria for the diagnosis of myocardial infarction were based on a history of chest pain supported by unequivocal electrocardiographic findings accompanied by a transient rise in creatine kinase activity. The myocardial infarction was presumed to be due

to coronary atherosclerosis. The presence of diabetes mellitus, hypertension, hyperlipidaemia (serum cholesterol > 6.5 mmols/l) and history of coronary heart disease in first degree relatives were obtained from all patients. None of the patients were insulin dependent diabetics. Patients with valvular heart disease and cardiomyopathy were excluded. Of the 103 patients 48 were of North Indian origin (Aryans), 43 of South Indian origin (Dravidians) and 12 could not be classified into either of these two groups.

HLA-A, B and C antigens were determined in all patients and in 876 controls. The latter comprised of 323 Aryans, 478 Dravidians, and 75 could not be classified. The HLA-DR antigens were determined in 93 patients (Aryans = 41; Dravidians = 41, unclassified = 11) and 165 controls (Aryans = 36; Dravidians = 121; unclassified = 8).

The patients were typed for HLA-A, B and C antigens using 180 antisera in a two-stage lymphocytotoxic test (Terasaki & McClelland 1964). HLA-DR antigens were detected on B lymphocytes with 120 antisera in a long incubation two-stage lymphocytotoxic test (Terasaki et al. 1978). Lymphocytes were isolated on a Ficoll-Hypaque density gradient (Boyum 1968) and T and B cells separated by the nylon wool method (Terasaki et al. 1978).

All the patients and controls were typed in the laboratories of the Natal Institute of Immunology with antisera that have been used in International Workshops or obtained by serum exchange from other laboratories. (Hammond et al. 1975, 1977, 1980 a–e, 1984 a–e, 1986 a–c).

Statistical analysis was performed using the chi-squared test. The resulting probabilities were multiplied by the number of antigens tested to determine the corrected p value.

Table 1.
Frequency of HLA-A and B antigens in Indian males with myocardial infarction.

HLA antigen	Frequency (%) Controls (n=876)	Patients (n=103)
A1	28.1	28.2
A2	30.9	31.1
A3	13.6	13.6
A11	28.5	33.0
A23	1.3	1.9
A24	28.9	28.2
A25	1.7	1.0
A26	6.2	3.9
A28	12.3	13.6
A29	1.6	0.0
A30	2.3	1.9
A31	3.8	5.8
A32	4.9	2.9
A33	16.2	14.6
One antigen	19.8	20.4
B7	13.4	7.8
B8	6.4	6.8
B13	6.4	6.8
B14	0.6	0.0
B15	10.4	12.6
B16	3.8	1.9
B17	21.2	19.4
B18	2.9	3.9
B21	3.5	3.9
B22	5.0	2.9
B27	1.9	3.9
B35	20.4	14.6
B37	6.1	5.8
B41	0.2	1.0
B42	0.1	0.0
B44	13.5	16.5
B45	0.2	0.0
B47	0.1	0.0
B51	16.4	17.5
B52	14.3	14.6
B53	0.5	0.0
B60*	10.3	20.4
B61	19.0	20.4
B70	4.0	4.9
One antigen	15.9	12.6

* = $p < 0.005$.

Results

The frequencies of the HLA antigens in patients with myocardial infarction and in the control group are shown in Tables 1 and 2. At the A and DR loci no antigen showed an observed frequency significantly different from the control population. At the B locus B60 has a significantly greater frequency in the patients than the controls ($p < 0.005$). This difference was not significant when correction was made for the number of antigens tested (50 antigens). Furthermore there was no significant difference in the frequency of HLA-A, B, C and DR antigens between patients with a history of diabetes, hypertension, hyperlipidaemia and coronary artery disease in first degree relatives as compared to those patients without these risk factors. Similarly, the frequency of HLA antigens studied was not significantly different in the Aryan and Dravidian patients and their respective control populations. However B7 and DR1 antigens were observed to occur with decreased frequency in the Aryans with myocardial infarction as compared to the Aryan control group (0% vs 11.2% and 2.4% vs 19.5%, respectively) but this was not statistically significant when the p value was corrected for the number of antigens tested.

Table 2.
Frequency of HLA-DR antigens in Indian males with myocardial infarction.

HLA antigen	Frequency (%) Controls (n=165)	Patients (n=93)
DR1	8.5	8.6
DR2	37.6	47.3
DR3	13.9	15.1
DR4	23.0	19.4
DR5	15.8	11.8
DR6	18.8	21.5
DR7	31.5	30.1
DR8	3.6	3.2
DR9	1.2	0.0
DR10	10.3	12.9
DR12	0.6	0.0
One antigen	35.2	30.1

Discussion

Although associations between specific diseases and HLA antigens have been well documented for certain disorders (Ritzman 1976), no such clear association has yet been established between coronary artery disease and the HLA antigens. Stone et al. (1981) demonstrated a statistically significant frequency of HLA-BW 38 in patients with premature coronary artery disease but this statistical significance was lost when allowance was made for the number of antigens tested. The findings of Mathews (1975) were based on mortality figures.

Our data, in accordance with others have also failed to demonstrate a significantly increased incidence of any HLA antigens in patients with myocardial infarction (Scott et al. 1976, Logan et al. 1977). Although the frequency of the antigen HLA-B60 in the patients (20.4%) was significantly higher than the controls (10.3%) the significance was lost when the p value was multiplied by the number of antigens (50) tested. Comparing the HLA profiles of North and South Indians revealed a similar trend viz no difference in the frequency of the HLA antigens between patients and their respective controls.

Our failure to demonstrate a significant increase in the frequency of any HLA antigen in patients with myocardial infarction probably serve to highlight the heterogenicity of factors involved in the genesis of coronary artery disease. Also it is possible that since we are dealing with a common disease the control population included individuals who would have later in life developed a myocardial infarction.

Also the genetic influence on any common disease may be affected by environmental factors (Rose 1977). Hence without proper control of these factors it may be difficult to identify genetically the high risk group for coronary artery disease.

In conclusion this study has demonstrated that there appears to be no clear association between the HLA antigens and myocardial infarction in young Indian men and hence gives no support for either a genetic predisposition or for an immunological basis for myocardial infarction in our patients.

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References

Boyum, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand J Lab Invest* **21**, 97–98.

Epstein, F. H. (1964) Hereditary aspects of coronary heart disease. *Am Heart J* **67**, 445–456.

Hammond, M. G., Appadoo, B. & Brain, P. (1975) HLA antigens in Bantu and Indians. In *Histocompatibility Testing 1975*, ed. Kissmeyer-Nielsen, F. p. 173, Munksgaard, Copenhagen.

Hammond, M. G., Appadoo, B. & Brain, P. (1977) HLA in non-Caucasian populations. In *Histocompatibility Testing 1977*, eds. Bodmer, W. F. et al. p. 407, Munksgaard, Copenhagen.

Hammond, M. G. (1980a) Further splits of HLA B5. In *Histocompatibility Testing 1980*, ed. Terasaki, P. I. p. 758. UCLA Tissue Typing Laboratory, Los Angeles.

Hammond, M. G. & Appadoo, B. (1980b) Confirmation of ST-1 in Asian Indians. In *Histocompatibility Testing 1980*, ed. Terasaki, P. I. p. 844. UCLA Tissue Typing Laboratory, Los Angeles.

Hammond, M. G. (1980c) Hetetogeneity of HLA B40. In *Histocompatibility Testing 1980*, ed.Terasaki, P. I. p. 782. UCLA Tissue Typing Laboratory, Los Angeles.

Hammond, M. G. & Lamm, L. (1980d) A/C crossover in a South African Indian family. In *Histocompatibility Testing 1980*, ed. Terasaki, P. I. p. 794. UCLA Tissue Typing Laboratory, Los Angeles.

Hammond, M. G. (1980e) HLA-Bw53. In *Histocompatibility Testing 1980*, ed. Terasaki, P. I. p. 429. UCLA Tissue Typing Laboratory, Los Angeles.

Hammond, M. G. (1983a) HLA-Bw35. In *Proceedings of the Second Asia-Oceania Histocompatibility Workshop*, eds. Simons, M. J. & Tait, B. D. p. 112. Melbourne, Australia.

Hammond, M. G. (1983b) HLA-Bw53. In *Proceedings of the Second Asia-Oceania Histocompatibility Workshop*, eds. Simons, M. J. & Tait, B. D. p. 65. Melbourne, Australia.

Hammond, M. G. (1983c) HLA-Cw4. In *Proceedings of the Second Asia-Oceania Histocompatibility Workshop*, eds. Simons, M. J. & Tait, B. D. p. 138. Melbourne, Australia.

Hammond, M. G. (1983d) Subdivision of HLA-B15 in Indians. In *Proceedings of the Second Asia-Oceania Histocompatibility Workshop*, eds. Simons, M. J. & Tait, B. D. p. 413. Melbourne, Australia.

Hammond, M. G. (1983e) Anomalous reactions with Bw4 sera in Indian families. In *Proceedings of the Second Asia-Oceania Histocompatibility Workshop*, eds. Simons, M. J. & Tati, B. D. p. 411. Melbourne, Australia.

Hammond, M. G., Betuel, H. & Gebuhrer, L. (1984a) HLA-A29. In *Histocompatibility Testing 1984*, eds. Albert, E. D. et al. p. 126, Springer-Verlag, Berlin.

Hammond, M. G. (1984b) Definition of Bw53 in South African Indians. *Ninth International Histocompatibility Workshop Newsletter* **VI**, 6.

Hammond, M. G. (1984c) The HLA-A10 and Aw19 complex in South African Indians and Negroes. *Ninth International Histocompatibility Workshop Newsletter* **VII**, 6.

Hammond, M. G. (1984d) HLA B15 complex in South African Indians. *Ninth International Histocompatibility Workshop Newsletter* **VIII**, 4.

Hammond, M. G. (1984e) Short Bw41. *Ninth International Histocompatibility Workshop Newsletter* **VIII**,, 9.

Hammond, M. G. (1986a) Antigen report A3. *Pro-*

ceedings of the Third Asia-Oceania Histocompatibility Workshop (in press).

Hammond, M. G. (1986b) Antigen report A11. *Proceedings of the Third Asia-Oceania Histocompatibility Workshop* (in press).

Hammond, M. G. & Appadoo, B. (1986c) HLA antigens in African Blacks. *Proceedings of the Third Asia-Oceania Histocompatibility Workshop* (in press).

Kannel, W. B. & McGee, D. L. (1979) Diabetes and cardiovascular risk factors: the Framingham study. *Circulation* **59**, 8–13.

Logan, R. L., Oliver. M. F., McTavish, J., Darg, C. & White, A. G. (1977) Histocompatibility antigens and myocardial infarction. *Tissue Antigens* **10**, 361–363.

Mathews, J. D. (1975) Ischaemic heart disease: possible genetic markers. *Lancet* **ii**, 681–682.

McKwick, V. A. (1959) Genetic factors in cardiovascular disease: I The four major types of cardiovascular disease. *Mod Concepts Cardiovasc Dis* **28**, 535–542.

Ritzman, S. E. (1976) HLA patterns and disease associations. *JAMA* **236**, 2305–2309.

Rose, G. (1977) Ischaemic heart disease. *J Med Genet* **14**, 330–331.

Schweitzer, M. D., Clark, G., Gearing, F. R. & Perera, G. A. (1962) Genetic factors in primary hypertension and coronary artery disease: a reappraisal. *J Chronic Dis* **15**, 1093–1108.

Scott, B. B., McGuffin, P., Rajah, S. M., Stoker, J. B. & Losowsky, M. S. (1976) Histocompatibility antigens and myocardial infarction. *Tissue Antigens* **7**, 187–188.

Stone, P. H., Sherrid, M. V. & Cohn, K. E. (1981) Correlation of HLA types in premature coronary artery disease: an attempt to define independent genetic risk factors. *Chest* **79**(4), 381–385.

Terasaki, P. I. & McClelland, J. D. (1964) Microdroplet assay of human serum cytotoxins. *Nature* **204**, 998–1000.

Terasaki, P. I., Bernoco, D., Park, M. S., Ozturr, G. & Iwaki, Y. (1978) Microdroplet testing for HLA-A, B, C, and D antigens. *Am J Clin Pathol*, **69**, 103–120.

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HLA-A, B, DR, and DQ antigens in black patients with severe chronic rheumatic heart disease

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ABSTRACT To determine whether genetic factors could be involved in the pathogenesis of rheumatic heart disease, we performed HLA-A and HLA-B typing in 120 black patients with severe chronic rheumatic heart disease requiring cardiac surgery, and HLA-DR and HLA-DQ typing in 103 and 97 of these patients, respectively. The HLA typing was done by a standard microlymphocytotoxicity method. Patients were 12 to 60 years old (mean 27.6 ± 14.5). No differences in HLA-A, HLA-B, and HLA-DQ frequencies between patients and controls were noted. HLA-DR 1 antigen was present in 12.6% of patients compared with 2.7% of normal control subjects (corrected $p<.045$; relative risk = 5.2) and the HLA-DRw6 antigen was present in 31.1% of patients compared with 15% of control subjects (corrected $p<.045$; relative risk = 2.6). These findings suggest that genetically determined immune-response factors may play a role in the pathogenesis of severe chronic rheumatic heart disease.
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THE HLA antigens, which are encoded by closely arranged genes on the short arm of the sixth chromosome, influence the predisposition to several diseases.[1] Some diseases with initially weak associations with HLA-A and HLA-B antigens have been found to have stronger associations with HLA-DR antigens.[2] Since a genetic predisposition to the development of rheumatic fever has been documented,[3,4] and since there is little information on the relationship between antigens at the DR-locus of the HLA system and chronic rheumatic heart disease, we performed HLA typing in a group of black patients with this disease to determine whether genetic factors could be involved in the pathogenesis of rheumatic heart disease.

Patients and methods

HLA-A and HLA-B typing was carried out in 120 black patients with severe chronic rheumatic heart disease, as defined by the World Health Organization,[5] who required cardiac surgery at the Cardiothoracic Surgical Unit, Wentworth Hospital, Durban, and HLA-DR and HLA-DQ typing was performed in 103 and 97 of these patients, respectively. The distribution of valvular lesions was as follows: isolated mitral stenosis, 36 patients; mitral stenosis plus aortic incompetence, one patient; mitral stenosis and aortic incompetence with aortic stenosis (mixed aortic valve disease), one patient; mitral incompetence alone, five patients; mitral plus aortic incompetence, 18 patients; mitral stenosis with mitral incompetence (mixed mitral valve disease), 32 patients; mixed mitral valve disease plus aortic incompetence, 17 patients; mixed mitral valve disease plus mixed aortic valve disease, three patients; mixed aortic valve disease, three patients; isolated aortic incompetence, four patients. In each case the rheumatic etiology of the valve lesions was confirmed by inspection of the valve at surgery or on histologic examination of the valve. Many patients were having their second or third operation. There were 80 female and 40 male patients between 12 and 60 years old. Their mean age was 27.6 ± 14.5 years; 60% of the patients fell within the 12 to 25 year age group. The control group consisted of 1416 normal adults for the HLA-A and HLA-B typing, 220 for the HLA-DR and 64 for the HLA-DQ typing. Although over 2000 individuals have been tested for the HLA-DQ locus in our laboratory the majority were Caucasoid or patients with selected diseases.[6] As a consequence, only 64 normal healthy black individuals had undergone DQ typing.

The HLA-A and HLA-B antigens were identified with a two-stage lymphocytotoxicity test.[7] These antigens were defined with 180 antisera, which consisted of local serum samples that had been requested for use in International Histocompatibility Workshops, local samples that had been verified by use in parallel with the International Workshop samples, and samples that had been exchanged with other laboratories worldwide.[8–12] Similarly, 120 serum samples were used to define the HLA-DR and HLA-DQ antigens in B cell–enriched lymphocyte suspensions prepared with the use of straws packed with nylon wool.[13]

The differences in frequency of the various antigens between patients and controls were tested for significance by means of the chi-square test (without Yates' correction). The resulting probabilities were multiplied by the number of HLA specific-

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TABLE 1
Frequencies of HLA-A antigens (%)

Antigen	Patients (n = 120)	Control subjects (n = 1416)
A1	9.2	6.4
A2	25.0	21.4
A3	14.2	12.6
A11	0	0.1
A23	15.8	18.3
A24	2.5	4.9
A25/34	14.2	13.5
A26	14.2	10.5
A28	21.7	20.9
A29	18.3	17.1
A30	25.8	37.4
A31	7.5	6.0
A32	2.5	2.3
Aw33	2.5	2.2
One antigen	26.7	26.4

p = NS for all comparisons.

ities tested to determine the corrected value. Relative risk was calculated according to the method of Svejgaard et al.[14]

Results

The percentage of frequencies of the HLA-A, HLA-B, HLA-DR, and HLA-DQ antigens in patients with chronic rheumatic heart disease and the control subjects are shown in tables 1 to 4. There was no difference in the frequency of any of the antigens at the A, B, and DQ loci between patients and control subjects.

TABLE 2
Frequencies of HLA-B antigens (%)

Antigen	Patients (n = 120)	control (n = 1416)
B5	1.7	1.3
B7	20.8	20.4
B8	12.5	12.9
B13	3.3	3.8
B14	8.3	5.7
B15	5.0	4.0
B16	1.7	3.3
B17	42.5	38.6
B18	7.5	5.2
B21	4.2	1.8
Bw22	0	0.1
B27	0	0.3
B35	6.7	6.7
B37	0	0.1
B40	0	0.6
Bw41	1.7	1.5
Bw42	16.7	23.5
B44	12.5	15.0
B45	11.7	8.6
Bw53	0.8	1.6
Bw70	30.8	24.6^
One antigen	11.7	20.4

p = NS for all comparisons.
^n = 220.

TABLE 3
Frequencies of HLA-DR antigens (%)

Antigen	Patients (n = 103)	Control subjects (n = 220)	p value	pc value
DR1	12.6	2.7	<.0001	<.045[B]
DR2	23.3	23.6	NS	
DR3	34.0	33.6	NS	
DR4	13.6	12.3	NS	
DR5	30.1	32.3	NS	
DRw6	31.1	15.0	<.0001	<.045[C]
DR7	15.5	12.3^	NS	
DRw8	2.9	8.7^	NS	
DRw9	0	1.5^	NS	
DRw10	2.9	2.6	NS	
One antigen	34.0	42.0	NS	

pc = corrected p value.
^n = 138.
[B]Relative risk = 5.2.
[C]Relative risk = 2.6.

The HLA-DR1 antigen was found in 12.6% of patients compared with 2.7% of normal control subjects. This increased frequency of DR1 remained significant after correcting the p value (relative risk: 5.2). The frequency of HLA-DRw6 was also increased in patients compared with controls (31.1% vs 15%), and this difference also remained significant after correcting the p value (relative risk: 2.6) (table 3).

Discussion

Associations between disease and the HLA system may involve class I (HLA-A, HLA-B or HLA-C) or class II (HLA-DR or HLA-DQ) antigens. In this study, no differences in frequency of any of the HLA-A, B, or DQ antigens in black patients with severe chronic rheumatic heart disease and control subjects were

TABLE 4
Frequencies of HLA-DQ antigens (%)

Antigen	Patients (n = 97)	Control subjects (n = 64)
DQw1	65.0	68.8
DQw2	21.7	23.4
DQw3	21.7	31.3
One antigen	92	76.5

p = NS for all comparisons.

found. Our observations support the impression obtained from analysis of previous studies[15–21] that no association exists between rheumatic heart disease and any of the antigens at the A or B loci. Confirmation of a lack of an association between this disease and the HLA-DQ antigens will have to await further studies since these antigens were not tested in previous investigations.

However, we found an increased frequency of both HLA-DR 1 and HLA-DRw6 antigens in our patients with severe rheumatic heart disease; the differences in frequencies in patients and controls remained significant after correcting for the total number of HLA antigens tested. The corrected p value would be $<.01$ in each case if a correction were made only for the number of DR antigens tested, as is done by some workers.[21–23]

Our study shows that severe chronic rheumatic heart disease in blacks is associated with certain DR antigens. This implies that genetically determined immune-response factors may play a role in the pathogenesis of chronic rheumatic heart disease in some individuals. Support for this conclusion is provided by a recent report of an association between certain HLA-DR antigens and rheumatic fever;[4] the majority of patients in this study developed mitral and/or aortic incompetence.

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References

1. Stastny P, Ball EJ, Dry PJ, Nunez G: The human immune response region (HLA-D) and disease susceptibility. Immunol Rev **70:** 113, 1980
2. Bodmer WFL, editor: The HLA system. Br Med Bull **34:** 213, 1978
3. Patarroyo ME, Winchester RJ, Vejerano A, Gibofsky A, Chalem F, Zabriskie JB, Kunkel HG: Association of B-cell alloantigen with susceptibility to rheumatic fever. Nature **278:** 173, 1979
4. Ayoub EM, Barrett DJ, MacLaren NK, Krischer JP: Association of class II human histocompatibility leucocyte antigens with rheumatic fever. J Clin Invest **77:** 2019, 1986
5. World Health Organization: Prevention of rheumatic fever. WHO Technical Report Series, No. **342**
6. Azaiwa M: HLA in Asia—Oceania 1986 (in press)
7. Mittal KK, Mickey MR, Singal DP, Terasaki PI: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. Transplantation **6:** 913, 1968
8. Hammond MG, Appadoo G, Brain P: HLA antigens in Bantu and Indians. *In* Kissmeyer-Nielsen F, editor: Histo-compatibility testing. Copenhagen, 1975, Munksgaard, p 173
9. Hammond MG, Appadoo G, Brain P: HLA in non-Caucasian populations. *In* Bodmer WF, editor: Histocompatibility testing. Copenhagen, 1977, Munksgaard, p 407
10. Hammond MG: HLA Bw53. *In* Terasaki PI, editor: Histocompatibility testing 1980. Los Angeles, 1980. UCLA Tissue Typing Laboratory, p 429
11. Hammond MG: HLA Bw35. *In* Simons MJ, Tait BD, editors: Proceedings of the Second Asia-Oceania Histocompatibility Workshop. Toorak, 1983, Immunopublishing, p 112
12. Hammond MG, Betuel H, Gebuhrer L: HLA A29. *In* Albert ED, Baur MP, Mayr WR, editors: Histocompatibility testing. Berlin, 1984, Springer-Verlag, p 126
13. Danilov JA, Ayoub G, Terasaki PI. Joint report: B lymphocyte isolation by thrombin-nylon wool. *In* Terasaki PI, editor: Histocompatibility testing 1980. Los Angeles, 1980, UCLA Tissue Typing Laboratory, p 287
14. Svegaard A, Platz P, Ryder LP, Staub-Nielsen L, Thomsen M: HLA and disease associations. Transplant Rev **22:** 3, 1975
15. Falk JA, Fleischman JL, Zabriskie JB, Falk RE: A study of HLA antigen phenotype in rheumatic fever and rheumatic heart disease patients. Tissue Antigens **3:** 173, 1973
16. Caughey DE, Douglas R, Wilson W, Hassal IB: HLA-antigens in Europeans and Maoris with rheumatic fever and rheumatic heart disease. J Rheumatol **2:** 319, 1975
17. Leirisalo M, Laitinen O, Tiilikainen A: HLA phenotypes in patients with rheumatic fever, rheumatic heart diseases and Yersinia arthritis. J Rheumatol **4**(suppl 3): 78, 1977
18. Joysey VC, Roger JH, Ashworth F. Parallel studies of HLA antigens in patients with rheumatic heart disease and scleritis: comparisons with three control populations. J Rheumatol **4**(suppl 3): 84, 1977
19. Ward C, Gelsthorpe K, Doughty RW, Hardisty CA: HLA antigens and acquired valvular heart diseases. Tissue Antigens **7:** 227, 1976
20. Haffejee IE, Hammond MG, Moosa A: HLA antigens in black South African children with rheumatic heart disease. Ann Trop Paedr **2:** 17, 1982
21. Naito S, Kitajima K, Arakawa K: HLA and rheumatic heart disease in Japanese. Am Heart J **106:** 1164, 1983
22. Fiorito S, Autore C, Fragola PV, Purpura M, Cannata D, Sangiorgi M: HLA DR 3 linkage in patients with hypertrophic cardiomyopathy. Am Heart J **111:** 91, 1986
23. Orren A, Taljaard D, du Toit E: HLA A, B, C and DR antigens in insulin dependent diabetes mellitus (IDDM) in South African Negro (black) and Cape coloured people. Tissue Antigens **26:** 332, 1985

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HLA Class I and II Antigens in South African Blacks with Graves' Disease

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A study was done to evaluate the relationship between Graves' disease and the HLA system in South African Blacks of Zulu descent. One hundred and three patients with Graves' disease and 1416 control subjects were typed for HLA A, B, and C antigens while HLA DR antigens were done on 63 of the former and 330 of the latter. There was a significant increase in the frequency of HLA DR3 in patients compared to control subjects (57.1% vs 36.1%; P corrected = 0.014). A relationship was also seen at the DR1 locus (14.3% vs 4.6%; P corrected = 0.023).

INTRODUCTION

The association between Graves' disease and the HLA system has aroused considerable interest in recent years. High frequencies of HLA B8 and DR3 have been found in White Caucasoids with the disease, whereas associations with HLA B35 and HLA B46 have been shown in Japanese and Chinese, respectively (1–5). A previous study involving a South African Black group with Graves' disease could not establish any definite relationship at the HLA Class I locus (6). Thus it can be seen that there appears to be an ethnic variability in the association between HLA antigens and Graves' disease.

The present study was undertaken to evaluate the relationship between HLA Class I and Class II antigens and Graves' disease in South African Blacks of Zulu descent.

PATIENTS AND METHODS

The patients studied were all Blacks of Zulu descent who had Graves' disease diagnosed on the basis of history, clinical, and biochemical signs of hyperthyroidism, the presence of a diffuse goiter on examination, and the finding of diffuse uptake of radiolabeled ^{131}I on a thyroid scan. The HLA status of the patients was compared to a group of unrelated healthy Black control subjects of Zulu descent. The control group comprised randomly selected staff and blood donors, many of whom have been typed for international histocompatibility workshops. There were 103 unrelated patients and 1416 unrelated control subjects who were typed for HLA A, B, and C antigens by means of a two-stage microlymphocytotoxicity test (7) using a total of 180 antisera.

HLA DR antigens were determined on 63 of these patients and 330 unrelated control subjects by means of a microlymphocytotoxicity test using B cell-enriched

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lymphocytes prepared with the aid of straws packed with cotton wool (8). These Class II antigens were defined using 120 antisera.

The sera used for typing the various Class I and II antigens consisted of local sera that have been requested for use in international histocompatibility workshops, local sera that have been verified by using in parallel with international workshop sera, and sera that have been exchanged with other laboratories worldwide.

Differences in HLA frequencies were tested for significance with the χ^2 test (without Yate's correction) and the probability was corrected by multiplying the P value by the number of comparisons made, i.e., the number of different antigens tested (9). Relative risk was calculated according to the formula recommended by Woolf (10).

RESULTS

The results are shown in Tables 1–3. There was an increase in the frequency of HLA B8 in patients compared to control subjects (23.3% vs 12.9%) but the P value was not significant after a correction was made for the number of antigens tested. Similarly, the association with HLA B13 (9.7% vs 3.8%) loses significance once the P value is corrected.

At the DR locus there is a significant increase in the frequency of DR3 (57.1% vs 36.1%; P corrected 0.014), even after correction for the number of antigens

TABLE 1

FREQUENCY OF HLA ANTIGENS IN PATIENTS AND CONTROL SUBJECTS

Antigen (n = 103)	Patients (n = 103) %	Controls (n = 1416) %	Antigen	Patients (n = 103) %	Controls (n = 1416) %	Antigens	Patients (n = 63) %	Controls (n = 330) %
A1	7,8	6,4	B5	1,0	1,3	DR 1[c]	14,3	4,6
A2	19,4	21,4	B7	22,3	20,4	DR 2	20,6	24,2
A3	9,7	12,6	B8[a]	23,3	12,9	DR 3[d]	57,1	36,1
			B13[b]	9,7	2,3	DR 4	19,1	9,9
A11	0	0,1	B14	11,7	5,7	DR 5	22,2	35,1
A23	27,2	18,3	B15	2,9	4,0	DRW 6	14,3	14,3
A24	3,9	4,9	B16	4,9	3,3	DR 7	12,7	15,3
A25/34	15,5	23,5	B17	38,8	38,6	DRW 9	0	0,5
A26	9,7	10,5	B18	3,9	5,2	DRW10	1,6	2,4
A28	24,3	20,9	B21	1,0	1,8			
A29	13,6	17,1	BW22	0	1,0			
A30	39,8	37,4	B27	1,0	0,3			
A31	5,8	6,0	B35	4,9	6,7			
A32	1,0	2,3	B37	0	0,1			
AW33	2,9	2,2	B40	1,0	0,6			
One antigen	19,4	26,4	BW41	1,9	1,5			
			BW42	16,5	23,5			
			B44	12,6	15,0			
			B45	7,8	8,8			
			BW53	0	1,6			
			BW70	19,4	24,6[a]			
			One antigen	15,5	20,4			

Note. puc, P uncorrected; pc, P corrected. RR, relative risk.

[a] puc = 0.003; pc 0.12, RR 2.03.

[b] puc = 0.004; pc 0.13, RR 2.69.

[c] puc = 0.002; pc 0.023, RR 3.48.

[d] puc = 0.001; pc 0.014, RR 2.35.

tested. In addition, a significant association is seen with DR1 (14.3% vs 4.6%; P corrected 0.023).

The occurrence of specific DR antigens together with certain B locus antigens in the same halotype is shown in Table 2. There is a significant linkage disequilibrium between DR3 B8. The high frequency of this haplotype in the patients with Graves' disease is due to the association of DR3 with the disease, while the increased frequency of HLA B8 can be explained by it being in linkage disequilibrium with DR3. In fact, as can be seen in Table 3, the primary association is with HLA DR3 since the strongest relationship is seen in HLA B8 negative patients.

Linkage disequlibrium was also seen between DR3 BW42, but not between DR3 B17.

DISCUSSION

The association between Graves' disease and HLA B8 and DR3 has been firmly established in White Caucasoids (1–3, 9). Moreover, it has now become clear that the presence of DR3 is far more important in determining susceptibility to Graves' disease than HLA B8 which is then associated with the disease by virtue of being in linkage disequilibrium with DR3 (1).

South African Blacks with Graves' disease, as shown in this study, certainly show a significant relationship with HLA DR3 and DR1. It appears that to date no other non-Caucasoid groups studied, viz: Japanese, Chinese, Thai, and American Blacks, has shown an association between Graves' disease and HLA DR3 (4, 5, 11, 12). These observations provide further support for the existence of heterogeneity in HLA associations relevant to Graves' disease.

A high frequency of HLA B8 was found in patients with Graves' disease compared to controls. Although the corrected P value was greater than 0.05, this association does become significant when it is considered in the light of a priori hypothesis since the same antigen has been found to be significantly increased (P uncorrected < 0.01) in a previous study involving another group of South African Blacks (6, 9). Moreover, the association could well be a secondary phenomenon as this antigen occurs in linkage disequilibrium with DR3 as shown in this study.

Since both South African Blacks and American Blacks presumably have the same origin, the lack of any relationship at the DR locus among the latter is

TABLE 2
LINKAGE DISEQUILIBRIUM BETWEEN SELECTED HLAB8 LOCUS ANTIGENS AND HLA DR LOCUS ANTIGENS

Haplotype	Control subjects			Patients with Graves' disease		
	Haplotype frequency	Delta $\times 10^3$	Delta SE	Haplotype frequency	Delta $\times 10^3$	Delta SE
DR3 B8[a]	30	18	2.5	86	39	1.1
DR3 BW42[a]	62	42	4.5	88	53	1.9
DR3 B17[b]	24	−19	−1.4	50	−30	−0.6

[a] Significant linkage disequilibrium.
[b] No significant linkage disequilibrium.

TABLE 3
OCCURRENCE OF HLA DR3 TOGETHER WITH SELECTED HLA B LOCUS ANTIGENS IN PATIENTS WITH GRAVES' DISEASE

	DR 3 positive patients	DR 3 negative patients
B8 positive patients	10 (15.9%)	4 (6.3%)
B8 negative patients	26 (41.3%)	23 (36.5%)
BW 42 positive patients	11 (17.4%)	1 (1.6%)
BW 42 negative patients	25 (39.7%)	26 (41.3%)
B17 positive patients	13 (20.6%)	13 (20.6%)
B17 negative patients	23 (36.5%)	14 (22.2%)

somewhat surprising (12). Of interest also is the absence of any negative association with HLA B7 or BW42, as has been found in the previous study on South African Blacks (5). In addition, this study did not find an increase in HLA B17, which is associated with Graves' disease in South Nigerians, another African Black group (2).

A previous study defined a clear association between insulin-dependent diabetes mellitus and HLA DR4 in South African Blacks of Zulu descent (14). However, no association was found with HLA DR3 (14). In contrast, Graves' disease affecting the same population group is associated with HLA DR3.

In conclusion, this study, having been the only one thus far to show a significant relationship between HLA DR3 and Graves' disease involving a non-Caucasoid group, underlines the need for more population-based studies involving groups other than Caucasoids to evaluate such associations.

REFERENCES

1. Farid, N. R. (Ed.), HLA in endocrine and metabolic disorders. *In* "Graves' Disease," p. 85, Academic Press, New York, 1981.
2. Farid, N. R., and Bear, J. C., The human major histocompatibility complex and endocrine disease. *Endocr. Rev.* 2, 50–86, 1981.
3. McKenna, R., Kearns, M., and Sugrue, D., HLA and hyperthyroidism in Ireland. *Tissue Antigens* 19, 97–99, 1982.
4. Nakao, Y., Kishihara, M., and Baba, Y., HLA antigens in autoimmune thyroid diseases. *Arch. Int. Med.* 138, 567–570, 1978.
5. Hawkins, B. R., Ma, J. T. C., Lam, K. S. L., Wang, C. C. L., and Young, R. T. T., Association of HLA antigens with thyrotoxic Graves' disease and periodic paralysis in Hong Kong Chinese. *Clin. Endocrinol.* 23, 245–252, 1985.
6. Kalk, W. J., Maier, G., and Van Drimellen, M., HLA antigens and Graves' disease in Black South Africans. *Tissue Antigens* 2, 7–15, 1983.
7. Terasaki, P. I., and McClelland, J. D., Microdroplet assay of human serum cytotoxins. *Nature (London)* 204, 998, 1954.
8. Danilovs, J. A., Ayoub, G., and Terasaki, P. I., Joint report: B lymphocyte isolation by thrombin-nylon wool. *In* "Histocompatibility Testing" (P. I. Terasaki, Ed.), UCLA Press, Los Angeles, 1980.
9. Svejgaard, A., Jersild, L., Staub-Nielsen, L., and Badmer, W. F., HLA antigens and disease: Statistical and genetic considerations. *Tissue Antigens* 4, 95–105, 1974.
10. Woolf, B., On estimating the relation between blood group and disease. *Ann. Hum. Genet.* 19, 251–253, 1955.

11. Tiwari, J. L., and Terasaki, P. I., "HLA and Disease Associations," p. 20, Springer-Verlag, New York, 1985.
12. Sridama, V., Hara, Y., Fauchet, R., and DeGroot, L. J., HLA immunogenetic heterogeneity in Black American patients with Graves' disease. *Arch. Intern. Med.* 147, 1, 1987.
13. Rapaport, B., Approaching an understanding of the genetic basis for autoimmune thyroid disease. *Arch. Intern. Med.* 147, 213, 1987.
14. Omar, M. A. K., Hammond, M. G., and Asmal, A. C., HLA A, B, C and DR antigens in South African blacks with Type I diabetes mellitus. *Diabetologia* 26, 30–33, 1984.

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HLA-A, B, DR, and DQ Antigens in Black Patients with Idiopathic Dilated Cardiomyopathy

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The HLA antigens, which are encoded by closely arranged genes on the short arm of the sixth chromosome, influence the predisposition to several diseases.[1] Some with initially weak associations with HLA-A and HLA-B antigens have been found to have stronger associations with HLA-DR antigens.[2] Since a genetic predisposition to the development of idiopathic dilated cardiomyopathy has been postulated, and since there is little information on the relation between antigens at the DR and DQ loci of the HLA system and idiopathic dilated cardiomyopathy,[3-5] we performed HLA typing in a group of black patients with this disease to determine if immunogenetic factors could be involved in the pathogenesis of idiopathic dilated cardiomyopathy.

HLA-A and HLA-B typing was carried out in 62 black patients with idiopathic dilated cardiomyopathy who had been admitted and evaluated at King Edward VIII Hospital, Durban, and HLA-DR and HLA-DQ typing was performed in 57 of these individuals; all had evidence of global hypokinesis on echocardiography. None had any disease other than idiopathic dilated cardiomyopathy; habitual alcoholics and hypertensives were excluded. Coronary angiography was not performed in any of the patients because coronary artery disease is rare in the black population of South Africa.[6,7] Patients were aged between 17 and 63 years. The control group consisted of 1,416 normal adults for the HLA-A and HLA-B typing, 220 for the HLA-DR typing and 198 for the HLA-DQ typing. Although over 2,000 individuals have been tested for the HLA-DQ locus in our laboratory, the majority were caucasoid or patients with

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TABLE I Frequencies of HLA-A Antigens.(%)

Antigen	Pts (n = 62)	Control Subjects (n = 1,416)
A1	11.3	6.4
A2	27.4	21.4
A3	16.1	12.6
A11	0.0	0.1
A23	12.9	18.3
A24	3.2	4.9
A25/34	14.5	13.5
A26	9.7	10.5
A28	19.4	20.9
A29	12.9	17.1
A30	35.5	37.4
A31	4.8	6.0
A32	3.2	2.3*
Aw33	8.1	2.2
One antigen	21.0	26.4

Difference not significant for all comparisons.
* Uncorrected p <0.005.

TABLE II Frequencies of HLA-B Antigens (%)

Antigen	Pts (n = 62)	Control Subjects (n = 1,416)
B5	0.0	1.3
B7	29.0	20.4
B8	9.7	12.9
B13	1.6	3.8
B14	1.6	5.7
B15	6.5	4.0
B16	4.8	3.3
B17	37.1	38.6
B18	3.2	5.2
B21	1.6	1.8
Bw22	1.6	0.07
B27	1.6	0.3
B35	11.3	6.7
B37	0.0	0.01
B40	0.0	0.6
Bw41	1.6	1.5
Bw42	21.0	23.5
B44	14.5	15.0
Bw47/47	0.0	0.1
Bw53	0.0	1.6
Bw70	25.8	14.2
One antigen	17.7	30.7

Difference not significant for all comparisons.

TABLE III Frequencies of HLA-DR Antigens (%)

Antigen	Pts (n = 57)	Control Subjects (n = 412)	p Value	Corrected p Value
DR1	12.3	4.6	<0.025	NS
DR2	29.8	24.0	NS	NS
DR3	28.1	36.2	NS	NS
DR4	7.0	10.0	NS	NS
DR5	38.6	35.2	NS	NS
DRw6	24.6	14.6	NS	NS
DR7	14.0	15.3	NS	NS
DRw8	5.3	2.9	NS	NS
DR9	0.0	0.7	NS	NS
DRw10	8.8	2.2	<0.01	NS
DR1 + DRw10	21.1	6.8	<0.0005	<0.02*
One antigen	31.6	54.4	<0.002	NS

* Relative risk = 3.7.
NS = not significant.

TABLE IV Frequencies of HLA-DQ Antigens (%)

Antigen	Pts (n = 57)	Control Subjects (n = 198)
DQw1	66.7	58.1
DQw2	14.0	23.4
DQw3	35.1	29.8
One antigen	84.2	88.9

Difference not significant for all comparisons.

selected diseases.[8] Only 198 normal healthy black individuals had undergone DQ typing.

The HLA-A and HLA-B antigens were identified with a 2-stage lymphocytotoxicity test.[9] These antigens were defined with 180 antisera, which consisted of local serum samples that had been requested for use in International Histocompatibility Workshops, local samples that had been verified by use in parallel with the International Workshop samples and samples that had been exchanged with other laboratories worldwide.[10-14] Similarly, 120 serum samples were used to define the HLA-DR and HLA-DQ antigens in B cell-enriched lymphocyte suspensions prepared with the use of straws packed with nylon wool.[15] The difference in frequency of the various antigens between patients and control subjects was tested for significance by means of the chi-square test (without Yates' correction). The resulting p values were multiplied by the number of HLA antigens tested to determine the corrected p value. Relative risk was calculated according to the method of Svejgaard et al.[16]

The percentage frequencies of the HLA-A, HLA-B, HLA-DR and HLA-DQ antigens in patients with idiopathic dilated cardiomyopathy and the control subjects are listed in Tables I to IV. With respect to the differences noted, only the increased frequency of the closely related antigens, HLA-DR1 and DRw10 (21.1 vs 6.8%), remained significant after correcting the p value (relative risk 3.7).

Associations between disease and the HLA system may invoive class I (HLA-A, B or C) or class II antigens (D-locus antigens). In this study, no differences in frequency of any of the HLA-A, B or DQ antigens between black patients with idiopathic dilated cardiomyopathy and control subjects were detected. However, we found an increased frequency of the HLA-DR1 and DRw10 antigens in our patients with idiopathic dilated cardiomyopathy; the differences in frequencies between patients and control subjects remained significant after correcting for the total number of HLA antigens tested. The corrected p value would be <0.005 if a correction were made only for the number of DR antigens tested, as is done by some investigators.[17,18]

Zerbe et al[4] were unable to find an association between this disease and any of the class I and class II antigens in white patients. Another group observed an increased frequency of HLA-B27 and HLA-DR4 antigens and an underrepresentation of the HLA-DR6 in caucasoid patients with idiopathic dilated cardiomyopathy[3]; it is not stated whether the p values were corrected for the number of antigens tested. An increased frequency of HLA-DR4 antigen was also noted in another group of caucasoid patients with this disease.[5] Confirmation of a lack of an association between this disease and the HLA-DQ antigens will have to await further studies since these antigens were not tested in previous investigations.

Our study shows that idiopathic dilated cardiomyopathy in blacks is associated with certain DR antigens. This, together with the results of the study by Anderson et al[3] and those of Limas and Limas,[5] implies that genetically determined immune-response factors may play a role in the pathogenesis of this condition in some individuals.

1. Stastny P, Ball EJ, Dry PJ, Nunez G. The human immune response region (HLA-D) and disease susceptibility. *Immunol Rev 1980;70:113-153.*

2. Bodmer WF. The HLA system. *Br Med Bull 1978;34:213-216.*

3. Anderson JL, Carlquist JF, Lutz JR, DeWitt CW, Hammond EH. HLA-A, B and DR typing in idiopathic dilated cardiomyopathy. A search for immune response factors. *Am J Cardiol 1984;53:1326-1330.*

4. Zerbe TR, Kaufmann C, Colson Y, Duquesnoy R. Associations of HLA-A, B, DR antigens with primary disease in cardiac allograft recipients. *Am J Cardiol 1988;61:1359-1361.*

5. Limas CJ, Limas C. HLA antigens in idiopathic dilated cardiomyopathy. *Br Heart J 1989;62:379-383.*

6. Powell SJ, Wright R. Cardiomyopathy in Durban. *S Afr Med J 1965;39:1062-1066.*

7. Adams EB. A Companion to Clinical Medicine in the Tropics and Subtropics. *Oxford: University Press, 1979:109.*

8. Hammond MC, Appadoo B. HLA antigens in African blacks. In: Azaiwa M, Natori T, Wakisaka A, Konoeda Y, eds. HLA in Asia-Oceania, 1986. *Sapporo: Hokkaido University Press, 1986:316-319.*

9. Mittal KK, Mickey MR, Singal DP, Teraski PI. Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation 1968;6:913-927.*

10. Hammond MG, Appadoo G, Brain P. HLA antigens in Bantu and Indians. In: Kissmeyer-Nielsen F, ed. Histocompatibility Testing. *Copenhagen: Munksgaard, 1975:173-178.*

11. Hammond MG, Appadoo G, Brain P. HLA in non-caucasian populations. In: Bodmer WF, ed. Histocompatibility Testing. *Copenhagen: Munksgaard, 1977:407.*

12. Hammond MG. HLA Bw53. In: Terasaki PI, ed. Histocompatibility testing 1980. *Los Angeles: UCLA Tissue Typing Laboratory, 1980:429-432.*

13. Hammond MG. HLA Bw35. In: Simons MJ, Tait BD, eds. Proceedings of the Second Asia-Oceania Histocompatibility Workshop. *Toorak: Immunopublishing, 1983:112-114.*

14. Hammond MG, Betuel H, Gebuhrer L. HLA A29. In: Albert ED, Baur MP, Mayr WR, eds. Histocompatibility Testing. *Berlin: Springer-Verlag, 1984:126.*

15. Danilov JA, Ayoub G, Tersaki PI. Joint report: B lymphocyte isolation by thrombin-nylon wool. In: Terasaki PI, ed. Histocompatibility Testing 1980. *Los Angeles: UCLA Tissue Typing Laboratory, 1980:287-288.*

16. Svejgaard A, Platz P, Ryder LP, Staub-Nielsen L, Thomsen M. HLA and disease associations. *Transplant Rev 1975;22:3-43.*

17. Fiorito S, Autore C, Fragola PV, Purpura M, Cannata D, Sangiorgi M. HLA DR 3 linkage in patients with hypertrophic cardiomyopathy. *Am Heart J 1986;111:91-94.*

18. Orren A, Taljaard D, du Toit E. HLA-A, B, C and DR antigens in insulin dependent diabetes mellitus (IDDM) in South African Negro (black) and Cape coloured people. *Tissue Antigens 1985;26:332-339.*

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HLA antigens in donovanosis (granuloma inguinale)

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Abstract

***Objective*—To compare the frequencies of HLA antigens in patients with donovanosis and in controls.**

***Design*—HLA Class I, Class II and DQ antigens were detected in patients with genital ulceration caused by donovanosis and in a control group.**

***Setting*—City Health STD Clinic, King Edward VIII Hospital, Durban, South Africa.**

***Participants*—Sixty (47 men, 13 women) patients with donovanosis.**

***Results*—HLA B57 was detected in nine of 60 (15%) with donovanosis and 75 of 1478 (5·1%) controls (RR = 3·3 χ^2 = 11·0, p = 0·001, p corrected = 0·026).**

***Conclusions*—A possible link between donovanosis and HLA B57 could be explained by co-existing alleles or immune response genes in linkage disequilibrium altering disease susceptibility.**

Introduction

Donovanosis is a genital ulcerative disease (GUD) found in diverse geographical locations where poor socio-economic conditions prevail and is commoner in dark-skinned races.[1] Donovanosis is generally regarded as a sexually transmitted disease (STD) but the modes of infection and transmission are not yet established with certainty. The causative agent, *Calymmatobacterium granulomatis*, has been isolated from faeces, and transmission through auto-inoculation is suggested.[2] The organism possesses a capsule and is similar to klebsiella strains but its biochemical and bacterial characteristics are not well defined.[3]

Although previously thought to be uncommon in Southern Africa, donovanosis has recently emerged as a significant cause of GUD in Durban. In 1988[4] 171 cases were diagnosed by the presence of Donovan bodies on direct microscopy using the RapiDiff technique,[5] a simple bench diagnostic staining method.

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Most bacterial STDs are readily transmitted from male to female and female to male. However, variable transmission rates of infection with *C granulomatis* are reported from different populations. The prevalence of disease amongst regular sexual partners varies from 1% in the USA[6] and 1% in Papua and New Guinea[7] to 50% in India.[8] The apparent racial predominance amongst blacks and variability in transmission suggests that host susceptibility factors may be relevant in the disease process.

No clear association between a single HLA antigen and a particular STD has been described but donovanosis has been suggested as one STD with a reasonable chance of such a link.[9] We therefore investigated the frequency of HLA antigens amongst Zulu patients with donovanosis attending a STD clinic in Durban.

Patients and Methods

Sixty Zulu patients (47 men, 13 women) attending the City Health STD Clinic at King Edward VIII Hospital, Durban with genital ulcerative lesions of donovanosis were entered into the study. Donovanosis was diagnosed by the detection of Donovan bodies on tissue smears stained with RapiDiff[5] and examined by direct microscopy. Specific (TPHA) and non-specific (RPR) serological tests for syphilis were performed. Laboratory facilities for identifying herpes simplex virus, chancroid and lymphogranuloma venereum infections were unavailable.

The control group consisted of 1478 normal subjects who were either staff or randomly selected blood donors of the same ethnic origins as the patients. HLA Class I antigens were determined in all patients and control subjects by a two-stage microlymphocytotoxicity test[10] with 180 sera consisting of: 1. local sera requested for use in international histocompatibility workshops; 2. local sera verified with international workshop sera; 3. sera exchanged with other laboratories worldwide.

Similarly 120 sera were used to define the Class II antigens on B-lymphocyte enriched lymphocyte suspension prepared with the aid of straws packed with nylon wool.[11] Class II antigens were determined in 53 patients and 513 controls except that HLA DQ antigens were tested in 129 controls.

Statistics

Differences in HLA frequencies were tested for significance with the χ^2 test and the probability

Table 1 Frequency of HLA Class I antigens in patients with donovanosis and normal controls

HLA	Controls N = 1478	(%)	Donovanosis N = 60	(%)	χ^2	Relative risk
A1	123	(8·32)	3	(5·00)	0·85	0·6
A36	12	(0·81)	1	(1·67)	0·50	2·1
A2	358	(24·22)	15	(25·0)	0·02	1·0
A3	165	(11·16)	12	(20·0)	4·42	2·0
A23	281	(19·01)	3	(5·00)	7·52	0·2
A24	81	(5·48)	6	(10·00)	2·21	1·9
A25	55	(3·72)	0	(0·00)	2·32	0·0
A26	173	(11·71)	5	(8·33)	0·64	0·7
A34	124	(8·39)	6	(10·00)	0·19	1·2
A28	319	(21·58)	21	(35·00)	6·03	2·0
A29	213	(14·41)	7	(11·67)	0·35	0·8
A30	455	(30·78)	16	(26·67)	0·46	0·8
A31	57	(3·86)	3	(5·00)	0·20	1·3
A32	28	(1·89)	2	(3·33)	0·62	1·8
A33	58	(3·92)	2	(3·33)	0·05	0·8
A43	2	(0·14)	0	(0·00)	0·08	0·0
A66	1	(0·07)	0	(0·00)	0·04	0·0
B7	348	(23·55)	15	(25·00)	0·07	1·1
B8	189	(12·79)	10	(16·67)	0·77	1·4
B13	45	(3·04)	1	(1·67)	0·38	0·5
B14	88	(5·95)	1	(1·67)	1·94	0·3
B18	80	(5·41)	1	(1·67)	1·62	0·3
B21	29	(1·96)	2	(3·33)	0·55	1·7
B22	1	(0·07)	0	(0·00)	0·04	0·0
B27	5	(0·34)	0	(0·00)	0·20	0·0
B35	109	(7·37)	8	(13·33)	2·91	1·9
B37	2	(0·14)	0	(0·00)	0·08	0·0
B38	29	(1·96)	2	(3·33)	0·55	1·7
B39	24	(1·62)	1	(1·67)	0·00	1·0
B41	27	(1·83)	2	(3·33)	0·71	1·9
B42	296	(20·03)	12	(20·00)	0·00	1·0
B44	233	(15·76)	11	(18·33)	0·29	1·2
B45	139	(9·40)	1	(1·67)	4·17	0·2
B47	1	(0·07)	0	(0·00)	0·04	0·0
B48	1	(0·07)	0	(0·00)	0·04	0·0
B51	16	(1·08)	0	(0·00)	0·66	0·0
B52	20	(1·35)	2	(3·33)	1·60	2·5
B53	20	(1·35)	2	(3·33)	1·60	2·5
B57	75	(5·07)	9	(15·00)	11·00	3·3
B58	471	(31·87)	21	(35·00)	0·26	1·2
B60	1	(0·07)	0	(0·00)	0·04	0·0
B62	10	(0·68)	0	(0·00)	0·41	0·0
B63	40	(2·71)	1	(1·67)	0·24	0·6
B70	407	(27·54)	9	(15·00)	4·59	0·5

corrected by multiplying the p value by the number of comparisons made, that is, the number of antigens tested.[12] Relative risks were calculated according to the formulae recommended by Woolf.[13]

Results

The frequencies of HLA A and B antigens in the patients and controls are shown in table 1 and of HLA DR and DQ antigens in table 2. HLA B57 was detected in nine of 60 (15%) with donovanosis and 75 of 1478 (5·1%) controls (RR = 3·3, χ^2 = 11·0, p = 0·001, p corrected = 0·026). HLA A23 was detected in three of 60 (5%) with donovanosis and 281 of 1478 (19·0%) controls (RR = 0·2, χ^2 = 7·5, p < 0·01, p not significant after correction).

Positive serological tests for syphilis (TPHA and RPR were detected in 14 (10 men and four women).

Table 2 Frequency of HLA Class II antigens in patients with donovanosis and normal controls

HLA	Controls N = 513	(%)	Donovanosis N = 53	(%)	χ^2	Relative risk
DR1	24	(4·68)	1	(1·89)	0·89	0·4
DR2	124	(24·17)	16	(30·19)	0·93	1·4
DR3	181	(35·28)	23	(43·40)	1·37	1·4
DR4	49	(9·55)	5	(9·66)	0·87	0·6
DR5	165	(32·16)	13	(24·53)	2·03	0·6
DR6	92	(17·93)	6	(11·32)	1·47	0·6
DR7	79	(15·40)	10	(18·87)	0·44	1·3
DR8	20	(3·90)	3	(5·66)	0·38	1·5
DR9	4	(0·78)	2	(3·77)	4·11	5·0
DR10	11	(2·14)	4	(7·55)	5·44	3·7
	N = 176		N = 53			
DQW1	122	(69·32)	36	(67·92)	0·04	0·9
DQW2	59	(33·52)	5	(9·62)	0·67	1·3
DQW3	42	(23·86)	16	(30·19)	0·86	1·4

Discussion

There are few reports linking HLA antigens and STDs. Amongst Chinese prostitutes in Singapore HLA AW19 and HLA B17 were associated with syphilis and gonorrhoea and HLA A11 and HLA B15 conferred relative resistance.[14] Behcet's disease, although not a STD, does cause genital ulceration and is associated with HLA B5.[15] The development of disease may be related to early sexual intercourse or adolescent infection.[16] Our findings of a possible link between HLA B57 and donovanosis and a trend towards resistance to disease with HLA A23 could be explained by co-existing alleles or immune response genes in linkage disequilibrium altering disease susceptibility.

Donovanosis is a STD about which little is known despite its recognition in the nineteenth century. It differs from most bacterial STDs in having a long incubation period and a variable transmission rate to regular sexual partners thereby suggesting inherent differences in host susceptibility. The causative organism *C granulomatis* shares some features of klebsiella strains including a prominent capsule but its bacterial characteristics are still not yet clearly defined. Klebsiella extracts are more likely to interact with HLA B27 than other HLA antigens producing an altered-self major histocompatibility complex that may trigger reactive arthritis.[17]

Donovanosis has only recently been recognised as a significant cause of GUD amongst the local Zulu population.[4] Whether this reflects a new epidemic or increased awareness following the introduction of a rapid diagnostic test is uncertain. Elsewhere in South Africa donovanosis occurs in East Transvaal amongst the Swazis[18] but is otherwise uncommon.

The highest prevalence of donovanosis worldwide is in Dutch New Guinea and Papua New Guinea.[19] However, HLA B57 was not identified amongst

natives of the Highlands and Coastal Areas.[20] Further studies of HLA status and donovanosis are required amongst population groups from endemic areas to clarify possible immunopathological mechanisms of disease and assess the role of genetic factors.

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1 D'Aunoy R, von Haam E. Granuloma inguinale. *Am J Trop Med* 1937;17:747–63.
2 Goldberg J. Studies on granuloma inguinale: VII. Some epidemiological considerations of the disease. *Br J Venereal Dis* 1964;40:140–5.
3 Kuberski T. Granuloma inguinale (Donovanosis). *Sex Transm Dis* 1980;7:29–36.
4 O'Farrell N. Donovanosis (Granuloma inguinale) in Durban: a risk factor for HIV-1 in men. MSSVD Spring Meeting. 30 May–2 June 1991, Heidelberg, West Germany.
5 O'Farrell N, Hoosen AA, Coetzee K, van den Ende J. A rapid stain for the diagnosis of granuloma inguinale (Donovanosis). *Genitourin Med* 1990;66:200–1.
6 Packer H, Goldberg J. Studies of the antigenic relationship of D granulomatis to members of the tribe Escherichiae. *Am J Syphilis* 1950;34:343–50.
7 Maddocks I, Anders EM, Dennis E. Donovanosis in Papua New Guinea. *Br J Venereal Dis* 1976;52:190–6.
8 Lal S, Nicholas C. Epidemiological and clinical features in 165 cases of granuloma inguinale. *Br J Venereal Dis* 1970;46: 461–3.
9 Kuberski T. Histocompatibility antigens and the sexually transmitted diseases. *Sex Transm Dis* 1980;7:203–5.
10 Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. *Nature* 1964;204:998–1000.
11 Danilovs JA, Ayoub G, Terasaki PI. B lymphocyte isolation by thrombin-nylon wool. In: Terasaki PI, Ed. *Histocompatibility Testing 1980*. Los Angeles, University of California Press, 1980.
12 Svejgaard A, Jersild C, Nielsen LC, Bodmer WF. HLA antigens and disease: statistical and genetical considerations. *Tissue Antigens* 1974;4:95–105.
13 Woolf B. On estimating the relation between blood group and disease. *Ann Hum Gen* 1955;19:251–3.
14 Chan SH, Tan T, Kamarudin A, Wee GB, Rajan VS. HLA and sexually transmited diseases in prostitutes. *Br J Venereal Dis* 1979;55:207–10.
15 Ersoy F, Berkel I, Firat T, Kazokoglu H. HLA antigens associated with Behcet's disease. *Arch Dermatol* 1977;113:1720–1.
16 Cooper C, Pippard EC, Sharp H, Wickham C, Chamberlain MA, Barker DJP. Is Behcet's disease triggered by childhood infection? *Ann Rheum Dis* 1989;48:421–3.
17 Batchelor JR, Welsh KI. Association of HLA antigens with disease. In: Lachmann PJ, Peters DK, eds. *Clinical Aspects of Immunology, Vol 1*, Oxford: Blackwell Scientific Publications, 1982.
18 Wistrand R, Wegerhoff F. Granuloma inguinale in the Eastern Transvaal. *S Afr Med J* 1985;67:13–15.
19 Vogel LC, Richens J. Donovanosis in Dutch South New Guinea: History, evolution of the epidemic and control. *Papua New Guinea Med J* 1989;32:203–18.
20 Crane G, Bhatia K, Honeyman M, *et al.* HLA studies of Highland and Coastal New Guineans. *Hum Immunol* 1985;12:247–60.

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onset of activity (lorazepam). Similarly, for acute behavioural episodes such as violence the choices have generally been intramuscular lorazepam or antipsychotics such as haloperidol or chlorpromazine. In addition to delayed onset of effects, intramuscular antipsychotics have been associated with acute extra pyramidal side effects, orthostatic hypotension, and extreme sedation.

Over the past year we have used the benzodiazepine midazolam intramuscularly to treat patients with acute seizures or extreme behavioural episodes. In the United States midazolam is currently approved for use as a pre-anaesthetic agent, and not for treatment of seizures.[1] It has, however, been successfully used in clinical situations to treat acute seizures including status epilepticus, and severe behavioural problems often with almost immediate effects.[2-9] Midazolam is a highly lipophilic water soluble drug which allows for excellent intramuscular absorption and rapid CNS penetration. Intramuscular (IM) administration can result in sedation within five to 15 minutes with peak effects noted within 30–60 minutes. The drug possesses a short half-life of 1·5 to 3·5 hours, although in some patients residual psychomotor effects may be noted for up to eight hours. Although there have been reports of respiratory problems with the intravenous administration of midazolam, especially in elderly patients, this has not been reported after intramuscular use. Warnings of respiratory problems specifically only mention intravenous administration.

After previously published reports of success with IM midazolam for the treatment of acute seizures and behavioural emergencies, we have been treating patients with this medication. We present four cases involving clinical use of IM midazolam, two for acute seizures and two for behavioural control.

Case 1: A 26 year old white male suffered a head injury on the 9 February 1985 secondary to a motor vehicle accident. The patient has had persistent problems with late onset prolonged seizures which often needed admission to hospital for acute treatment despite receiving intramuscular lorazepam. These admissions averaged at least one per month between 1989–90. In early 1990 lorazepam was switched to IM midazolam 10 mg. Since the change to midazolam, no further admissions have been necessary for treatment of acute seizures, despite no significant changes in the primary anticonvulsant drug treatment.

Case 2: A 22 year old white male suffered a head injury on 2 January 1986 when he was hit by a car. He developed frequent and prolonged late-onset seizures, both focal and generalised. On 3 April 1990 he developed right-sided twitching of the face and extremities for seven to 10 minutes, without secondary generalisation. IM midazolam 15 mg stopped the seizures "within five minutes." On 25 June 1990 he developed prolonged generalised tonic-clonic seizures. IM midazolam 15 mg was administered and the seizures ceased within five minutes with the patient falling asleep. Sedation was the only reported adverse effect.

Case 3: A 52 year old black male suffered a head injury in May 1987 secondary to a fall. Post traumatically he developed seizures, and paranoid psychosis with prolonged agitated, aggressive, and combative behaviours. On 6 April 1990 he became euphoric, paranoid, very agitated and threatened physical abuse to staff members. He refused medications and also cigarettes. After IM midazolam 5 mg he fell asleep for one hour and awoke amnesic about the episode.

Case 4: A 39 year old black male, had primary behavioural problems including chronic violence to others and agitation. The patient has had a chronic idiopathic seizure disorder since 1980. In 1988, he developed an episode of status epilepticus leading to anoxic encephalopathy with resulting severe cognitive impairment, chronic paranoid psychosis, aggressive behaviours, and visual and auditory hallucinations. Intramuscular midazolam has been used on numerous occasions to treat agitation resulting in alleviation of agitation and violence as well as a reduction in psychosis without significant sedation or long term "after effects." These positive effects have lasted for a day, sometimes for eight to 12 hours.

Although seizures after brain injury can sometimes be self-limiting, the known rapid onset of midazolam and our knowledge of these patients' seizure histories makes this possibility unlikely. While some patients (such as case 4) may respond to very low doses, the general dosage guideline for midazolam is 0·15 to 0·30 mg/kg.[2]

Side effects were reported ranging from slight lethargy to sleep. In most cases, this lasted for one to two hours and the patients' recovery was uneventful. Only case 4 demonstrated prolonged effects—even at a very low dose. Intramuscular midazolam appears to be a safe, rapidly effective drug for treatment of both acute seizures and behavioural emergencies and deserves further study.

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1 Versed: Product information. Nutley NJ, Hoffman-LaRoche, 1986.
2 Egli M, Albani C. Relief of status epilepticus after IM administration of the new Short-Acting benzodiazepine midazolam. *Twelth World Congress of Neurology*, 20–25 September 1981, Kyoto, Japan. Princeton, Excerpta Medica, 44 (abs 137).
3 Jawad S, Oxley J, Wilson J, Richens A. A pharmacodynamic evaluation of midazolam as an antiepileptic compound. *J Neurol Neurosurg Psychiatry* 1986;49:1050–4.
4 Galvin GM, Jelinek GA. Midazolam: an effective intravenous agent for seizure control. *Arch Emerg Med* 1987;4:169–72.
5 Mayhue FE. IM midazolam for status epilepticus in the Emergency Department. *Ann Emerg Med* 1988;17:643–5.
6 Von Albert HH. Midazolam for the treatment of states of restlessness and excitation. In: Steffens J, ed. Midazolam in anesthesiology. Int Symposium Darmstadt, 28–29 October 1983. Basle, Switzerland: Roche Scientific Service, 1986:255–58.
7 Mendoza R, Djenderedjian AH, Adams J, Ananth J. Midazolam in acute psychotic patients with hyperarousal. *J Clin Psychiatry* 1987;48:7, 291–2.
8 Bond WS, Mandos LA, Kurtz MB. Midazolam for aggressivity and violence in three mentally retarded patients. *Am J Psychiatry* 1989;146:7, 925–6.
9 Dundee JW, Halliday NJ, Harper KW. Midazolam. A review of its pharmacological properties and therapeutic use. *Drugs* 1984;28:519–43.

HLA profile and HTLV-I associated myelopathy (HAM/TSP) in Natal, South Africa

Myelopathy associated with HTLV-I (HAM/TSP) is an important cause of neurological disability in the Zulus in Natal.[1] To explore the role of host factors in the pathogenesis of this disorder we examined the HLA profiles in 40 HAM/TSP patients. The results were also compared with two antibody positive patients with adult T-cell leukaemia/lymphoma (ATLL). The control group consisted of normal adults who were either staff or randomly selected blood donors of the same ethnic origin as the patients. Class I antigens were tested in 1848 controls, DR antigens in 556 and DQ in 340.

Standard techniques[2,3] using 180 antisera for Class I antigens and 120 antisera for Class II antigens, were employed. Differences in HLA frequencies were tested for significance with the Chi square test (without Yates's correction) and the probability was corrected by multiplying the P-value by the number of comparisons made, that is, the number of antigens tested.[4] Relative risks were calculated according to the formulae recommended by Woolf.[5] The difficulties of establishing negative correlations which may indicate a "protective" antigen have been discussed by Svejgaard *et al.*[6] Haplotype frequencies were estimated by the method of Mattiuz *et al.*[7]

The HLA frequencies of the large number of controls was typical of the Southern African black population. There was virtual absence of A11, B22, B40, Bw54, Bw52, Cw1 and DR9 whilst high frequencies of A23, A30, Bw42, B58, B70, Cw2 and DR5 were observed. In the patient group an increased frequency of only one antigen—Bw57—reached statistical significance (table) at the 1% level after correction for the number of Class I antigens tested. The increased frequencies of A24 (12·5% vs 6·0 %), B7 (32·5% vs 23·4%) and DR2 (37·1% vs 24%) were of borderline significance.

There were no significant differences in the frequencies of HLA C and HLA DQ antigens. The joint occurrence of A24, B7, DR2, DQw1 was found in 3/35 patients (8·6%) but was present in only 3·1% of the control group. The two patients with lymphoma/leukamia had the following antigens: HLA A2, A30, B8, B-, Cw2, Cw-, DR7, DR-, DRw53, DQw1 and DQw- and HLA Aw31, A-, B35, B45, Cw-, DRw8, DRw52, DQw3, DQw-. There were no significant differences in the estimated haplotype frequencies between patients and controls.

In contrast to our largely negative findings Usuku *et al*[8] found specific HLA haplotypes in 70% of their HAM patients. Furthermore, none of the HAM associated HLA hapolotypes were seen in ATLL. The joint occurrence of A24, B7, DR2, DQw1 found in 8·8% of our patients, has been reported by the Japanese,[9] although DR2 was usually found with different B-locus antigens. The other HLA antigens associated with HAM/TSP in the Japanese[8]—A11, Bw54, Bw52, are not found in the Zulus. Also those antigens associated with ATLL in Japanese are rare in the local black population.

There is accumulating evidence that the neurological injury in HAM/TSP is immune mediated.[10] A more refined examination of the HLA system may yet prove fruitful. The recent molecular genetic study by Usuku *et al*[11] showed a relationship between a particular amino acid sequence of the HLA-DR 1

Table MHC Class I antigen frequencies in Zulu control subjects and patients with HAM/TSP

	Control *N = 1848*	%	*HAM/TSP* N = 40	%	CHI-SQ	R-R
B7	432	23·3	13	32·5	1·81	1·6
B8	235	12·7	7	17·5	0·80	1·5
B13	62	3·3	2	5·0	0·32	1·5
B14	112	6·0	2	5·0	0·08	0·8
B18	95	5·1	3	7·5	0·44	1·5
B21	35	1·8	0	0·0	0·77	0·0
Bw22	1	0·0	0	0·0	0·02	0.0
B27	8	0·4	0	0·0	0·17	0·0
B35	135	7·3	3	7·5	0·0	1·0
B37	2	0·1	0	0·0	0·04	0·0
B38	32	1·7	1	2·5	0·13	1·5
B39	29	1·5	0	0·0	0·64	0·0
Bw41	33	1·7	0	0·0	0·73	0·0
Bw42	368	19·9	6	15·0	0·60	0·7
B44	303	16·4	8	20·0	0·37	1·3
B45	174	9·4	1	2·5	2·23	0·2
Bw47	2	0·1	0	0·0	0·04	0·0
Bw48	1	0·0	0	0·0	0·02	0·0
B51	20	1·0	1	2·5	0·72	2·3
Bw52	1	0·0	0	0·0	0·02	0·0
Bw53	29	1·5	0	0·0	0·64	0·0
Bw57	88	4·7	7	17·5	13·29	4·2
Bw58	585	31·6	8	20·0	2·47	0·5
Bw60	1	0·0	0	0·0	0·02	0·0
Bw61	0	0·0	0	0·0	—	—
Bw62	12	0·6	0	0·0	0·26	0·0
Bw63	43	2·3	0	0·0	0·95	0·0
Bw70	512	27·7	4	10·0	6·18	0·3

N = number; R-R = relative risk.

chain and the succeptibility to HAM. We have already established control frequencies in the Zulus for HLA polymorphism using PCR amplified DNA, dot-blots and oligonucleotide probes and hope to embark on a project to determine if any of these DNA markers are relevant to HAM/TSP.

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1 Bhigjee AI, Kelbe C, Haribhai HC *et al.* Myelopathy associated with human T cell lymphotropic virus type I (HTLV-I) in Natal, South Africa. *Brain* 1990;113:1307–20.
2 Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. *Nature* 1964; 204:998.
3 Danilovs JA, Ayoub G, Terasaki PI. B lymphocyte isolation by throbin-nylon wool. In: Terasaki PI, ed. *Histocopatibility testing.* Los Angeles: University of California Press, 1980:287–8.
4 Svejgaard A, Jersild L, Staub-Nielsen L, Bodmer WF. HLA antigens and disease: statistical and genetical considerations. *Tissue Antigens* 1974;4:95–105.
5 Woolf B. On estimating the relation between blood group and disease. *Ann Hum Gen* 1955;19:251–3.
6 Sveigaard A, Platz P, Ryder LP *et al.* HLA and disease associations—a survery. In: Moller G, ed. *HLA and disease,* (Transplant Rev 22). Copenhagen, Munksgaard 1975:3–44.
7 Mattiuz PL, Ihde D, Piazza A, *et al.* New approaches to the population genetics and segregation analysis of the HLA system. In: Terasaki PI, ed. *Histocopatibility testing.* Copenhagen: Munksgaard, 1970:193–206.
8 Usuku K, Sonoda S, Osame M, *et al.* HLA haplotype-linked high immune responsiveness against HTLV-I in HTLV-I associated myelopathy: Comparison with adult T-cell leukemia/Lyphoma. *Ann Neurol* 1988;23 (Suppl):S143–50.
9 Kawai H, Nishida Y, Takagi M *et al.* HTLV-I associated myelopathy with adult T-cell leukemia. *Neurology* 1989;39:1129–31.
10 Dalgleish A, Richardson J, Matutes E, *et al.* HTLV-I infection in tropical spastic paraparesis: lymphocyte culture and serological response. *AIDS Res Hum Retroviruses* 1988; 4(6):475–85.
11 Usuku K, Nishizawa M, Matsuki K, *et al.* Association of a particular amino acid sequence of the HLA-DR I chain with HTLV-I associated myelopathy. *Eur J Immunol* 1990;20:1603–6.

Extrapyramidal symptoms in a patient treated with fluvoxamine

A 77 year old woman had a longstanding history of recurrent major depressive episodes. She was treated with several tricyclic and heterocyclic antidepressants. Approximately six months before she came under our care, she started taking neuroleptics for the first time in her life. Flupenthixol 1 mg three times daily was prescribed in combination with the tricyclic antidepressant melitracene for a major depressive episode with psychotic features.

After a few months, marked orofacial involutary movements were noted. All psychoactive drugs were discontinued and the orofacial dyskinesia disappeared gradually over the following month. The depression relapsed, however, and treatment was started with fluvoxamine, a serotonin-reuptake inhibiting antidepressant. The initial dose of 50 mg was gradually increased to 200 mg. By the time she had been on fluvoxamine for six weeks, she was transferred to our psychogeriatric ward.

On initial neurological examination, a mild akinetic-rigid syndrome and hyperactive tendon reflexes were found. Blood pressure fell from 130/80 to 90/50 mm Hg when the patient changed from the supine to standing position. A CT scan showed mild generalised brain atrophy with a slightly more pronounced cerebellar atrophy. The akinetic-rigid syndrome deteriorated considerably over the following eight months, eventually leading to multiple falls. No tremor was noted. Meanwhile, the depressive symptoms had substantially improved. As an explanation for her neurological symptoms, a multiple system atrophy was suspected, although Parkinson's disease was also considered.

Before starting a drug trial with levodopa, we wanted to rule out the possibility that the Parkinsonism was drug-induced. Fluvoxamine was therefore reduced to a daily dose of 100 mg. The extrapyramidal symptoms had already markedly decreased one week later. The fluvoxamine was now completely withdrawn, resulting in an almost complete disappearence of the extrapyramidal symptoms over a period of two weeks. One month after the cessation of fluvoxamine, only a mild decrease in arm swing was left; the hyperactive tendon reflexes were unchanged. The orthostatic hypotension had also disappeared. An MRI scan of the brain showed mild atrophic changes and some periventricular and deep subcortical white matter hyperintensities. There was no signal attenuation in the putamen on T2-weighted images as has been described in striatonigral degeneration and other multisystem atrophies. Neither were there changes in the posterior fossa suggestive of olivopontocerebellar atrophy. A rechallenge with the offending drug was considered unacceptable because of the risk of serious injury when falls reoccurred.

Our patient presented with a severe akinetic-rigid syndrome and orthostatic hypotension almost completely reversible after withdrawal of the antidepressant fluvoxamine which she had been taking for several months. Extrapyramidal and autonomic side effects are not usually described with this selective serotonin-reuptake inhibitor. As far as we know, the occurrence of orthostatic hypotension is very unusual with this drug that has no known antagonist activity for alfa-adrenergic receptors. The association between selective serotonin-reuptake inhibitors and extrapyramidal side-effects as well as akathisia has already been reported, however, although mainly for fluoxetine.[1–3] A possible explanation is that increased serotonergic activity may exert an inhibitory action on nigrostriatal dopaminergic neurons.[4] Pre-existing compromised nigrostriatal function caused by Parkinson's disease , other degenerative neurological disorders or dopamine-blocking agents might predispose patients to this adverse effect. Our patient had no such conditions. A causative role for the neuroleptics she had taken some months before the treatment with fluvoxamine is very unlikely because the extrapyramidal syndrome reached its maximum severity almost one year after the complete withdrawal of the antipsychotics. Finally, it could be argued that a dose of 200 mg of fluvoxamine is relatively high for an elderly patient, adding to the risk of developing side-effects.

Clinicians should be aware of this rare but potentially serious neurological complication of treatment with selective serotonin-reuptake inhibiting antidepressants, especially in patients with pre-existing neurological disease or already compromised extrapyramidal function due to neuroleptic medication.

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1 Citaulo DA, Shader RI. Fluoxetine drug-drug interactions: I. Antidepressants and antipsychotics. *J Clin Psychopharmacology* 1990; 10:48–50.

PATERNITY CALCULATIONS IN COURT

To the Editor:

Li and Chakravarti [1] introduce their article with the observation that "the purpose of conducting genetic marker tests ... is to ascertain whether the accusation (of paternity) is true or false". This is the crux of the issue and the results of any tests should only be used to help the court make its decision.

If the tests exclude the accused man then the court uses this information to decide that the accusation is false. If the tests do not exclude the accused, then the court still has to decide whether the accusation is true or false. If the accusation is indeed false, but not revealed by the tests, then the accused man has been chosen at random as far as his genetic make-up is concerned. Thus, the question to be asked is "what is the probability that a man, chosen at random and subjected to the same tests, would not be excluded". This is not the same as Steinberg's [2] contention that the question to be asked is "what is the probability that an accused male among the non-excluded males is the father". In fact, it is not reasonable to ascribe different probabilities to different non-excluded males, when ANY of the non-excluded males could be the father. I must emphasise that "not reasonable" is in the context of court proceedings and not in an academic environment.

Dodds [3] states that "... the legal profession prefer the probability that a random man would not be excluded...". The legal correspondent of the British Medical Journal [4] describes a case where "The evidence was to the effect that 998 out of 1000 men tested at random would have been excluded from paternity by the test".

If several men are tested to determine the paternity of one child, and none of the men can be excluded, then the "probability of a random man not being excluded" applies equally to all the men. Therefore, from a legal point of view, none of the men can be considered more likely than any of the other men because of his phenotype. Jaffee [5] makes the point that you only need to cast a reasonable doubt on the case against the accused man. The court will have to decide on other grounds or ask for additional tests to be carried out.

The probability of paternity, the likelihood of paternity, the paternity index and/or other statistics are based on gene frequencies. The probability that a random man would not be excluded should be based on phenotype frequencies. In the HLA system, haplotype frequencies are often used but even when gene frequencies at each locus are used the calculations are not a true reflection of "a random man not being excluded".

The complexity of the HLA system and the phenomenon of linkage disequilibrium within this complex gives rise to incorrect interpretations if gene frequencies or haplotype frequencies are used.

The difficulty is best illustrated with an example:
My database consists of over 26,000 HLA typed people.

Random Caucasian population

N = 2268
A2 = 48.63%
B7 = 26.15%

Observed (direct count) number of people with both antigens = 272 / 2268 = 11.99%

Estimated HF = 3.39% = 77 / 2268

Haplotype frequencies were estimated by the method of Mattiuz et al. [6].

If a child receives A2 and B7 from his father then any man with these two antigens can not be excluded

i.e. a probability of 272 / 2268 = 0.1199

= 11.99% Ratio 1 : 8.3

Using estimated HF's the probability is = 0.0339

= 3.39% Ratio 1 : 29.5

After many paternity investigations, it is likely that several cases would have occurred where the biological father must have possessed the antigens HLA A2 and HLA B7 and the accused men would all have been assigned a probability of 3.39% (the frequency of men with the A2 and B7 antigens on the same chromosome), whereas, in fact, some of those men should have been excluded because they do not carry the A2 and B7 antigens on the same chromosome. We know this is so because 11.99% of random men have both antigens but the haplotype frequency is only 3.39%.

Now, the question arises, which of the men should be excluded? Probability theory tells us that

$$(272\text{-}77)/272 = 71.69\%$$

of the accused men carry the A2 and B7 alleles on different chromosomes, so that seven out of ten men should be excluded by probability theory. The probabilty using HF's could be modified, which would result in a probability somewhere between 3.39% and 11.99% but then seven of the ten men would be worse off while the other three would be better off. Aicken and Kaye [7] quote Essen-Moller to make the point that every individual decision must be granted perfect independence.

The judge should not be presented with a probability that is "on the average" correct, because he has to decide each case individually. It is therefore preferable to use the probability that a random man possesses these two alleles (on any chromosome) for all cases.

Therefore, the probability of 11.99% should be used for all the men in these cases. Alternatively, full family studies must be done on each man to determine his haplotypes and then, and only then, can estimated haplotype frequencies be used.

Naturally, in each individual case the true probability is 3.39% and some theorists modify this result with the probability of the alleles being found on the same chromosome but this is only of academic interest and should not be used in a court of law.

Many laboratories do not have large databases of phenotype date and rely on published tables of allele and haplotype frequencies. However, using the formulae of Mayr and Pausch [8] it is possible to calculate backwards from frequency tables to phenotypes.

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REFERENCES

1 Li CC and Chakravarti A (1988) **An expository review of two methods of calculating the paternity probability.** *Am J Hum Genet.* 43:197-205

2 Steinberg AG (1987) **Letter to the editor.** *Am J Hum Genet* 41:900-901

3 Dodds BE (1980) **When blood is their argument** *Medicine, Science and the Law* 29: 231

4 Legal Correspondent Medicolegal (1986) **Blood tests and paternity** *BMJ* 292:262

5 Jaffee LR (1978) **Comment on the judicial use of HLA paternity test results and other statistical evidence: a response to Terasaki** *J Family Law* 17: 457

6 Mattiuz PL, Ihde D, Piazza A, Ceppellini R and Bodmer WF (1970) **New approaches to the population genetic and segregation analysis of the HLA system.** In: *Histocompatibility Testing 1970* (ed PI Terasaki) Munksgaard, Copenhagen p193-206

7 Aicken M and Kaye D (1983) **Some mathematical and legal considerations in using serological tests to prove paternity** In: *Inclusion probabilities in paternity testing* (ed RH Walker) American Association of Blood Banks, Arlington p155 - 169.

8 Mayr WR and Pausch V (1975) **Die berechnung der vaterschaftsaus-schluffchance im HLA system** *Z Immun-Forsch* 150: 447