The Association of various HLA-A, -B and -DR loci with Membranous Glomerulonephritis , IgA Nephropathy and Focal Segmental Glomerulosclerosis in KwaZulu-Natal Renal Patients

By

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Glória in excélsis Deo

To Emmanuel and Elizabeth

✣

Eternal rest grant unto them O Lord
And let perpetual light shine upon them
May their souls and the souls of all the faithful departed
Through the Mercy of God
Rest In Peace

✣

In Nomine Patris, et Filii, et Spiritus Sancti. Amen.

Declaration

This study presents original work by the author and has not in any form been submitted to this or any other university. The research described here was carried out in the Department of Medicine, Faculty of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, under the Supervision of Professor AGH Assounga. Where use is made of the work of others, it has been appropriately documented in the text.

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ABBREVIATIONS

Å – Angstrom Unit

ATP – Adenosine Triphosphate

β2-m – β2-microglobulin

CLIP – Class II-associated *i*nvariant chain *p*eptide

cm – Centimeter

CMV – Cytomegalovirus

COX-2 – Cyclooxygenase-2

CRF – Chronic Renal Failure

DNA – Deoxyribonucleic Acid

e.g. – For Example

EBV – Epstein-Barr Virus

EDTA – Ethylenediamine Tetra-Acetic Acid

ER – Endoplasmic Reticulum

ESRD – End Stage Renal Disease

FSGS – Focal Segmental Glomerulosclerosis

g – Gram

GBM – Glomerular Basement Membrane

GD – Glomerular Disease

GFR – Glomerular Filtration Rate

GN – Glomerulonephritis

H_2O – Water

HBVMN – Hepatitis B Virus-Associated Membranous Nephropathy

HIV – Human Immunodeficiency Virus

HIVAN – HIV Associated Nephropathy

HLA – Human Leukocyte Antigen

hp – Hewlett-Packard

HSP – Henoch-Schönlein purpura

HSV – Herpes Simplex Virus

H_v Regions – Hypervariable Regions

IALCH – Inkosi Albert Luthuli Central Hospital

IgA – Immunoglobulin A

IgAD – IgA Deficiency

IgAN – IgA Nephropathy

IgG – Immunoglobulin G

IgM – Immunoglobulin M

Ii – Invariant Chain

KZN – KwaZulu-Natal

MCGN – Minimal Change Glomerulonephritis

MGN/MN – Membranous Glomerulonephritis/ Membranous Nephropathy

MHC – Major Histocompatibility Complex

mm – Millimeter

mmHg – Millimeter Mercury

NK Cells – Natural Killer Cells

nm – Nanometer

NS – Nephrotic Syndrome

P – Probability of error

P_c – Corrected P value

PAS – Periodic Acid-Schiff

PCR – Polymerase Chain Reaction

PE – Plasma Exchange

pIgA1 – Polymeric IgA1

PMP – Pattern Matching Program

PP – Plasmapheresis

RER – Rough Endoplasmic Reticulum

SA-HRP – Streptavidin-Horseradish Peroxidase

SANBS – South African National Blood Services

SLE – Systemic Lupus Erythematous

SSO – Sequence Specific Oligonucleotide

STR – Single Tetranucleotide Repeat

TAP – Transporter associated with Antigen Processing

TAPBP – Tapasin

TB – Tuberculosis

T_C – Cytoxic T Cells ($CD8^+$)

T_H – T Helper Cells ($CD4^+$)

μm – Micrometer

viz. – Namely

VZV – Varicella-Zorster Virus

WHO – World Health Organization

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ABSTRACT

This KwaZulu-Natal (KZN) based study investigates hypertension, glomerulonephritides and the rarity of IgA Nephropathy (IgAN) in Africans in association with the Human Leukocyte Antigen (HLA). A retrospective hypertensive study found a positive association with HLA-B40 (P_c<0.05) and HLA-B15 (P_c<0.02) in Indians and Africans respectively. No association was found in Whites. A prospective study showed glomerulonephritides to be positively associated with HLA-A33 in Indians (P_c<0.049). No associations were found with glomerulonephritides in Africans and Whites. Combined Race groups show no HLA associations. HLA-A30; HLA-A34; HLA-A29; HLA-B42; HLA-B58; HLA-B70 and HLA-DR11 were extremely significantly higher in Africans compared to Indians and Whites (all P<0.0001). In conclusion, HLA-B40 and HLA-B15 are possible disease susceptibility markers in Indian and African hypertensives; HLA-A33 is a possible disease susceptibility marker for glomerulonephritides in Indians and alleles in linkage might be responsible for the rarity of IgAN in Africans but further studies need to be employed.

Keywords: hypertension, glomerulonephritides, HLA

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INTRODUCTION

Glomerular Disease (GD) (Remuzzi *et. al.*, 2006) and hypertension (Agnani *et. al.*, 2005) are the main contributors of end-stage renal disease (ESRD), requiring renal replacement therapy which is very expensive and, unlike the United Kingdom (Ellis *et. al.*, 1998), not always guaranteed in South Africa. This is not feasible in a developing country like South Africa where majority of the diseased renal population are poor but priority goes to HIV/AIDS which is a social disease and tuberculosis.

Patients with delayed presentation of renal failure increase the numbers for renal replacement therapy as pharmacological options are futile at this stage where ESRD is inevitable (Ellis *et. al.*, 1998). Kidney dysfunction is coupled with cardiovascular disease (Ritz, 2006) hence disease of the kidney will ultimately lead to disease of the heart.

Certain genes of the immune system are said to be disease susceptibility genes whereas certain genes are said to be protective (Mitchison *et. al.*, 1996). This is a result of either the disease susceptibility alleles being present in a higher proportion of patients when compared to controls (in the case of disease susceptibility) or vice versa (in terms of protection). The human major histocompatibility complex (HLA) is used to determine possible disease susceptibility or protective alleles. Carrying either a disease susceptibility or protective allele will either predispose or initiate immunity to that particular disease in an individual (Lipsitch *et. al.*, 2003).

Trowsdale (1995) described the major histocompatibility complex (MHC) as being the 'Centre of the Immune Universe' stimulating almost every immunological activity involving cell-to-cell interaction (Kimball, 1986). Genes of the human MHC are present on chromosome 6 and code for molecules that present processed antigens to cytotoxic T cells.

HLA differs between race and geographic location (Kukko *et. al.*, 2004). This is due to polymorphic residues present in the peptide binding region of the HLA molecule. Polymorphism is postulated to be a result of gene conversion and genetic drift (Parham and Ohta, 1996), infectious disease outcome (Jeffery and Bangham, 2000) as well as environmental factors (Kukko *et. al.*, 2004). The MHC is extremely sensitive that even a single amino acid substitution due to mutation can alter the antigen it presents (Mellins *et. al.*, 1988).

The aim of this study was to investigate associations between HLA and hypertension and glomerular diseases in this diverse KZN setting. Although there is an immunological genetic basis for each population responding differently to GD, a socio-economic racial predisposition also exists (Seedat *et. al.*, 1984).

IgAN has been found to be uncommon amongst the African race group (Jennette *et. al.*, 1985; Seedat *et. al.*, 1984; Seedat *et. al.*, 1988). No reason for this occurrence has been found to date. It could be argued that the rarity of IgAN in Africans could be due to late presentation with ESRD when diagnostic renal biopsies may not be available and this

could be true in poor settings however, IgAN has also been shown to be rare in an African American population (Jennette *et. al.*, 1985). America is a first world, resource rich country with a greater standard of health care. Therefore another goal of this study was to attempt to find possible alleles present or absent in the HLA complex of the African population that protects them against IgAN.

Associations have been found between HLA and the diseases under investigation in this study in other settings (Freedman *et. al.*, 1994a and b; Klouda *et. al.*, 1979; Kobayashi *et. al.*, 1985; Lagueruela *et. al.*, 1990; Ruder *et. al.*, 1990). In KZN, studies aiming to find associations between HLA and nephrotic syndrome (NS) (Adhikari *et. al.*, 1985) as well as hepatitis B virus-associated membranous nephropathy (HBVMN) (Bhimma *et. al.*, 2002) have been carried out in the pediatric patient population. Significant associations were found between the Class II HLA-DQB1*0603 and HBVMN in Black African children, HLA-B*w*44 in Indian and HLA-B*w*21 in African children with NS.

HLA associations with HIV in KZN have suggested insight into vaccine design (Kiepiela *et.al.*, 2004). HLA studies may therefore provide the foundation on which other studies are built upon towards the development of therapeutic measures.

With regards to HLA studies it is difficult to build upon earlier investigations as each population (in terms of ethnicity as well as geographic location) has a unique HLA profile although HLA-B15 has been shown to be associated with hypertension in various populations (Gudbrandsson *et. al.*, 1980; Hilme *et. al.*, 1993; Kristensen, 1981). It

therefore becomes feasible to rather aim to prove similar associations or detect novel disease susceptibility and protective alleles in populations where these studies have not been carried out.

Another difficulty presented is the fact that the HLA type of a population is determined by genetic recombination and environmental factors implying that each generation of the same population in the same geographic area might have a different HLA profile. This indicates that making use of information derived from HLA studies carried out in the past may not at present be valid in the development of therapeutic measures as the HLA type of a population is continually changing.

About the Study

The HLA alleles under investigation in this study *viz.* HLA-A, -B and -DR were chosen because they are they most polymorphic Class I and Class II HLA alleles. The importance of studying the association of HLA with hypertension and renal diseases stems from the fact that these are major contributors of ESRD. Detecting an allele that is highly represented in the patient population is not sufficient to make any conclusions. It is therefore important to compare the diseased individuals with healthy controls to determine possible disease susceptibility or protective alleles which either predispose or protect an individual from a particular disease. If a an allele is present in the diseased population in amounts that greatly exceed the normal population and a significant association is found after correcting the *P* value, that allele becomes a possible disease susceptibility marker. This information can now be used as a basis for various other studies including those towards the development of therapeutic measures.

This is primarily an aim driven study which is divided into three parts with distinct aims.

1. The first part of the study, Part A, aims to find associations (if any) of HLA alleles with hypertension amongst the African, Indian and White populations in KZN. In addition since HLA-B15 has been shown to be associated with various hypertensive populations, it was hypothesized that a similar association would be found in KZN.

2. The second part of the study, Part B, aims to find associations between HLA alleles and glomerulonephritides in KZN. These diseases include IgA

nephropathy (IgAN), membranous glomerulonephritis (MGN) and focal segmental glomerulosclerosis (FSGS). These diseases were chosen for the following reasons. IgA nephropathy is the most prevalent primary glomerulonephritides worldwide, FSGS is very common especially in African males and MGN is known to be one of the most common causes of NS

3. The third part of the study, Part C, aims to investigate the reason for the rarity IgAN in the African population by observing the genetic profile of the African Race as a whole (combining diseased and normal populations) and comparing it to the White and Indian populations.

CHAPTER 1

LITERATURE REVIEW

In order for the adaptive immune system to destroy a foreign antigen it first needs to detect its presence (Turka, 1997). The body does not destroy its own antigens or that of an identical twin (Jawetz *et al.*, 1982) because they are recognized as "self" (except for autoimmune disorders such as systemic lupus erythematosis) but when a foreign cell enters the body by organ transplant (Wolfe, 1995) for example, its proteins are degraded either cytosolically or endocytically and presented to the T Cell Receptors of T lymphocytes bound to cell surface glycoproteins called the Major Histocompatibility Complex proteins (Brodsky, 2001).

1.1 General Introduction of the Major Histocompatibility Complex

All nucleated (Barrett, 1988) vertebrate (Austyn and Wood, 1993) somatic (Hughes and Yeager, 1998) cells have a set of molecules present on its surface which are responsible for lymphocyte recognition (Schwartz, 1987) and antigen presentation (Brodsky, 2001; Zacharias and Springer, 2004) termed The Major Histocompatibility Complex (MHC) molecules. These molecules are polymorphic glycoproteins (Austyn and Wood, 1993) which can be found in serum, saliva and urine (Braun, 1979) and are encoded by a closely linked set of genes (Sayegh *et al.*, 2000) that form a "complex" on chromosomes called the MHC. The human MHC is present on the short arm of chromosome 6 (Williams, 2001), the rat MHC on chromosome 20 and the mouse MHC on chromosome 17 (Barrett, 1988; Margulies, 1999). The molecules of the MHC include the highly

polymorphic class I and class II molecules (Pattison *et al.*, 1997; Rees, 1997) and "the not so polymorphic" class III molecules (Rees, 1997). Class I and class II molecules are specialized membrane proteins that facilitate T cells to recognize antigen (Austyn and Wood, 1993) thus playing a vital role in graft rejection.

MHC was christened as such due to its discovery during initial transplant studies (Gorer, 1936a, b) and was acknowledged as an important ("Major") set of genes ("Complex") which predispose the regulation of the acceptance of grafts between individuals whose tissues are genetically similar ("Histcompatible") or rejected by individuals that are not ("Histoincompatible") (Austyn and Wood, 1993). In addition, minor histocompatibility antigens which are derived from polymorphic cellular proteins (Simpson *et. al.*, 2002) do not mount a rapid rejection response as MHC but play a role in tissue compatibility (Austyn and Wood, 1993).

Discovery of the MHC antigens initially occurred during tumour transplant studies between different strains of inbred mice (Gorer, 1936b) and were thus characterized as transplant antigens (Hoecker, 1986). MHC antigens are the strongest and most important transplant antigens as they provide the strongest incompatibilities for any type of tissue and organ transplant in both animals and humans and are able to stimulate a primary immune response without being sensitized (Sayegh *et al.*, 2000). Other transplant antigens include the minor histocompatibility antigens, ABO blood group antigens and monocyte/endothelial cell antigens (Sayegh *et al.*, 2000).

Inbred strains, unlike outbred populations (e.g. humans) which are made up of a great number of MHC haplotypes (Heise, 1984), are composed of genetically identical individuals hence tissue transplantation between the same inbred strain was possible without risk of rejection (Gorer, 1936a). Thus the tissues of one inbred strain are deemed histocompatible in relation to each other but histo**in**compatible with another inbred strain (Austyn and Wood, 1993).

In 1958, Dausset discovered agglutinating antibodies as a result from a reaction between the sera of multi-transfused individuals as well as that of multiparous women and leukocytes from blood donors. These serological studies demonstrated that agglutination does not occur between the sera and leukocytes of the same individual or between those of individuals who produce similar antibodies. It also demonstrated that leukocyte antigens are genetically determined by Dausset's observation that the pattern of agglutination from a series of antisera was identical in monozygotic twins but not in dizygotic twins.

1.1.1 General features of the Major Histocompatibility Complex

Three different families of glycoproteins are coded for by the MHC *viz*. Class I, class II and class III molecules (Austyn and Wood, 1993). Co dominant expression (Buckley, 2003), polygenicity and polymorphism are prominent features of the MHC (Janeway *et. al*., 2005) which work together to prevent pathogens from invading the host cell. Class I and class II molecules are alloantigens which are expressed on the cell surface as

membranous glycoproteins (Austyn and Wood, 1993). Class III molecules are soluble proteins which include soluble effector molecules (Austyn and Wood, 1993) such as enzyme steroid 21-hydroxylase (Hauptmann and Bahram, 2004), heat shock proteins HSP70 (Porto *et. al.*, 2005) and components of the complement system (Barrett, 1998).

The genes of the MHC are most common, divergent, evenly distributed (Parham and Tomoko, 1996) and are highly polymorphic (Turka, 1997). In fact they are known to be the most polymorphic cluster of genes that exist in the mammalian genome (Just, 1995; Wooley and David, 1984).

Class I and Class II molecules are involved in antigen processing and presentation of peptides within the cell or those from extracellular space (Stevanović, 2002) via the cytosolic and endocytic pathways respectively (Brodsky, 2001).

1.1.2 Nomenclature

MHC nomenclature is extremely complicated due to the presence of different nomenclature systems between species and not as a result of any fundamental distinctions between the structure and function of the MHC molecules (Austyn and Wood, 1993). Due to the rapid increase of new alleles (Braun, 1979) and novel findings (Margulies, 1999) further complications are discovered bringing about a need for greater precision from historical systems (Margulies, 1999).

1.1.2.1 Nomenclature in terms of species

MHC in the mouse is designated *H-2* (Panayi and David, 1984) and is maintained for historical reasons as it was the second of the four blood group antigens that Gorer discovered to have an association with tumour transplants. Except for *B* used to describe the chicken MHC (Götze, 1981) and *RTI*, which replaces the old nomenclature AgB used to describe the rat MHC, most of the nomenclature systems were designed around that assigned to humans – *HLA* which represents human leukocyte antigen or more specifically human leukocyte associated antigen A (Austyn and Wood, 1993). Hence *ChLA* is used to describe the chimpanzee leukocyte antigen (Margulies, 1999); *RLA*, the rabbit leukocyte antigen (Austyn and Wood, 1993); *BoLA*, the cattle (bovine) leukocyte antigen (Götze, 1981) *DLA*, the dog leukocyte antigen (Margulies, 1999); *GPLA*, the guinea pig leukocyte antigen (Austyn and Wood, 1993); *SLA*, the swine (pig) leukocyte antigen (Margulies, 1999) and *RhLA*, the rhesus monkey leukocyte antigen (van Rood *et al.*, 1981).

1.1.2.2 Nomenclature in terms of the gene map

The earliest genes of the MHC that were recognized are classified as MHC genes because the cell surface molecules they encode can be detected by antibodies and transplantation responses (Margulies, 1999). At present there are hundreds of mouse and human MHC genes but MHC molecules refer distinctively to MHC-I and MHC-II structurally and functionally related molecules (Margulies, 1999).

As of April 2007, the international ImMunoGeneTics (IMGT) Database currently contains 545 Class I HLA-A alleles, 894 Class I HLA-B alleles, 307 Class I HLA-C alleles, 3 Class II HLA-DRA alleles, 577 Class II HLA-DRB alleles, 34 Class II HLA-DQA1 alleles, 83 Class II HLA-DQB1 alleles, 23 Class II HLA-DPA1 alleles and 126 Class II DPB1 alleles.

Specific MHC genes are denoted by a letter for the locus after the prefix e.g., for the mouse MHC, H-2K where H-2 is the prefix and K is the locus (Margulies, 1999). An example of the human MHC is HLA-A where HLA is the prefix and A is the locus (Margulies, 1999).

Alleles of the mouse MHC have been designated by adding a subscript to the locus for e.g., H-2K^{b} and H-2K^{d} where K^{b} and K^{d} are two different alleles present at the same locus (Margulies, 1999). Human MHC class II genes are designated HLA-D but murine MHC class II genes are denoted as H-2IAa, H-2IAb, H-2IEa, H-2IEb where a and b respectively denote the alpha (α) and beta (β) coding chains (Margulies, 1999).

The nomenclature system for the human MHC is developed by the HLA Nomenclature Committee of the World Health Organization (WHO) (Schwartz, 1987). The classical HLA MHC class I genes officially recognized by the WHO are HLA-A, HLA-B and HLA-C (Schwartz, 1987) and the MHC class II region is HLA-D which comprises HLA-DR, HLA-DP and HLA-DQ loci (Austyn and Wood, 1993). Non classical MHC genes

with limited immune functions and a low degree of polymorphism and expression are the MHC-Ib genes *viz.* HLA-E, HLA-F and HLA-G (Williams, 2001).

Provisionally accepted antigens have a "*w*" placed between the locus and the number e.g., HLA-B*w*22 (Braun, 1979) and is a result of inaccurate serological assignments (Margulies, 1999). When it becomes officially recognized and accepted the '*w*', which stands for workshop, is eliminated (Schwartz, 1987) and the allele becomes HLA-B22.

MHC Class I loci only encompass a single gene (Austyn and Wood, 1993) whereas the Class II region contains the A and B genes which may confer antigen variability (Williams, 2001) e.g., HLA-DRA and HLA-DRB1 (Austyn and Wood, 1993).

The Human MHC is known to be the predominantly investigated (Janer and Geraghty, 1998) and most polymorphic set of genes present in the human genome (Jeffery and Bangham, 2000; McCluskey and Peh, 1999) and initial serological methods used to resolve alleles proved to be inadequate in detecting the variation of the alleles that antibodies were unable to gain access to thus bringing about a need for modern molecular methods (McCluskey and Peh, 1999). For example alleles identified by serology is denoted as HLA-A2 and those identified by DNA typing, HLA-A*0201.

In order to fully understand the way the human designation works, a conversion table (Table 1.1.1 and Table 1.1.2) which aligns the alleles that have been resolved

serologically with those that have been resolved by modern DNA typing methods needs to be consulted (Margulies, 1999).

Table 1.1.1 Classical HLA Class I Alleles and their Serological Designations.

HLA-A Locus		HLA-B Locus		HLA-C Locus	
Serological Designation	HLA Allele	Serological Designation	HLA Allele	Serological Designation	HLA Allele
A1	A*0101-A*0125	B7	B*0702-B*0755	Cw1	Cw*0102-Cw*0118
A2	A*0201-A*0299	B8	B*0801-B*0833	Cw2	Cw*0202-Cw*0218
A3	A*0301-A*0329	B13	B*1301-B*1318	Cw3	Cw*0302-Cw*0340
A11	A*1101-A*1130	B14	B*1401-B*1407	Cw4	Cw*0401-Cw*0427
A23	A*2301-A*2315	B15	B*1501-B*1599	Cw5	Cw*0501-Cw*0516
A24	A*2402-A*2476	B18	B*1801-B*1826	Cw6	Cw*0602-Cw*0616N
A25	A*2501-A*2506	B27	B*2701-B*2737	Cw7	Cw*0701-Cw*0748
A26	A*2601-A*2534	B35	B*3501-B*3557	Cw8	Cw*0801-Cw*0814
A29	A*2901-A*2916	B37	B*3701-B*3713	Cw12	Cw*1202-Cw*1221
A30	A*3001-A*3021	B38	B*3801-B*3816	Cw14	Cw*1402-Cw*1408
A31	A*3101-A*3117	B39	B*3901-B*3941	Cw15	Cw*1502-Cw*1520
A32	A*3201-A*3215	B40	B*4001-B*4075	Cw16	Cw*1601-Cw*1609
A33	A*3301-A*3310	B41	B*4101-B*4108	Cw17	Cw*1701-Cw*1704
A34	A*3401-A*3408	B42	B*4201-B*4209	Cw18	Cw*1801-Cw*1803
A36	A*3601-A*3604	B44	B*4402-B*4453		
A43	A*4301	B45	B*4501-B*4507		
A66	A*6601-A*6606	B46	B*4601-B*4610		
A68	A*6801-A*6838	B47	B*4701-B*4705		
A69	A*6901	B48	B*4801-B*4816		
A74	A*7401-A*7412	B49	B*4901-B*4905		
A80	A*8001	B50	B*5001-B*5004		
A92	A*9201-A*9216	B51	B*5101-B*5148		
		B52	B*5201-B*5211		
		B53	B*5301-B*5312		
		B54	B*5401-B*5412		
		B55	B*5501-B*5526		
		B56	B*5601-B*5620		
		B57	B*5701-B*5712		
		B58	B*5801-B*5815		
		B59	B*5901-B*5902		
		B67	B*6701-B*6702		
		B73	B*7301		
		B78	B*7801-B*7805		
		B81	B*8101-B*8102		
		B82	B*8201-B*8202		
		B83	B*8301		
		B95	B*9501-B*9529		

Adapted from http://www.anthonynolan.org.uk/HIG/lists/classIlist.html

Table 1.1.2 Classical HLA Class II Alleles and their Serological Designations.

HLA-DR Locus		HLA-DQ Locus		HLA-DP Locus	
Serological Designation	HLA Allele	Serological Designation	HLA Allele	Serological Designation	HLA Allele
DRA	DRA*0101-DRA*0202	DQA1	DQA1*0101-DQA1*0107	DPA1	DPA1*0103-DPA*0109
			DQA1*0201		DPA1*0201-DPA*0203
DRB1	DRB1*0101-DRB1*0116		DQA1*0301-DQA1*0303		DPA1*0301-DPA*0303
	DRB1*0301-DRB1*0335		DQA1*0401-DQA1*0404		DPA1*0401
	DRB1*0401-DRB1*0464		DQA1*0501-DQA1*0509		
	DRB1*0701-DRB1*0712		DQA1*0601-DQA1*0602	DPB1	DPB1*0101-DPB1*0102
	DRB1*0801-DRB1*0832				DPB1*0201-DPB1*0203
	DRB1*0901-DRB1*0906	DQB1	DQB1*0201-DQB1*0205		DPB1*0301-DPB1*0302
	DRB1*100101-DRB1*100103		DQB1*0301-DQB1*0320		DPB1*0401-DPB1*0403
	DRB1*11101-DRB1*1163		DQB1*0401-DQB1*0402		DPB1*0501-DPB1*0502
	DRB1*1201-DRB1*1215		DQB1*0501-DQB1*0505		DPB1*0601-DPB1*0602
	DRB1*1301-DRB1*1375		DQB1*0601-DQB1*0630		DPB1*0801-DPB1*0802
	DRB1*1401-DRB1*1466				DPB1*0901-DPB1*0902
	DRB1*1501-DRB1*1522				DPB1*1001-DPB1*1002
	DRB1*1601-DRB1*1611				DPB1*1101-DPB1*1102
					DPB1*1301-DPB1*1302
DRB2	DRB2*0101				DPB1*1401-DPB1*1402
					DPB1*1501-DPB1*1502
DRB3	DRB3*0101-DRB3*0111				DPB1*1601-DPB1*1602
	DRB3*0201-DRB3*0222				DPB1*1701-DPB1*1702
	DRB3*0301-DRB3*0303				DPB1*1801-DPB1*1802
					DPB1*1901-DPB1*1902
DRB4	DRB4*0101-DRB4*0107				DPB1*2001-DPB1*2002
	DRB4*0201N				DPB1*2101-DPB1*2901
	DRB4*0301N				DPB1*3001-DPB1*3901
					DPB1*4001-DPB1*4901
DRB5	DRB5*0101-DRB5*0113				DPB1*5001-DPB1*5901
	DRB5*0202-DRB5*0205				DPB1*6001-DPB1*6901
					DPB1*7001-DPB1*7901
DRB6	DRB6*0101				DPB1*8001-DPB1*8901
	DRB6*0201-DRB6*0202				DPB1*9001-DPB1*9901
DRB7	DRB7*010101-DRB1*010102				
DRB8	DRB8*0101				
DRB9	DRB9*0101				

Adapted from http://www.anthonynolan.org.uk/HIG/lists/class2.html

In the human MHC, alleles are denoted by adding a letter and a number e.g., HLA-A2 where 2 is the allele at locus A (Margulies, 1999). Human MHC genes can be more specifically denoted by adding a superscripted asterisk (McCluskey and Peh, 1999) to the prefix-locus combination followed by a four digit number (Margulies, 1999) to the locus which corresponds to a specific allele e.g., HLA-A*0201 (McCluskey and Peh, 1999).

The first two digits following the locus represent the fundamental serological specificity of the allele and the following two a specific allele sequence but the addition of a fifth digit indicates a silent polymorphism (Leffell, 2002; Williams, 2001). Variations that take place outside the coding region result in the addition of a sixth and seventh digit (Williams, 2001). The presence of a null allele sequence is denoted by the addition of an N to the allele number (Williams, 2001). A simplified illustration can be seen by Table 1.1.3.

Private antigens are HLA antigens which are resolved by a single allele alone as opposed to public antigens which are familiar to a number of HLA molecules and which bears its own private antigen (Schwartz, 1987). Excellent examples of HLA public antigens are HLA-Bw4 and HLA-Bw6 (Schwartz, 1987). HLA antigens that were originally considered to be single private antigens were identified as being part of a group of 2 to 3 closely related antigens, each of narrow specificity and characterized as 'splits' of the original antigen of broad specificity (Schwartz, 1987).

Nomenclature of split antigens occurs as follows. The broad antigen of which the split is made follows the split in parenthesis (Schwartz, 1987) for example, HLA-DR15(2) and HLA-DR16(2) implies that both HLA-DR15 and HLA-DR16 are splits of the broad antigen HLA-DR2 (Margulies, 1999) indicating that HLA-DR2 could possibly be regarded as a public HLA antigen which bears HLA-DR15 and HLA-DR16 as private antigens (Schwartz, 1987).

Blank alleles are indicative of homozygous alleles or alleles that cannot be defined by the available reagents (Schwartz, 1987). This could be due to the great polymorphism of the MHC whereby reagents produced in one country for a certain allele may not be able to detect alleles of an entirely different population. Blank alleles present with resolved alleles may be defined by family studies (Schwartz, 1987).

Table 1.1.3 A Simplification of HLA Nomenclature.

HLA Designation	Indicative of
HLA	Human MHC (Human Leukocyte Antigen)
HLA-A	The A locus at Human MHC
HLA-A2	Allele at the A locus
HLA-A*0201	Specific Designation
HLA-A*0201N	Null allele
HLA-A*02011	Silent Mutation
HLA-A*0201101	Variation outside the coding region
HLA-A*0201101N	Null allele outside the coding region

* Adapted from McCluskey and Peh, 1999.

1.1.3 Inheritance of MHC In Humans

Humans inherit their HLA genes by a Mendelian-dominant method (Buckley, 2003; Sayegh *et.* al., 2000; Sullivan and Amos, 1986). Inheriting a specific combination of the six MHC loci (*viz.* Class I and Class II) is referred to as a haplotype (Brodsky, 2001) which is passed on to the offspring by one of the parents (Payne, 1977) due to linkage of the HLA genes and low crossover rates (Buckley, 2003; Sayegh *et. al.*, 2000). Each individual has two haplotypes each inherited from one parent (Buckley, 2003; Sullivan and Amos, 1986) thus constituting the genotype (Payne, 1977).

1.1.4 Structure of The Human Major Histocompatibility Complex

1.1.4.1 Gene Structure of Human MHC (HLA)

The genes which encode the HLA are present on the short arm of autosome (Buckley, 2003) chromosome 6 (Williams, 2001) *in the distal portion* (Rees, 1997) of the 6p21.3 (Alper *et. al.*, 2006; Rees, 1997) band between 6p21.31 and 6p21.32 (Margulies, 1999). The HLA complex comprises more than 200 genes, extends over 4×10^6 nucleotides (Janeway *et. al.*, 2005) inhabiting a segment of about 4 centimorgans (cM) (Schwartz, 1987) and forms a linkage complex with phosphoglucomutase-3 ($PGM\text{-}_3$), glyoxalase (GLO) and urine-pepsinogen-5 (Pg-5) (Götze, 1981).

The genes are divided into three regions (see Figure 1.1.1) and are arranged from the centromeric to the telomeric end (Porto *et. al.*, 2005) as Class II, Class III and Class I

genes respectively (Rees, 1997). Class I genes are strictly α-chain genes whereas Class II genes are heavy α- and light β-chain genes (Janeway *et. al.*, 2005).

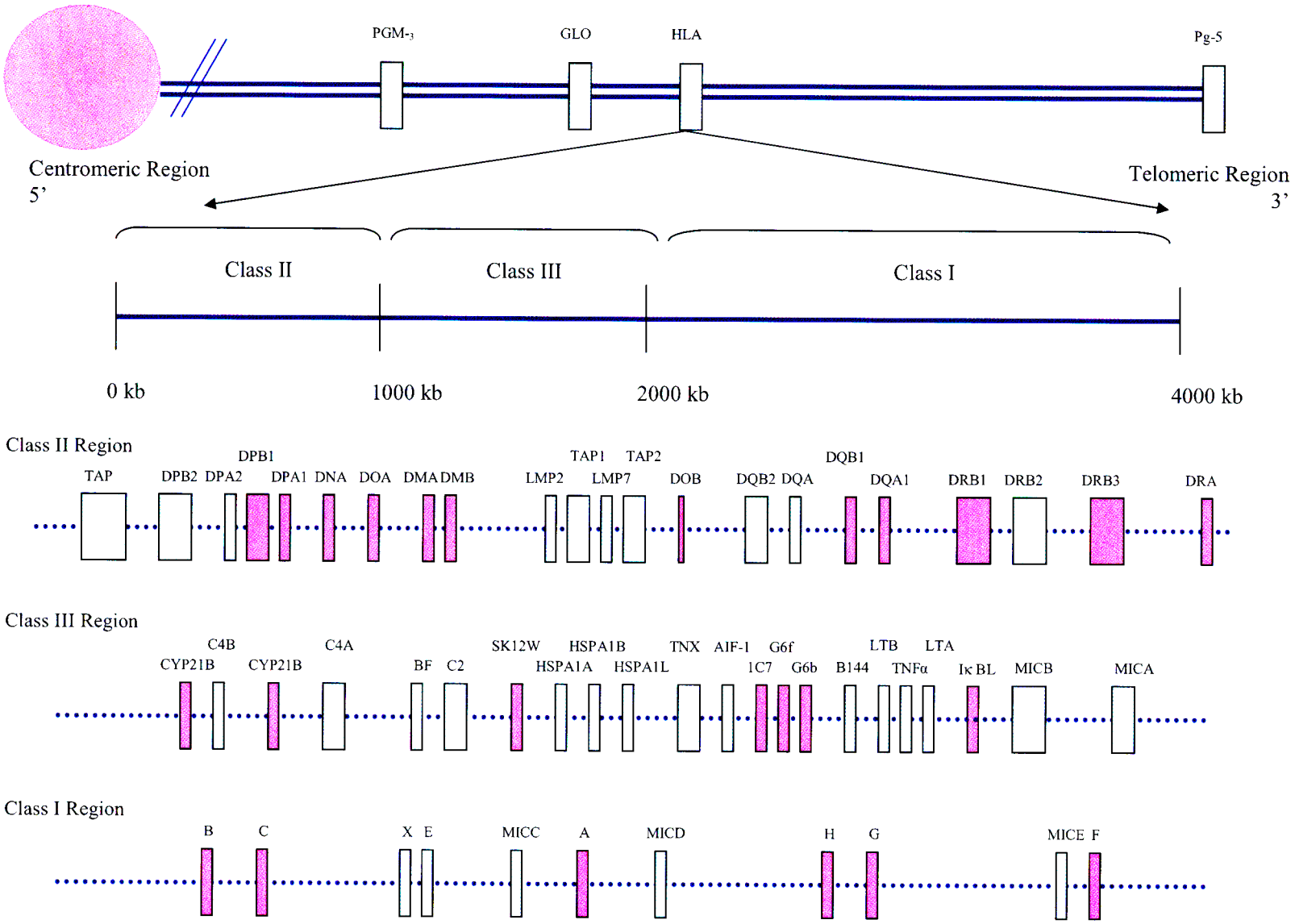

Figure 1.1.1 Gene structure of the Class I, Class II and Class II regions of the HLA complex present on the short arm of chromosome 6. Abbreviations explained in text. Adapted from Alvarado-Guerri *et. al.*, 2005; Austyn and Wood, 1993; Barret, 1988; Bennetts *et. al.*, 1999; Chevrier *et. al.*, 1998; Edwards and Hedrick, 1998; Hauptmann and Bahram, 2004; Hviid *et. al.*, 1999; Götze, 1981; Gruen and Weissman, 1997; Janeway *et. al.*, 2005; Margulies, 1999; Porto *et. al.*, 2005; Pozzi *et. al.*, 1998; Rees, 1997; Sayegh *et. al.*, 2000; Schwartz, 1987; Trowsdale, 2005; van Rood *et. al.*, 1981.

The HLA Class II region is divided into three subregions *viz.* HLA-DR, HLA-DP and HLA-DQ (Austyn and Wood, 1993). HLA-DP and HLA-DQ both have two α (α_1 and α_2) and two β (β_1 and β_2) genes (Austyn and Wood, 1993) whereas HLA-DR has only one monomorphic α gene (Sayegh *et al.*, 2000) and three β (β_1, β_2 and β_3) genes whose product is able to couple with the α chain resulting in four MHC Class II molecules from the three subregions (Janeway *et. al.*, 2005; Schwartz, 1987).

The position of the genes are ordered as follows, the HLA-D (Class II) region (Götze, 1981) closest to the centromere (van Rood *et. al.*, 1981) followed by the diverse Class III region which spans 120kb (Rees, 1997) thereafter HLA-B, HLA-C, HLA-E, HLA-A, HLA-H, HLA-G and HLA-F (Margulies, 1999) respectively followed approximately 4 megabases telomerically from HLA-A by HFE (Gruen and Weissman, 1997; Janeway *et. al.*, 2005; Porto *et. al.*, 2005) which is a hemochromatosis related gene (Porto *et. al.*, 2005).

HLA-A, -B and -C are classical Class I MHC antigens (Schwartz, 1987) and non-classical Class I antigens include HLA -E, -F, -G (Hviid *et. al.*, 1999; Pozzi *et. al.*, 1998). In addition to the Class III region, the genes controlling the expression of DR molecules are present between the HLA-D and HLA-B region (Götze, 1981).

Proteins that are responsible for antigen processing and presentation of Class I molecules (LMP2; LMP7 and TAP1; TAP2) as well as Class II molecules (HLA DM) have the genes that encode them in the Class II region (Bennetts *et. al.*, 1999; Chevrier *et. al.*, 1998; Rees, 1997). TAP genes lie between DQB1 and DPA1 (Alvarado-Guerri *et. al.*, 2005). In close

proximity to the centromere lies TAPBP which codes for tapasin (Janeway *et. al.*, 2005). Also present in the Class II region encoding the DO molecule, are the DNα and DOβ genes (Janeway *et.al.*, 2005).

The Class III region, centromeric to the Class I region (Kimball, 1986), known as the Central MHC (Gruen and Weissman, 1997; Hauptmann and Bahram, 2004; Noble *et. al.*, 2006) is located between the Class II and Class I regions (Wright *et. al.*, 2001) and contains genes unrelated to the Class I and Class II regions *viz.* complement protein genes C4; Factor B (Bf) and C2 (Barret, 1988; Janeway *et. al.*, 2005), extracellular matrix protein genes (Tenascin [TNX]) and Immunoglobulin superfamily gene proteins (1C7; G6f; G6b) (Hauptmann and Bahram, 2004), hormonal synthesis genes (steroid 21-hydroxylase; CYP21), cytokine encoding genes (Tumor Necrosis Factor α (TNF-α) and lymphotoxins; LTA (TNF-β) and LTB) (Gruen and Weissman, 1997; Janeway *et. al.*, 2005 and Makhatadze, 1998), cellular stress genes (MICA; MICB) (Janeway *et. al.*, 2005) and Heat Shock Proteins (HSPA1A; HSPA1B and HSPA1L) (Hauptmann and Bahram, 2004). Other cellular stress genes (MICC; MICD and MICE) are located in the MHC Class I region (Janeway *et. al.*, 2005).

Other genes involved in inflammatory responses are B144, AIF-1, I κ B-like and SKI2W (Gruen and Weissman, 1997). In 1997, Gruen and Weissman suggested that genes which are involved in inflammation, stress and infection be categorized into a distinct region called the Class IV region.

MHC-X lies between HLA-C and HLA-E (Edwards and Hedrick, 1998 and Trowsdale, 2005), occupies 7.6 megabases and is an extension of the Class I and Class II regions and contains genes that encode histones and tRNA (Trowsdale, 2005).

1.1.4.2 Physical Structure of HLA Class I and Class II Molecules

Chapter 1.1.4.1 focused on the genes which encoded the MHC in humans. I will now concentrate on the products of the Class I and Class II region of the HLA complex. Class I and Class II molecules are cell surface receptors (Maenaka and Jones, 1999) which are structured differently to facilitate different functions in antigen presentation, peptide binding and T-cell activation (Janeway *et. al.*, 2005).

Both Class I and Class II molecules are heterodimeric (Maenaka and Jones, 1999) glycoproteins (Austyn and Wood, 2003) which both span the cell membrane (Brodsky, 2001 and Turka, 1997) are composed of four domains which are paired (Janeway *et. al.*, 2005) into proximal constant and distal variable regions (Wolfe, 1997) and possess a single peptide binding groove which is able to accommodate a variety of peptides (Abbas and Lichtman, 2003).

1.1.4.2.1 Structure of Class I Molecules

The Class I molecules are made up of two chains, a heavy α chain (Bettinotti *et. al.*, 2003) with a molecular weight of 44 kDa (Anonymous, 2007b; Rees, 1997) and a light β chain with

a molecular weight of 12 kDa (Sayegh *et.al.*, 2000). The α chain is encoded by Exons 2, 3 and 4 of the MHC and can be distinguished into 5 structural domains, α_1 - α_5 (Austyn and Wood, 1993; Barret, 1988).

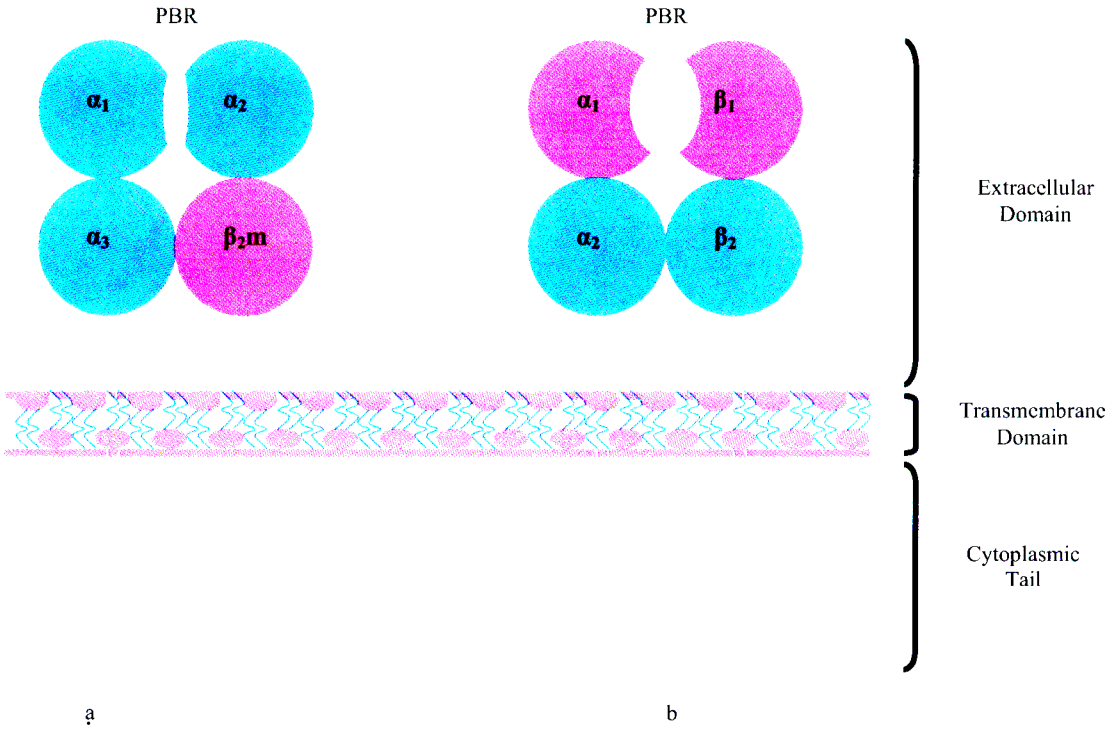

Figure 1.1.2 Diagrammatic representation of MHC Class I (a.) and Class II (b.) molecules. PBR= Peptide Binding Region. Adapted from Austyn and Wood, 1993; Brodsky, 2001; Sayegh *et. al.*, 2000.

Three of these domains α_1, α_2 and α_3 present as folded (Brodsky, 20001) extracellular domains (Hughes and Yeager, 1998) and form a noncovalent association (Kimball, 1986; Trinh *et. al.*, 2002) with a soluble (Maenaka and Jones, 1999) polypeptide β_2-microglobulin

(β_2-m) (Brodsky, 2001) which is encoded by a gene present on chromosome 15 (Janeway *et. al.*, 2005) (Figure 1.1.2a).

The α chain of the Class I molecule is comprised of three regions (Schwartz, 1987). The first region is a hydrophobic extracellular region (Schwartz, 1987) which is comprised of α_1, α_2 and α_3 domains (Brodsky, 2001) each comprised of approximately 90 amino acids (Rees, 1997; Schwartz, 1987). The second and third regions are comprised of the α_4 and α_5 domains (Austyn and Wood). These regions are the hydrophobic transmembrane region made up of about 25 amino acids (Schwartz, 1987) which spans the lipid bilayer (Ausytn and Wood, 1993) and an intracellular, hydrophilic (Schwartz, 1987) cytoplasmic tail made up of about 30 to 40 amino acids (Austyn and Wood, 1993) attached to the α_3 domain. The α_3 domain is the only Class I molecule chain that spans the membrane (Janeway *et. al.*, 2005) thus anchoring the molecule to the membrane by a carboxy-terminus (Brodsky, 2001).

As documented in Chapter 1.4.2, MHC Class I and Class II molecules are made up of four domains which are paired. In Class I molecules the relatively conserved (Rees, 1997) α_3 and β_2-m domains are paired, placed closest to the cell membrane (membrane proximal domain) (Austyn and Wood, 1993) and have a structure similar to that of an immunoglobulin domain (Janeway *et. al.*, 2005; Nikolich-Žugich *et. al.*, 2004). The highly polymorphic (Rees, 1997), variable (Sayegh *et. al.*, 2000; Williams, 2001) α_1 and α_2 domains are paired, placed distal to the membrane surface (Nikolich-Žugich *et. al.*, 2004) and folded in a particular way to form a cleft on the molecule surface (Janeway *et. al.*, 2005). This cleft is the site of peptide

binding (Williams, 2001) and is designated the peptide binding cleft/groove/region, the ends of which are closed up by specific hydrophobic residues (Maffei and Harris, 1998).

1.1.4.2.2 Structure of Class II Molecules

Class II molecules are heterodimers (Rees, 1997) comprised of two glycosylated (Austyn and Wood, 1993) polypeptide (Götze, 1981) chains (Janeway *et. al.*, 2005; Varney *et. al.*, 1999) *viz.* a heavy α chain made up of 229 amino acids (Schwartz, 1987) with a molecular weight of 34kDa (Sayegh *et. al.*, 2000) and a light β chain made up of 237 amino acids (Schwartz, 1987) with a molecular weight of 28kDa (Sayegh *et. al.*, 2000). Both chains of Class II molecules are encoded within the MHC (Janeway *et. al.*, 2005; Laurent and Welsh, 1984) by different genes (Varney *et. al.*, 1999) and are linked noncovalently (McCluskey and Peh, 1999).

Class II molecules are made up of three regions *viz.* an extracellular hydrophilic portion (Schwartz, 1987) where both the α and β chains fold to form two pairs of extracellular domains (Brodsky, 2001) each made up of about 100 amino acids (Austyn and Wood, 1993), a hydrophobic (Schwartz, 1987) transmembrane region made up of about 20-25 residues (Austyn and Wood) and an intracellular hydrophilic (Schwartz, 1987) cytoplasmic tail of about 3-15 residues in the α chain and 8-20 residues in the β chain (Austyn and Wood, 1993) (Figure 1.1.2b).

The α chain domains are designated α_1 and α_2 and the β chain domains are designated β_1 and β_2 (Brodsky, 2001). Unlike the Class I molecule, there is no association with a β_2-m domain (Götze, 1981); instead α_1 pairs with β_1 and α_2 pairs with β_2 (Austyn and Wood, 1993). Membrane proximal, Immunoglobulin-like (Schwartz, 1987) domains are α_2 and β_2 (Austyn and Wood, 1993). Both these chains span the membrane (Janeway, *et. al.*, 2005) and are anchored to the membrane by their carboxy termini (Brodsky, 2001).

Membrane distal domains are the α_1 and β_1 variable (Williams, 2001) domains. These domains fold in a specific way to form a peptide binding groove (Schafer *et. al.*, 1995) which appears open-ended (Maffei and Harris, 1998) and wider in the Class II molecule due to the type of peptide it binds (Janeway *et. al.*, 2005). Both β chain domains as well as the membrane proximal α domain encompass intrachain disulfide bridges (Sullivan and Amos, 1986).

1.1.5 Crystal Structure of HLA Class I and Class II Molecules

In order to determine HLA structures by x-ray crystallography at a resolution of 3·5Å (Bjorkman *et. al.*, 1987) and 3·3Å (Brown *et. al.*, 1993) for Class I and Class II molecules respectively, the extracellular domains of the molecule had to first be cleaved with papain from the cell surface before purification (Margulies, 1999). These experiments made it possible to determine the way the chains fold into a 3-Dimensional structure (Sayegh *et. al.*, 2000).

1.1.5.1 Structure of Class I Molecules

Bjorkman *et. al.* (1987) resolved an HLA Class I molecule (HLA-A2) by X-ray crystallography. Later a refined structure was determined by Saper *et. al.* (1991) at a resolution of 2·6Å. Briefly, the peptide binding groove occurs at the surface of the Class I molecule (Sayegh *et. al.*, 2000) at the NH_2 domains, α_1 and α_2 (Margulies, 1999) which have a similar tertiary structure (Austyn and Wood, 1993). External view of the cell indicates the α_3 and β_2-m domain pair asymmetrically (Margulies, 1999) proximal to the membrane surface (comparable to the constant domain of an immunoglobulin molecule) and the α_1 and α_2 domain pair distal to the membrane surface (Austyn and Wood, 1993) with the peptide binding site facing the exterior (Sayegh *et. al.*, 2000).

The α_3 and β_2-m domains are made up of two anti-parallel β-pleated sheets linked by a disulfide bond, one containing four β-strands and the other three β-strands (Bjorkman *et. al.*, 1987) (See Figure 1.1.3a). The α_1 and α_2 domains fold almost symmetrically (Sayegh *et. al.*, 2000) to form the peptide binding groove. The boundaries of this groove are formed by two segmented α-helices (Janeway *et. al.*, 2005) and is supported by the floor of the groove (Margulies, 1999) which is composed of eight anti-parallel β-strands (Janeway *et.* al., 2005; Margulies, 1999; Zacharias and Springer, 2004).

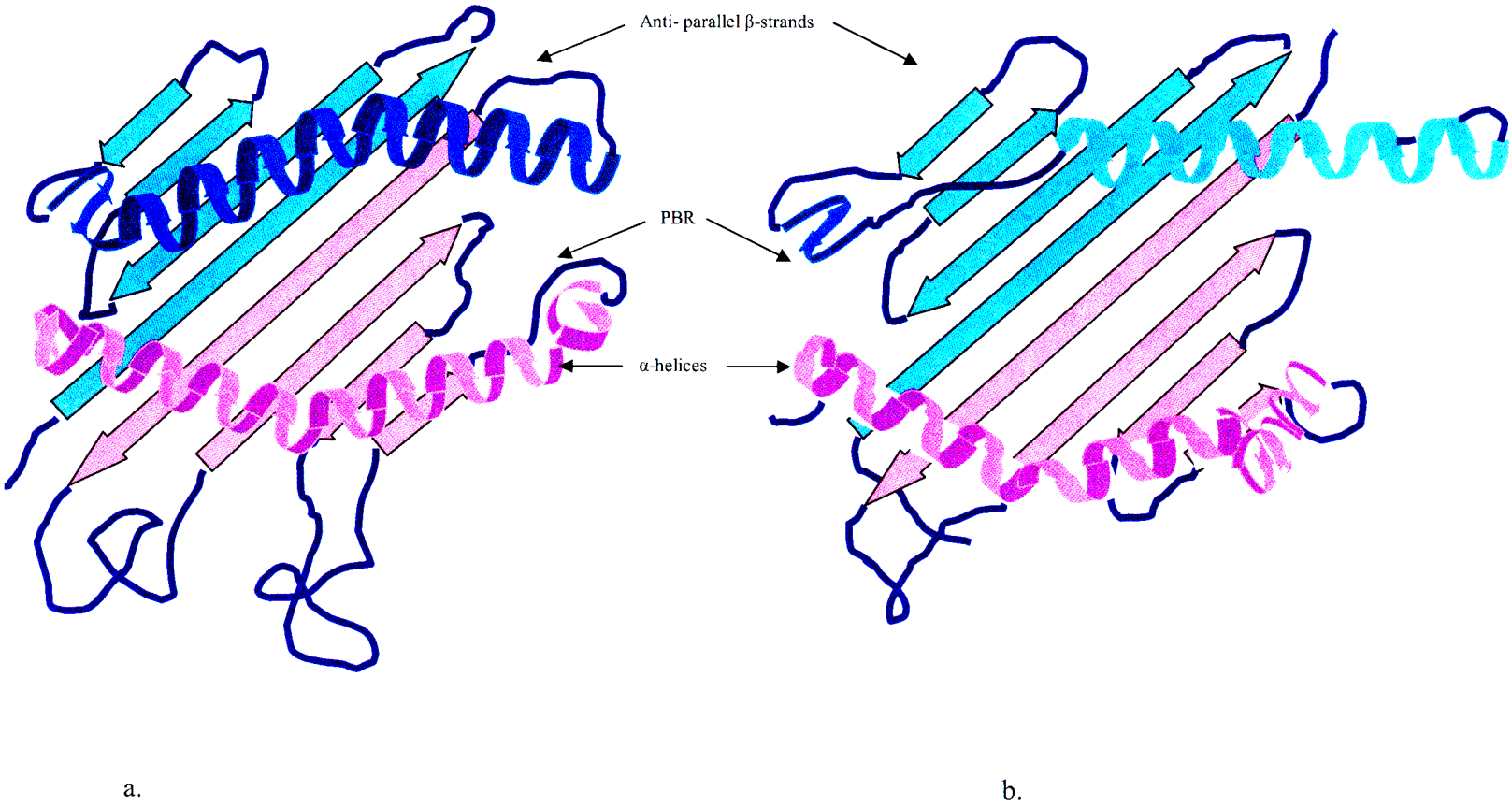

Figure 1.1.3 Diagrammatic representation of the crystal structures of HLA Class 1 (a.) and HLA Class II (b.) molecules. Explained in text. PBR = peptide binding region. Adapted from Bjorkman *et. al.*, 1987; Brown *et. al.*, 1993; Janeway *et. al.*, 2005.

1.1.5.2 Crystal Structure of Class II Molecules

X-ray crystallography indicates that the Class II molecules are folded almost identically to the Class I molecules (Brown, 1993) (See Figure 1.1.3b). The difference lies in the heavy and light chains of the Class II molecule which are joined symmetrically (Nikolich-Žugich *et. al.*, 2004) as opposed to an asymmetrical association between the Class I molecule domains (Margulies, 1999) and the space between the ends of the peptide binding groove being more ajar in the Class II molecules than the Class I molecules (Janeway *et. al.*, 2005; Sayegh *et. al.*, 2000). The antigenic peptides are bound to the MHC Class II molecules via hydrogen bonds (Stern *et. al.*, 1994).

1.1.6 Tissue Distribution and Expression of HLA Class I and Class II Molecules

Class I molecules are found on almost every (Schwartz, 1987) somatic (Austyn and Wood, 1993) nucleated (Barrett, 1988) cell as well as platelets (Sayegh *et.* al., 2000; Williams, 2001). The degree of expression of Class I antigens is determined by the type of cell it appears on (Austyn and Wood, 1993; Janeway *et.* al., 2005). For example, muscle cells; neurons (Austyn and Wood, 1993); kidney cells and even red blood cells display lower or no levels of expression than cells found in the immune system (Janeway *et. al.*, 2005). The level of expression however can be elevated by an immune response (Austyn and Wood) usually during inflammation and induced by cytokines Interferon-γ (INF-γ) and tumor necrosis factor (TNF) (Turka, 1997).

Class II antigens are found predominantly on antigen-presenting cells; B cells and their precursors (Laurent and Welsh, 1984) immunocompetent cells such as macrophages/monocytes (Schwartz, 1987) and dendritic cells (Germain, 1994) as well as endothelial (not generally expressed) (Sayegh *et. al.*, 2000) and epithelial cells (Laurent and Welsh, 1984), resting (Schwartz, 1987) and activated T cells (Turka, 1997). Cells expressing Class II molecules are involved in extracellular uptake of antigens as well as triggering immune responses (Austyn and Wood, 1993).

Expression of Class II molecules are increased in B cells, other antigen presenting cells and epithelial cells of the thymus but are found to be MHC II-negative in cells of the kidney, neutrophils and the brain although microglia of the brain are found to be MHC II positive (Janeway *et. al.*, 2005). The level of expression of Class II molecules are also increased by inflammatory responses by antigen (Turka, 1997) or cytokines such as INF-γ (Austyn and Wood, 1993). Cells that do not generally express Class II molecules, such as endothelium; renal proximal tubules and keratinocytes (Turka, 1997) can be stimulated to do so during inflammation (Sayegh, *et. al.*, 2000; Williams, 2001).

Cells that express both Class I and Class II molecules contain three Class I antigens and three or more Class II heterodimers (Buckley, 2003). Both MHC Class I and Class II molecules exist in the serum of healthy individuals and during disease are elevated (Zavazava and Eggert, 1997).

1.1.7 Function of The HLA Class I and Class II Molecules

The primary function of HLA Class I and Class II molecules is to bind to peptide derived fragments and present them extracellularly (Janeway *et. al.*, 2005) via the peptide binding groove (McCluskey and Peh, 1999) to the receptors of T cells (Battaglia and Gorski, 2002) enabling them to restrict and regulate specific antigenic responses (Caruso *et. al.*, 2001). Both Class I and Class II molecules are structured differently to present antigens via different pathways (Brodsky, 2001). Although antigen presentation occurs via different pathways, they share a similar antigen binding specificity (Stern *et. al.*, 1994). HLA molecules also enable the immune system to discriminate between self and non-self proteins (Hutter and Dohr, 1998).

As mentioned above Class I molecules are present on almost every cell. This makes it possible for any altered cell to be destroyed by T_C cells whereas the restricted distribution of MHC Class II molecules assures the exclusive response of T_H cells to antigens present on antigen presenting cells (Kimball, 1986).

1.1.7.1 Function of The Class I Molecule

The main antigens recognized by the host's immune system during tissue graft rejection are the Class I antigens (Schwartz, 1987) which by and large present peptides derived exogenously (Maffei and Harris, 1998) in the cytosol (Janeway *et. al.*, 2005). These peptides are generally are self (Bontrop and Watkins, 2005; Caruso *et. al.*, 2001) or derived from viruses or other intracellular pathogens that exploit the host's protein producing mechanisms

(Turka, 1997). These pathogens are degraded by catalytic proteases (Jeffery and Bangham, 2000) into small peptides (Abbas and Lichtman, 2003; McCluskey and Peh, 1999), transported for assembly to the endoplasmic reticulum (ER) (Germain, 1994; Jeffery and Bangham, 2000) by means of an active transport process (Tussey *et* al., 1995) by TAP (Faulkner *et al.*, 1998; Petrovsky and Brusic, 2004) (Transporter associated with Antigen Processing) molecules (Turka, 1997) and are presented to $CD8^+$ cytotoxic T lymphocytes (Hughes and Yeager, 1998; Li and Bouvier, 2005) bound to the peptide binding groove. The cytoplasmic and transmembrane domains however do not play a role in T cell activation (Gur *et. al.*, 1999).

Non-classical Class I molecules, HLA-E (Borrego *et.* al., 1998; Braud *et. al.*, 1998; Brooks *et. al.*, 1999; Lee *et. al.*, 1998) and HLA-G (Rajagopalan and Long, 1999), present peptides to Natural Killer (NK) cell receptors (McCluskey and Peh, 1999).

1.1.7.1.1 Antigen Processing and Presentation by The Cytosolic Pathway of Class I Molecules

Class I presentation of antigenic peptides to MHC $CD8^+$ restricted T cells occurs as follows. Proteins present in the cytosol are constantly degraded (Janeway *et. al.*, 2005) by a barrel shaped (McCluskey and Peh, 1999), 20S (Maffei *et. al.*, 1997) 700kDa (Maffei and Harris, 1997) multi-catalytic protease complex known as the proteasome (Janeway *et. al.*, 2005) (Figure 1.1.4a) into peptides with a length of approximately 8 to 10 amino acids (Janeway *et. al.*, 2005; Li and Bouvier, 2005; McCluskey and Peh, 1999).

Once degraded, these peptides are transported from the cytosol to the rough Endoplasmic Reticulum (RER) (Figure 1.1.4b) by TAP-1 and TAP-2 molecules (Turka, 1997) which form a channel in the membrane of the RER (Maffei and Harris, 1998) that pumps cytosolic peptides into the lumen of the RER against an ATP gradient (Brodsky, 2001).

Assembly of MHC Class I molecules occurs in the lumen of the RER and are only released once the molecule binds to peptide. In the lumen of the RER, the partly folded Class I α chains bind to a chaperone protein calnexin prior to assembly with β2-m (Janeway *et. al.*, 2005) (Figure 1.1.4c). Once association of the α chain and β2-m occurs, calnexin dissociates from the partly folded Class I molecule which thereafter binds to the MHC Class I loading complex (Janeway *et. al.*, 2005).

The first protein of the MHC Class I loading complex is calreticulin (Janeway *et. al.*, 2005) which together with calnexin facilitates initial folding and association of the Class I molecule (Brodsky, 2001). Tapasin (TAPBP) is the next MHC Class I loading complex protein that binds to the partly folded MHC Class I molecule forming a bridge between TAP and the Class I molecule and calreticulin (McCluskey and Peh, 1999) (Figure 1.1.4d).

Once antigenic peptides enter the lumen of the RER by the channel formed by the TAP molecules, the Class I molecule dissociates from its chaperone molecules (Turka, 1997) and the TAP molecule transports the appropriate peptide to the peptide binding groove of the MHC Class I molecule thus completing the folding process (Janeway *et. al.*, 2005) (Figure 1.1.4e).

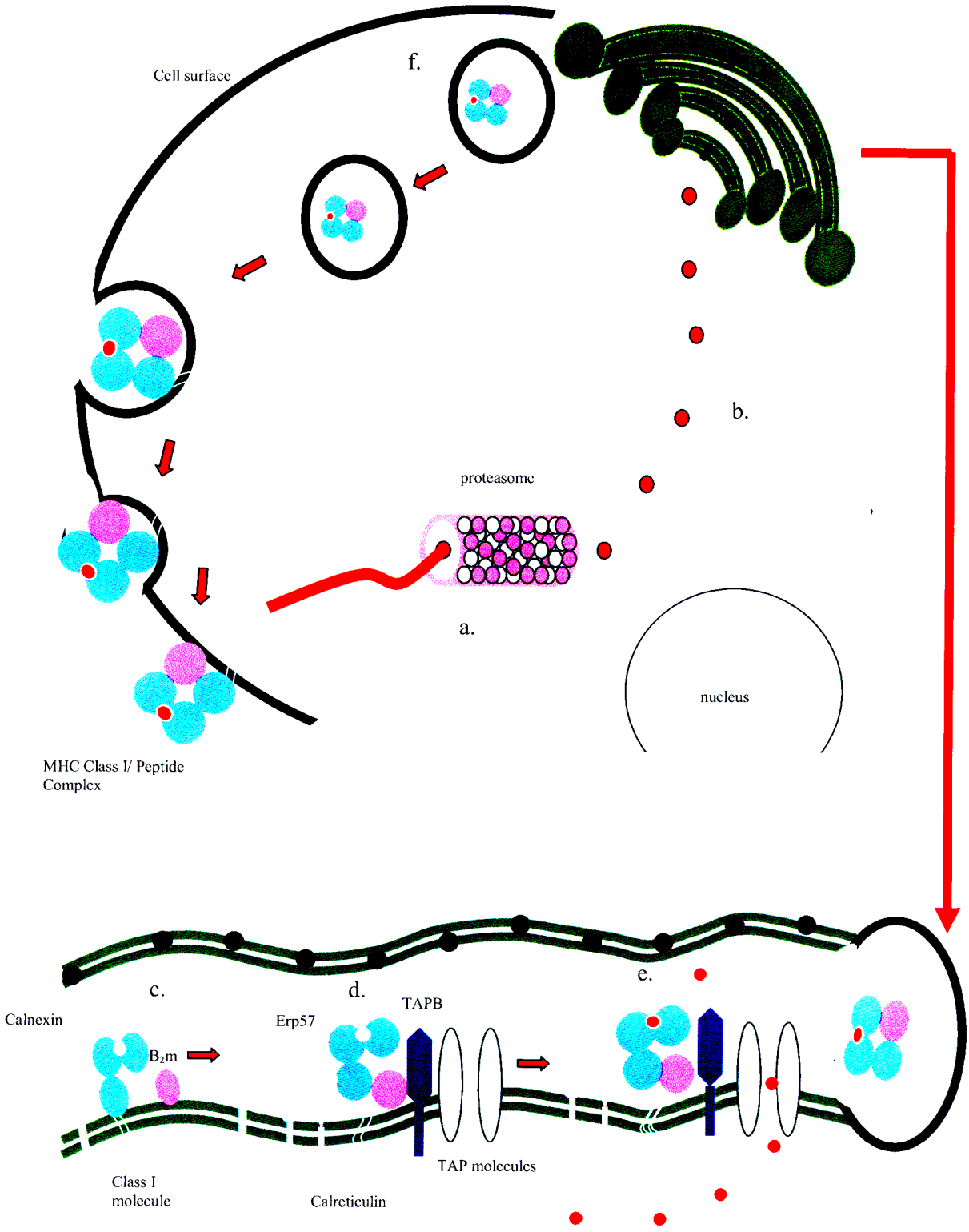

Figure 1.1.4 MHC Class I presentation by the Cytosolic Pathway. Explained in detail in text. Adapted from Brodsky, 2001; Janeway *et. al.*, 2005; Parham and Ohta, 1996; Sayegh *et. al.*, 2000.

The MHC Class I molecule/peptide complex is thereafter budded off into vesicles and transported to the cell surface (Thorsby, 1997) where they fuse with the cell membrane thus presenting the MHC Class I/peptide complex (Brodsky, 2001) (Figure 1.1.4f) to cytotoxic (Sayegh *et. al.*, 2000 Mitchison *et. al.*, 1996) $CD8^+$ T cells which bear MHC restricted Class I T cell receptors (Lederman and Suciu-Foca, 1999).

Another chaperon protein involved in Class I presentation is Erp57 which is postulated to play a role in the destruction and reconstruction of the disulphide bond in tapasin (Dick *et. al.*, 2002) present in the MHC Class I α_2 domain during peptide loading (Janeway *et. al.*, 2005).

1.1.7.2 Function of The Class II Molecules

Class II molecules function to maintain self tolerance as well as to induce and maintain immune responses against foreign invading pathogens (Friese *et. al.*, 2005). The Class II molecules are produced in the RER (Thorsby, 1997) and present extracellular peptides (Wright *et. al.*, 2001) which are derived endogenously (Maffei and Harris, 1998) in membranous endosomal vesicles (Brodsky, 2001) from proteins which are seized by and taken in to the cell via fluid-phase pinocytosis, phagocytosis or receptor-mediated endocytosis (Brodsky, 2001; Rodríguez-Pinto, 2005).

The peptides presented by MHC Class II molecules are up to 16 amino acids in length (Nikolich-Žugich *et. al.*, 2004) and could possibly be derived from parasites (Turka, 1997)

processed bacteria such as mycobacteria (the causative agent of tuberculosis) (Janeway *et. al.*, 2005) or intact viruses particles (Turka, 1997) and presented to $CD4^+$ T helper (T_H) cells (Bontrop and Watkins, 2005) which bear MHC class II restricted T cell Receptors (Lederman and Suciu-Foca, 1999).

Naïve T_H cells are differentiated into two classes of $CD4^+$ T helper cells (Middleton *et. al.*, 2002) *viz.* T_H1 and T_H2 cells (Janeway *et. al.*, 2005) by NK cells (Middleton *et. al.*, 2002). Once the MHC Class II molecule binds to the $CD4^+$ T cell it provokes the T cells to either release proinflammatory cytokines (Turka, 1997) which will either destroy the pathogen if the antigen presenting cell its bound to is a macrophage (Wright *et. al.*, 2001) in the case of disease-inducing (Mitchison *et. al.*, 1996) T_H1 cells (Janeway *et. al.*, 2005) or control the production of antibody (Bontrop and Watkins, 2005) by antigen presenting B cells in the case of protective (Mitchison *et. al.*, 1996) T_H2 cells (Janeway *et. al.*, 2005).

1.1.7.2.1. Antigen Processing and Presentation by The Endocytic Pathway of Class II Molecules

Antigen processing by the endocytic pathway occurs as follows. Antigenic proteins are protected from the cytosolic proteasomes by their initially neutral membrane bound vesicles (Janeway *et. al.*, 2005) (Figure 1.1.5a). As the vesicle moves into the cell the pH decreases to an acidic state resulting in protein degradation (Janeway *et. al.*, 2005; Turka, 1997) into peptides of various lengths and sequences (Brodsky, 2001) (Figure 1.1.5b).

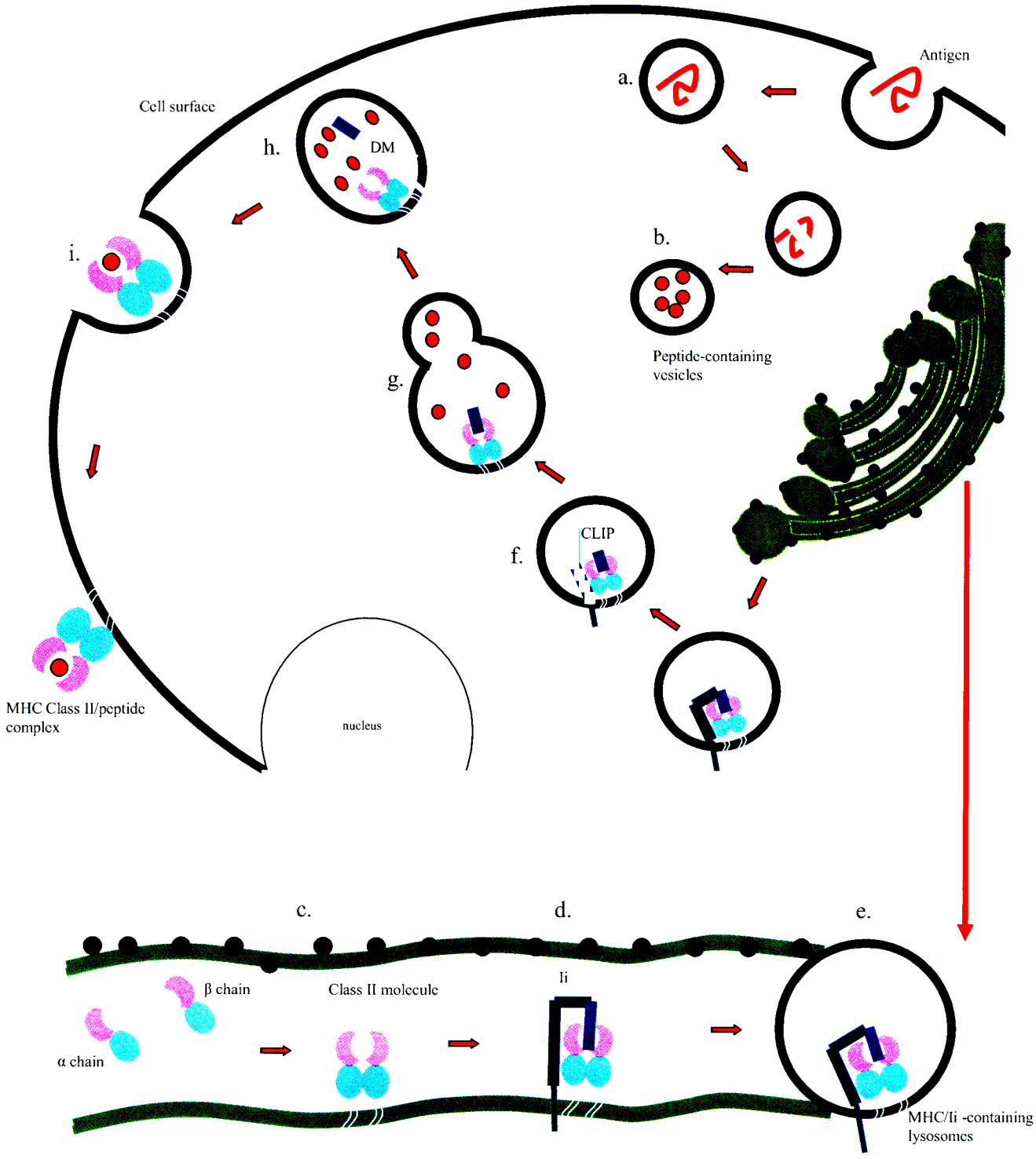

Figure 1.1.5 Diagrammatic representation of the MHC Class II pathway. Explained in detail in text. Adapted from Brodsky, 2001; Janeway *et. al.*,2005; Maenaka and Jones, 1999.

The MHC Class II α and β chains assemble to form the MHC Class II molecule in the RER (Brodsky, 2001; Schafer *et. al.*, 1995) (Figure 1.1.5c). In order to prevent the MHC Class II molecule from binding to other peptides in the ER (Turka, 1997) prior to fusion with an endocytic peptide containing vesicle, the peptide binding groove becomes occupied by a non polymorphic (Brodsky, 2001) invariant chain (Ii) (Maenaka and Jones, 1999) (Figure 1.1.5d). The MHCII/Ii complex is delivered to vesicles by the invariant chain (Janeway *et. al.*, 2005).

These MHCII/Ii complex containing vesicles bud out of the ER via the golgi apparatus into the endocytic pathway (Brodsky, 2001) (Figure 1.1.5e). The invariant chain becomes cleaved by acid proteases (Janeway *et. al.*, 2005) such as cathepsins (B, D and S) as they develop into prelysosomes (Brodsky, 2001) into a shorter peptide called CLIP (Class II-associated *i*nvariant chain *p*eptide) (Janeway *et. al.*, 2005) (Figure 1.1.5f).

Vesicles containing newly formed MHC molecules move towards the surface of the cell where they encounter vesicles containing antigenic peptides. Both the peptide containing endosomes and the MHCII/Ii-containing lysosomes fuse but the MHC Class II molecule still holds the CLIP molecule in its peptide binding groove and is thus unable to bind peptide (Janeway *et. al.*, 2005) (Figure 1.1.5g).

CLIP is dissociated from the MHC Class II molecule by the nonclassical HLA-DM molecule (Maenaka and Jones, 1999) which stabilizes the empty MHC Class II molecule (Watts, 2001) by preventing premature peptide binding (Figure 1.1.5h). This dissociation is negatively

(Janeway *et. al.*, 2005) controlled (Rodríguez-Pinto, 2005) by HLA-DO which inhibits the release of CLIP from MHC Class II molecules by HLA-DM (Janeway *et. al.*, 2005).

Once CLIP is released by HLA-DM from the peptide binding groove of the MHC Class II molecule, antigenic peptides from the now fused endosome/lysosome are able to bind to the empty groove (Janeway *et. al.*, 2005).

The entire vesicle thereafter moves to the cell surface where it fuses with the cell membrane resulting in the MHC Class II/peptide complex being presented at the cell surface of the antigen presenting cell (Schafer, 1995) to the T Cell Receptors of $CD4^+$ cells which binds to the β2 domain of the MHC Class II molecule (Brodsky, 2001) (Figure 1.1.5i). This interaction results in the activation and expansion of the $CD4^+$ cells which thereafter mount immune responses that act to destroy the pathogen whose antigens are presented by the MHC Class II molecules (Friese *et. al.*, 2005).

1.1.8 Linkage Equilibrium and Disequilibrium

Linkage equilibrium can be best described by the Hardy-Weinberg theorem ($(p+q)^2 = p^2 + 2pq + q^2$) whereby two conclusions can be derived *viz.* 1. After first generation mating, the progeny will present with a genotype frequency of p^2 *AA*: 2 pq *AB*: q^2*BB* for the two alleles (A and B) and, 2. The frequency of this distribution in migration-, mutation- and selection-free environments will remain constant during subsequent generations thus maintaining the population in equilibrium (Götze, 1981).

A deviation from this equilibrium results in disequilibrium. Linkage disequilibrium is a nonrandom association (Payne, 1977) of two different antigens (Braun, 1979) located at separate loci on the same chromosome (Mackay and Powell, 2006) in a population which are present in frequencies which greatly exceed the anticipated and calculated as the difference (Δ) between the expected and observed frequencies (Schwartz, 1987). Linkage disequilibrium is caused by genetic drift, mutation, population bottlenecks, founder effects, migration, selection and admixture (Mackay and Powell, 2006; Weeks and Lathrop, 1995).

Studying this phenomenon can only be possible in outbred populations which comprise large numbers of diverse haplotypes (van Rood *et. al.*, 1981). For example, in population *x*, the frequencies of HLA-A2 and HLA-DR7 occur with frequencies of 0.17 and 0.09 respectively. The expected frequency of the HLA-A2-DR7 haplotype would thus be 0.17×0.09, or 0.0153 but the actual frequency of the haplotype is 0.0812. Hence the linkage disequilibrium (Δ) is $0.0812 - 0.0153 = 0.0659$.

In general loci said to be in linkage disequilibrium are physically closely related. Palmer and Cardon (2005) and Wenda *et. al.* (2006) showed that lack of linkage disequilibria is due to the great physical distance between 6 STR loci examined.

Linkage disequilibrium has been demonstrated to be present between the classical Class II HLA-DR3 and the non-classical Class 1b HLA-G010102 (Vauvert *et. al.*, 2005).

Decay of linkage disequilibrium occurs with time in large populations (Park *et. al.*, 2007) due to meiotic (Palmer and Cardon, 2005) recombinations (Mackay and Powell, 2006; Palmer and Cardon, 2005; Weeks and Lathrop, 1995) at a rate determined by the distance between loci (Mackay and Powell, 2006; Weeks and Lathrop, 1995) over successive generations (Mackay and Powell, 2006; Palmer and Cardon, 2005) in the absence of disturbing factors such as migration (Park *et. al.*, 2007). Rapid decay of linkage disequilibrium occurs due to selection, drift or admixture and slower decay is due to closely linked loci (Weeks and Lathrop, 1995).

1.1.9 MHC Polymorphism

MHC polymorphism refers to the allelic variation derived from the MHC Class I and Class II genes which is present in a population at any given time in amounts that are greater than expected (Margulies, 1999). This variation may be due to gene conversion, recombination and exon shuffling events (Adams *et. al.*, 2004) and is considered to be aggravated and sustained by the ancient mêlée between our immune system and infectious pathogens (Beck *et. al.*, 1999)

Class I genes in humans display two alleles for each of the three classical HLA-A, HLA-B and HLA-C genes (Bettinotti *et. al.*, 2003) and Class II genes display three to four sets of genes resulting in expression of six distinct Class I molecules and eight distinct Class II molecules (Janeway *et. al.*, 2005).

Both Class I and Class II molecules are highly polymorphic and their polymorphism lies in their membrane distal NH_2 termini (Maffei and Harris, 1998; Sayegh *et. al.*, 2000) which fold to form the peptide binding groove thus signifying the importance of polymorphism which is required to allow the molecule and consequently the entire organism and species to react to a change in the antigenic environment (Margulies, 1999). Although Class II molecules are highly polymorphic, allelic variation of their α chains is limited (Turka, 1997). Their polymorphism thus lies in the β chain (Laurent and Welsh, 1984).

By and large polymorphisms arise from the variability of the amino acid side chains of the peptide anchor residues (Bontrop, 2006; Janeway *et. al.*, 2005) which bind to complementary polymorphic (Zhang *et. al.*, 1998) pockets lining the peptide binding groove (Bontrop and Watkins, 2005; Janeway *et. al.*, 2005; Marguiles, 1999). These pockets are formed by polymorphic residues (Li and Bouvier, 2005). There are 48 Class I MHC pockets which are classified into pocket families by similarities in their side-chain binding specificity and physical attributes (Zhang *et. al.*, 1998).

Polymorphisms in the Class I molecules line the floor of the peptide binding groove (Kostyu *et. al.*, 1997; Sullivan *et. al.*, 2006), are clustered in Hypervariable (H_v) regions (Male *et. al.*, 1996) present in the α_1 and α_2 domains (which are encoded by exon 2 and exon 3) (Tiercy, 2002) as well as the loops outside the peptide binding groove (Kostyu *et. al.*, 1997) and influence the binding specificity of the pockets by selecting the amino acids which bind to it. Even a single amino acid substitution at a given position may change the binding specificity altogether (Zhang *et. al.*, 1998).

Each MHC class I variant has a specific anchor residue (Janeway *et. al.*, 2005). This specific anchor residue is called the sequence motif (Janeway *et. al.*, 2005). Variability is shown to be greater in the α helix of the α_1 domain as compared to the α_2 domain but greater in the α_2 β-strand region as compared to the β-strand region of α_1 (Parham *et. al.*, 1988).

Polymorphic residues in Class II molecules rest in clusters that make up the H_v regions where they line the sides and floor of the peptide binding groove (Maffei and Harris, 1998). Polymorphism of Class II molecules are mostly exhibited on HLA-DRB, HLA-DQB, HLA-DPB and HLA-DQA as HLA-DPA is for the most part non-polymorphic and HLA-DRB is monomorphic/invariant (Male *et. al.*, 1996).

When polymorphism occurs outside the peptide binding groove, it does not affect antigen binding specificity as in the case of HLA-B*5133 (Lebedeva *et. al.*, 2003).

MHC polymorphism is beneficial due to the fact that allelic variation between individuals provides resistance to infectious agents (Leffell, 2002) and prevents a particular pathogen from causing extinction of an entire population (Bontrop, 2006; Bontrop and Watkins, 2005).

1.1.10 HLA and Disease Associations

HLA has been demonstrated to be associated with various diseases in different populations (Bassett *et. al.*, 1979; Rossman *et. al.*, 2002). HLA genes are also protective (by prevention of progression of HIV disease) (Migueles *et. al.*, 2000). These diseases encompass non-

infectious (Van Rood *et. al.*, 1981) autoimmune, infectious and the sequelae of certain infectious diseases which may be autoimmune (Götze and Burger, 1986). In order to determine disease susceptibility or resistance, population or family studies or both need to be conducted (Svejgaard, 1986).

Genes found to protect against a disease are on occasion found at lower frequencies in a diseased population as compared to disease susceptibility genes (Mitchison *et. al.*, 1996). The presence of an antigen is not indicative of disease susceptibility (Götze and Burger, 1986). Therefore in order to determine associations, the gene frequencies of the diseased population must be compared with a population of healthy controls (Mitchison *et. al.*, 1996).

In population studies, the relative risk is used to calculate the strength of the association between HLA and the population in question (Van Rood *et. al.*, 1981) thereafter statistical significance can be determined by the Fisher's exact or chi-square tests (Svejgaard and Ryder, 1977).

The relative risk is basically the cross-product ratio of patients and controls in a population with and without the antigen of interest (Svejgaard, 1986) and it indicates how more regularly the disease occurs among individuals bearing the antigen compared to those that are not (Van Rood *et. al.*, 1981). When the frequency of an antigen is greater in the diseased population as compared to the healthy controls, a relative risk greater than one is observed indicating an increased risk and vice versa but a relative risk equal to one indicates no difference between the control and diseased populations (Svejgaard, 1986).

Family studies are carried out in addition to population studies to better understand the genetics of the disease (Svejgaard, 1986) and could possibly indicate a linkage between disease susceptibility and HLA alleles between diseased family members that share haplotypes present at a greater frequency than would be expected by normal Mendelian genetics (Van Rood *et. al.*, 1981).

The difference between population and family studies is that population studies are employed to determine statistically significant associations between a HLA marker gene and a disease (Schwartz, 1987) whereas family studies are used to establish a linkage between the marker gene and a major disease-controlling gene (Svejgaard and Ryder, 1977). The terms associations and linkage cannot be used interchangeably because association does not consequentially signify linkage and vice versa (Schwartz, 1987). Association is possible without linkage between two loci and linkage is possible without an association (Svejgaard and Ryder, 1977).

Patients with steroid-responsive nephrotic syndrome were shown to have an increased association with HLA-DR7 (relative risk 4.5, $p < 0.001$) and decreased association with HLA-DR2 (relative risk 0.23, $p < 0.005$) when compared to a control group (Nuñez-Roldan *et. al.*, 1982). From the text we can therefore conclude due to the relative risk of HLA-DR7 being greater than 1 and the relative risk of HLA-DR2 being less than 1 that HLA-DR7 is a possible disease susceptibility gene and HLA-DR2 is a possible protective allele.

Another potent association has been found between idiopathic membranous nephropathy patients and the HLA-DRw3 allele (relative risk 12.0, *p* value = 0.00000034) when compared to a control group (Klouda *et. al.*, 1979). HLA-DRw3 was found in 75% of the patients and 20% of the controls suggesting together with a relative risk greater than 1, that it is a possible disease susceptibility gene.

An excellent example illustrating the importance of HLA associated studies was carried out by Kiepiela *et al.* (2004) demonstrating that HIV infected antenatal mothers carrying protective HLA-B alleles (HLA-B*57 and HLA-B*5801) pass on HIV to their children less frequently than infected antenatal mothers carrying the deleterious (HLA-B*5802 and HLA-B*18) alleles. These findings indicate that as the pandemic advances, the frequencies of HLA-B*5802 and HLA-B*18 will decrease and that of HLA-B*57 and HLA-B*5801 will increase rapidly.

1.2 The Kidney

The kidneys function to excrete nitrogenous (Wallace *et. al.*, 1996) waste products of metabolism, regulate the water and electrolyte balance, maintain the acid-base balance (Snell, 2004), regulate the body fluid osmolality, electrolyte concentration and arterial pressure and is involved in gluconeogenesis (Guyton and Hall, 2006) and play a role in erythropoiesis and calcium homeostasis. Generation of *de novo* nephrons is not possible hence nephrons that are destroyed due to renal injury, renal disease or natural ageing cannot be replaced (Guyton and Hall, 2006).

1.2.1 Functional Anatomy of The Kidney

From the outside both human kidneys appear to be smooth bean shaped organs (Harrison, 1981) which are located on the upper end of the posterior abdominal wall (Last, 1990) and positioned obliquely with the right kidney lying lower due to the magnitude of the right lobe of the liver (Snell, 2004). Normal dimensions of a kidney are usually 12 × 6 × 3 cm with a weight of 130 g (Last, 1990) and although both kidneys have roughly the same size and shape, the left kidney appears to be more elongated and slender (Drake *et. al.*, 2005).

The kidney encompasses upper (contiguous to the supra renal glands) and lower poles at the extremities (Green and Silver, 1981), it has smooth anterior and posterior surfaces in the presence of lobulation (Last, 1990) and is enclosed by a fibrous capsule (Drake *et. al.*, 2005) and medial which is a deep concavity that advances into a void which is the sinus of the kidney (Green and Silver, 1981). Entrance into the sinus occurs via the hilum (Green and Silver, 1981) which is a vertical slit fringed by thick lips of renal matter (Snell, 2004) extending almost half way up (Green and Silver, 1981) on the medial margin of the kidney (Drake *et. al.*, 2005) (see Figure 1.2.1).

In the renal sinus, two to three minor calyces come together to form a major calyx (Drake *et. al.*, 2005) and two to three major calyces make up the renal pelvis (Snell, 2004) which is the urine collecting (Wallace *et. al.*, 1996) upper end of the ureter displaying a funnel shaped conformation (Drake *et. al.*, 2005).

Passing through the hilum are lymph vessels, sympathetic fibres, the renal vein, two branches of the renal artery (Snell, 2004), the pelvis of the ureter (Harrison, 1981) and a third branch

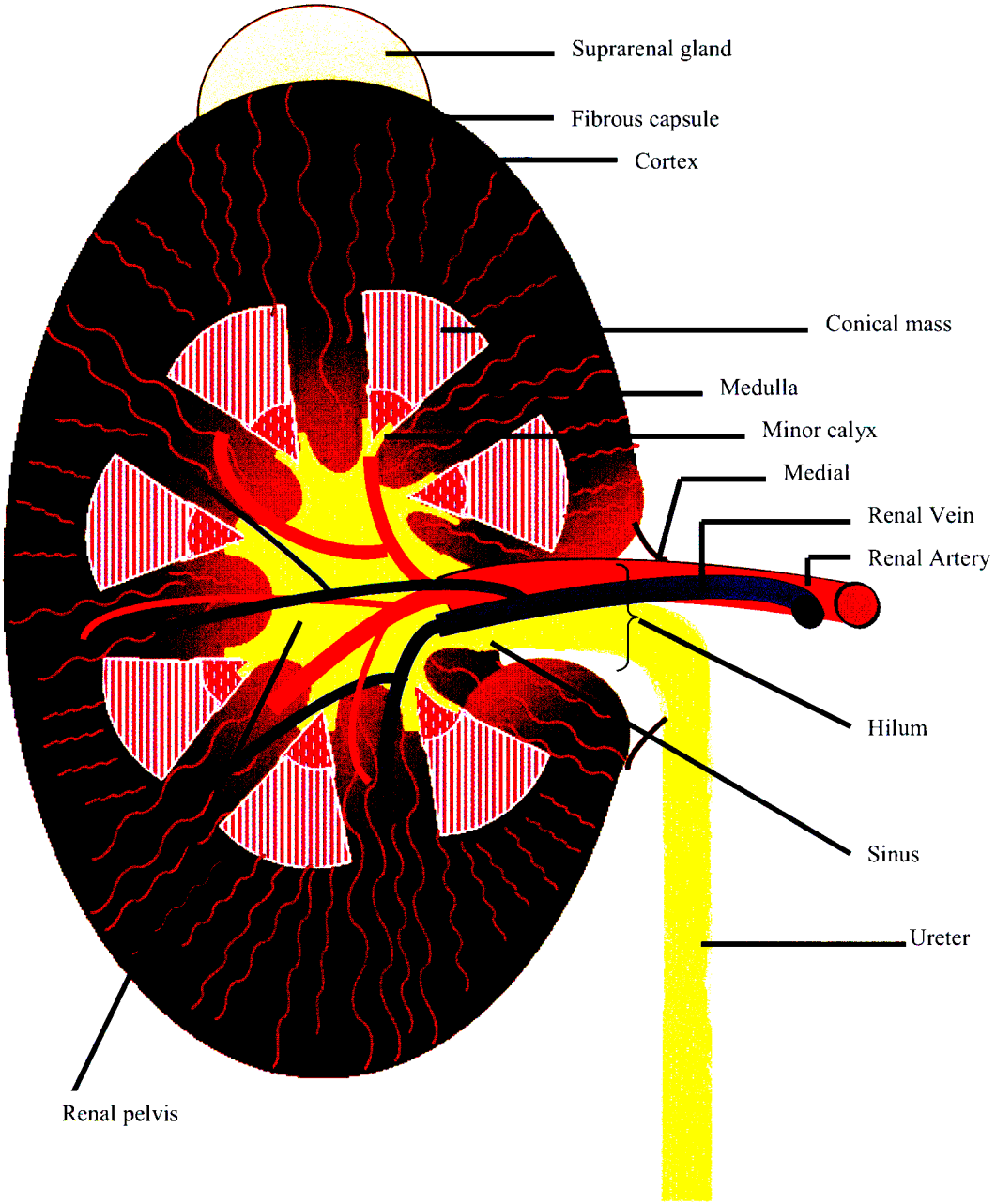

Figure 1.2.1 Diagrammatic representation of a human kidney. Functional anatomy explained in text. Adapted from Drake *et. al.*, 2005; Wallace *et. al.*, 1996.

of the renal artery (Snell, 2004). The renal artery enters the kidney via the hilum whilst the renal vein and pelvis leave the kidney via the same path (Harrison, 1981).

On the outer surface covering the easily removable fibrous capsule is perirenal (perinephric) fat surrounded by extraperitoneal (renal) fascia (Drake *et. al.*, 2005) which is condensation of connective tissue enclosing the kidney and suprarenal glands (Snell, 2004).

The kidney comprises a distinctively striated (Harrison, 1981) dark brown outer renal cortex and a light brown inner renal medulla (Snell, 2004). The outer renal cortex is an uninterrupted stretch of pale tissue which encircles the renal medulla entirely (Drake *et. al.*, 2005) extending into columns between pyramids in the inner renal medulla (Snell, 2004). Cells that make up the cortex are the cortical labyrinth and the medullary rays (Kriz and Kaissling, 1985). The medullary rays extend from the base of the pyramid into the cortex (Snell, 2004).

The inner medulla comprises several conical masses (Harrison, 1981) which employ the form of a pyramid (Drake *et. al.*, 2005). The base of the pyramid is comprised of alternate dark and light striations (Harrison, 1981) and faces the renal cortex whilst the apex which is known as renal papilla or crest (Kriz and Kaissling, 1985) is light coloured, faintly streaked (Harrison, 1981) encircled by a minor calyx and projects medially towards the renal sinus (Snell, 2004).

The cortex comprises the nephrons which are the structural (Kriz and Kaissling, 1985) filtering components of the kidney whilst the medulla consists mainly of the loop of Henle (de Wardener, 1985) and the collecting ducts (Wallace *et. al.*, 1996) several of which merge in the inner medulla forming the ducts of Bellini (Yaqoob, 2005) which drain their contents at the surface of the papilla (de Wardener, 1985) into the calices (Yaqoob, 2005).

There are three types of nephrons which are location determined *viz.* superficial, midcortical and juxtamedullary nephrons (Kriz and Kaissling, 1985). Each human kidney consists of approximately 1.3×10^6 nephrons (Ganong, 2005) with each having a width of approximately 25μm and a length of about 50 mm (de Wardener, 1985). The nephron is made up of a renal tubule and a glomerulus (Ganong, 2005) which is enveloped by epithelial cells (Guyton and Hall, 2006) and enclosed by a structure called the Bowman's capsule (Wallace *et. al.*, 1996) (see Figure 1.2.2a).

The tubule of the nephron is divided into a proximal convoluted tubule with a length of approximately 15 mm and a diameter of 55 µm (Ganong, 2005), the loop of Henle and a distal convoluted tubule which eventually leads into a collecting duct (de Wardener, 1985).

Proximal tubules that present towards the surface of the cortex contain shorter loops than those embedded deep within the cortex (de Wardener, 1985; Kriz and Kaissling, 1985) at a ratio of 1:4 respectively (de Wardener, 1985). The fluid filtered by the glomerulus is converted to urine that eventually drains into the pelvis of the kidney (Guyton and Hall, 2006).

The glomerulus is 200 μm in diameter (Ganong, 2005) and is made up of four vital cell types *viz.* 1. the endothelial cells fenestrated with pores 500-1000 Å (Yaqoob, 2005) with a diameter of 70-90 nm (Alpers, 2005); 2. mesangial cells which are contractile and are known to play roles in glomerular filtration and in the establishment of glomerular disease (Ganong, 2005); 3. epithelial cells of the visceral layer (Kriz and Kaissling, 1985) called podocytes which sustains the fragile glomerular basement membrane (basal lamina) via a pseudopodia network (Yaqoob, 2005) and interdigitate the capillary wall forming slits that are 50 × 120 Å approximately 25 nm wide (Ganong, 2005) which are smaller than an albumin molecule (Govan *et. al.*, 1995) and linked by a slit diaphragm (Kriz, 2005) and 4. epithelial cells which surround the Bowman's capsule (Yaqoob, 2005) called parietal epithelial cells (Kwoh *et. al.*, 2006).

The glomerular tuft contains a mass of approximately five thin walled capillary loops (de Wardener, 1985; Wallace *et. al.*, 1996) sourced at the afferent vessel and drained by a smaller efferent vessel (Ganong, 2005). The efferent vessel branches out into peritubular capillaries (Yaqoob, 2005) forming a network that extends over the whole nephron thereafter merging with peritubular capillaries from other nephrons forming the renal vein (Wallace *et. al.*, 1996).

In the Bowman's capsule there are two layers of cells that divide the blood from the glomerular filtrate *viz.* the capillary endothelium and the specialized epithelium of the capsule (Ganong, 2005) (see Figure 1.2.2). The endothelium and epithelium are divided by the basal lamina (Ganong, 2005). Mesangial cells are derived from mesenchymal

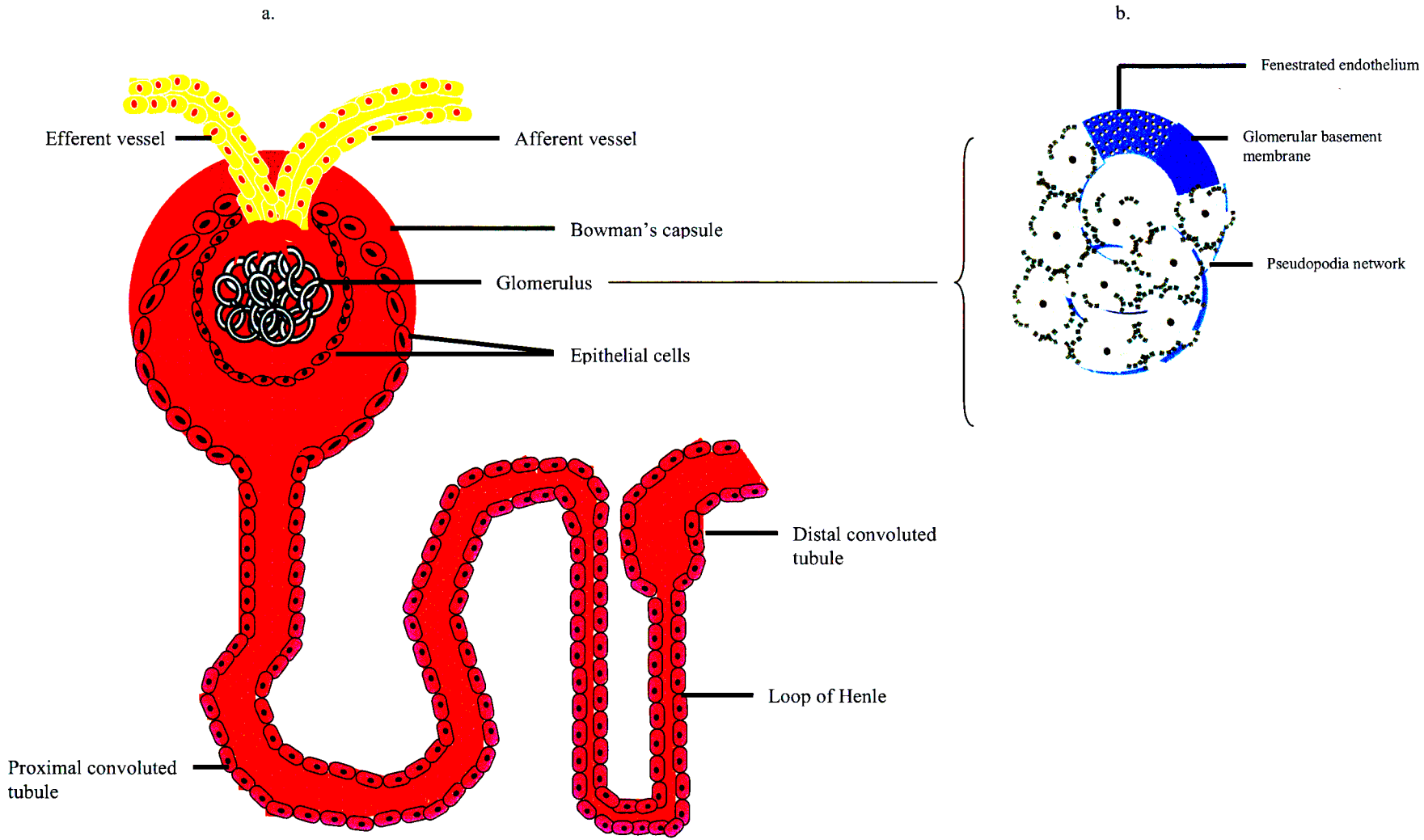

Figure 1.2.2 Simplified diagrammatic representation of the nephron (a) and detailed structure of a section of the glomerular tuft (b). Structure explained in detail in text. Adapted from Govan *et. al.*, 1995; Tryggvason *et. al.*, 2006; Wallace *et. al.*, 1996.

cells (de Wardener, 1985) and are found between the basal lamina and endothelium (Ganong, 2005) as well as the central stalk of the glomerulus which has a hollow external surface which forms the lumen of the capillary (de Wardener, 1985).

The glomerular membrane is composed of type IV collagen and negatively charged proteoglycans and has a thick central layer called the lamina densa which is surrounded by two layers called the lamina rara interna and the lamina rara externa (Yaqoob, 2005). It is selectively permeable and allows neutral substances with a diameter of up to 4 nm to traverse the membrane (Ganong, 2005). The lamina densa and the slit diaphragm covering the pores formed by the epithelial cell foot processes form the vital filtration barrier (Govan *et. al.*, 1995).

As mentioned above blood enters the glomeruli via the afferent vessels which are subdivisions of the renal artery (Yaqoob, 2005). Both renal arteries receive 25% of cardiac output (Yaqoob, 2005) from the most important artery in the body, the aorta (Wallace *et. al.*, 1996) hence there is a great hydrostatic pressure of approximately 60 mmHg (Guyton and Hall, 2006) in the capillaries of the glomerulus indicating that high pressure is essential for optimal functioning of the kidneys (Wallace *et. al.*, 1996).

1.2.2 Overview of Kidney Physiology

Everyday the kidneys function to produce urine by filtering more than 1700 liters of blood (Alpers, 2005). In addition they excrete the waste products of metabolism, regulate

salt and water concentration, maintain acid base balance and secrete hormones such as erythropoietin, renin and prostaglandins (Alpers, 2005).

As mentioned above the nephron is the functional unit of the kidney hence in this chapter kidney physiology will focus primarily on the physiology of the nephron. The nephron consists of several regions and each region has a distinct function (Govan *et. al.*, 1995).

The glomerulus functions as the filtration unit of the nephron due to the large surface area of the capillaries as well as its characteristic composition (Govan *et. al.*, 1995). The products of filtration are entirely free of blood cells and almost always free of protein due to the nature of the physiochemical and electrostatic charges of the filtration barrier (Brady *et. al.*, 2005). The production rate of the filtrate depends on the afferent arteriolar hydrostatic pressure, the plasma protein osmotic pressure and the pressure within the renal tubule (Govan *et. al.*, 1995).

The glomerular filtration rate (GFR) depends on blood flow within the glomerulus, ultrafiltration pressure and the composition of the filtration barrier which are coordinated by changes in the tone of the afferent and efferent arterioles and the contractility of the mesangial cells (Brady *et. al.*, 2005).

The tubule functions to eliminate the body of waste products, maintain normal acid-base balance and to conserve fluid, electrolytes as well as other vital substances via active

assimilation by the tubular epithelium, passive exchange between the filtrate and interstitial tissues and secretion by the tubular epithelium (Govan *et. al.*, 1995).

1.2.3 Glomerular Disease

Due to its complex anatomical structure, diseases of the kidney are divided into four types according to the morphology affected *viz.* those affecting the glomeruli, the tubules, the interstitium and the blood vessels (Alpers, 2005).

In the forthcoming chapters, IgAN, FSGS and MGN will be discussed. These are diseases predominantly affecting the glomeruli of the kidney.

Glomerular disease is the consequence of injury to glomeruli which may be primary or due to vascular (e.g. hypertension), metabolic (diabetes), hereditary (Fabry disease), systemic (SLE) diseases (Alpers, 2005) or genetic factors (some forms of steroid resistanr nephritic syndrome).

The consequence of glomerular disease is typically threefold *viz.* the impairment of glomerular function which results in the reduction in urinary output; the appearance of protein (proteinuria) and; blood (haematuria) in the urine (Brady *et. al.*, 2005; Govan *et. al.*, 1995).

The mechanisms underlying glomerular disease are as follows (Govan *et. al.*, 1995):

1. Swelling of the endothelium results in diminished blood flow which results in a reduction of urinary output.
2. Inflammation alters the permeability of the glomerular damage resulting in proteinuria.
3. Focal loss of epithelium and endothelium results in haematuria and proteinuria.

Both MGN and FSGS are causes of NS which is characterized by gross proteinuria (>3-3.5 g/d), hypoalbuminemia (<3 g/d) (Alpers, 2005), hyperlipidemia, lipiduria, thrombotic diathesis, generalized oedema and a slow decline in GFR (Brady *et. al.*, 2005) and MGN, FSGS and IgAN are glomerular diseases which result in chronic glomerulonephritis (GN) which result in ESRD (Alpers, 2005).

Hypertension is an increasing global problem (Kearny *et. al.*, 2004) defined as elevated blood pressure and it accelerates the progression to renal disease (Ritz and Fliser, 1993). Six million South African adults are already affected and a westernized lifestyle has been postulated to augment the numbers (Steyn *et. al.*, 2001). Renal disease is a secondary cause of hypertension. Race has been shown to affect the prevalence of hypertension and diet (fat and salt) has been argued to increase blood pressure. Hypertension has been shown to be the main cause of ESRD in KZN in the African and Indian populations (Seedat *et. al.*, 1984).

In view of the global problem of hypertension it becomes increasingly important to screen patients (Kearny *et. al.*, 2004). Although monitoring a patient's blood pressure over time and testing serum creatinine levels are the common methods employed, it requires the patient to be present on several occasions. This is not feasible in poor communities where patients are unable to attend public health care centres due to lack of travel money. Genome screens would involve analysis of the entire genetic profile (Chow *et. al.*, 2005) and would be futile if only certain genes were involved in disease progression. Therefore a practical alternative would be to first detect disease susceptibility markers present in the HLA complex and thereafter screen patients for these marker alleles.

1.2.4 IgA Nephropathy (IgAN)

IgA Nephropathy is an immune-complex mediated (Wardle, 2004) and the most widespread type of primary GN globally (Lai and Lai, 2005) resulting in fifteen to forty percent of patients (Donadio and Grande, 2002) progressing to ESRD (Olson, 2001). IgAN is also known as Berger's disease as it was initially discovered by Berger in 1968. The molecules that are produced by tonsillar lymphocytes during IgAN are under-o-glycosylated suggesting that tonsils play a major role in IgAN as they showed to provide the bulk of the underglycosylated IgA1 which are deposited into the glomeruli during the disease (Horie *et. al.*, 2003). Inflammation in IgAN is a result of the segregation of IgA1 in the mesangial cells of the renal glomeruli (Van Es, 1997; Wardle, 2004) therefore studies related to IgAN rarely involve its relation with the upper respiratory tract

(Wardle, 2004) yet rather they tend to center on reducing the damage of the glomeruli (Lai, 2002).

IgAN in the absence of a clinical precursor or association is referred to as primary IgAN whereas IgAN in association with other diseases is referred to as secondary IgAN (Floege and Feehally, 2000).

1.2.4.1 Aetiology

Aetiology of IgAN is unknown (Donadio and Grande, 2002) but the establishment or aggravation of disease is typically led by upper respiratory tract infections (Brake and Somers, 2006).

1.2.4.2 Morphology

Biopsy reveals focal and segmental proliferative glomerular lesions with polymeric IgA1 (pIgA1) deposited in mesangial cells (Yaqoob, 2005), interstitial fibrosis, arterial arteriolar changes (Nicholls *et. al.*, 1984) and crescents during rapid disease progression (Goddard *et. al.*, 2006).

1.2.4.3 Clinical Features

By and large patients are asymptomatic at the onset of the disease resulting in patients being ignorant of the disease until it is surmised to be IgAN during regular screening or

while examining some other disease (Donadio and Grande, 2002) e.g. hypertension (Nicholls *et. al.*, 1984). A number of patients may possibly display aggressive disease (Donadio and Grande, 2002).

Typical clinical features of primary IgAN include episodic macroscopic haematuria concurrent with an upper respiratory tract infection (Donadio and Grande, 2002) persisting proteinuria, persistent or intermittent microscopic haematuria, chronic renal failure (CRF) and hypertension and they occur prominently in patients greater than 25 years of age (Clarkson *et. al.*, 1999; Olson, 2001).

About 50% of patients with IgAN generally present with sudden commencement of gross haematuria which is occasionally associated with pain in the flank and increased protein excretion two to three days after an upper respiratory tract or gastrointestinal viral infection (Couser, 1999).

A minute proportion of patients with IgAN will present with azotemia a portion of which may require dialysis indicating the most aggressive type of the disease (Donadio and Grande, 1997) due to late diagnosis (Donadio and Grande, 2002).

Recurrent IgAN following renal transplantation is not a benign disease (Floege *et. al.*, 1998) and it presents as microscopic haematuria (Clarkson *et. al.*, 1999; Kishi *et. al.*, 2004) as macroscopic haematuria is uncommon (Clarkson *et. al.*, 1999) or proteinuria (Kishi *et. al.*, 2004).

Clinical features indicating poor prognosis include older patients with hypertension, heavy proteinuria and impaired renal function at diagnosis (Harper and Savage, 1999).

1.2.4.4 Diagnosis

Primary IgAN has no serological abnormalities (Couser, 1999) hence serological markers cannot be used in diagnosing disease. Although diagnosing IgAN is said to be strictly biopsy- and immunohistological examination-dependent (Donadio and Grande, 2002) analysis of protein patterns in urine with the aid of capillary electrophoresis on-line attached to a mass spectrophotometer (partly due to its sensitivity) could provide an alternative to invasive biopsies in diagnosing IgAN (Haubitz *et. al.*, 2005).

In biopsy, mesangial IgA deposits can be observed to be associated with IgAN by immunofluorescence (Kishi *et. al.*, 2004), focal or diffuse mild mesangial cell and matrix expansion by light microscopy (Donadio and Grande, 1997) and electron dense mesangial deposits by electron microscopy (McKay *et. al.*, 2000). The observations by light microscopy are commonly present in other renal diseases hence it cannot be used as a definitive tool to diagnose IgAN (Donadio and Grande, 1997).

There are a variety of diseases that present with IgA deposits in mesengial cells therefore the differential diagnosis of IgAN would be that IgAN occurs in the absence of systemic diseases such as systemic lupus erythematosus (SLE) (Harper and Savage, 1999).

1.2.4.5 Pathology

In general the most common deviation from the normal anatomy is the expansion of the mesangial region including the cells and matrix (Donadio and Grande, 2002; Olson, 2001) but this presentation cannot be used to distinctively diagnose IgAN as this disease process also occurs in other renal diseases (Donadio and Grande, 2002). Crescents involving in excess of 70% of the glomeruli are indicative of poor prognosis (Oslon, 2001).

1.2.4.6 Pathogenesis

The pathogenesis of IgAN is not yet known (Goddard *et. al.*, 2006) but it is postulated to be the consequence of a magnified tonsillar IgA1 and bone marrow immune response to antigens (Yaqoob, 2005). In addition an abnormality in the O-linked galactosylation of the hinge region of the Heavy chain of the IgA1 molecule is alleged to play a role in IgAN by playing a role in the development of macromolecular IgA resulting in its glomerular deposition in IgAN (Hiki *et. al.*, 1998).

Proinflammatory cytokines (IL-8) (Lai *et. al.*, 1996; Lim *et. al.*, 2001) and a high proportion of T_{H1} and T_{H2} have been shown to influence the severity of pathogenesis and clinical features of the disease (Lim *et. al.*, 2001). Also IgAN can be stimulated by a mycotoxin called deoxynivalenol and further promoted by the absence of the cyclooxygenase-2 (COX-2) gene (Jia and Pestka, 2005).

Oxidative stress levels are increased in patients with IgAN resulting in the oxidation of vital amino acids which in turn may well stimulate protein damage ultimately leading to loss of enzymatic activity and structural integrity (Túri *et. al.*, 1997).

IgAN can be transmitted from a living donor to recipient in a silent form via mesangial IgA deposits during a kidney transplant however prognosis for the recipient has proved to be more superior to that of the donor (Koselj *et. al.*, 1997).

1.2.4.7 Treatment

The type and option of treatment are restricted as it is difficult to differentiate patients with good and bad prognosis due to the frequent benign occurrence of IgAN (Harper and Savage, 1999). By and large treatment of IgAN is symptomatic (Couser, 1999).

Patients have been shown to be treated effectively with Mycophenolate mofetil (Nowack *et. al.*, 1997). Paediatric patients diagnosed early with severe IgAN displayed reduced proteinuria and serum IgA levels as well as a decreased concentration of mesangial IgA deposits when treated with a combination of prednisolone, azathioprine, heparin-warfarin and dipyridamole thus inhibiting immunologic renal injury as well as disease progression (Yoshikawa and Ito, 1999). This is postulated to be valid in the adult population as well (Yoshikawa and Ito, 1999).

Tonsillectomy (Akagi *et. al.*, 2003; Komatsu *et. al.*, 2005) and steroid therapy have been postulated to improve renal function and thus prognosis of patients with IgAN (Komatsu *et. al.*, 2005). In addition oral and intravenous administration of a long course of steroids proved to be effective in impeding the loss of renal function (Pozzi *et. al.*, 1999). The gold standard of treatment for patients progressing to ESRD is renal transplantation (Donadio and Grande, 2002).

Due to the increased oxidative stress, management of IgAN could be aided by antioxidant therapy and diet (Túri *et. al.*, 1997).

A non pharmacological alternative to treatment of IgAN is via the use of fish oil supplementation (Donadio *et. al.*, 1994; Grande and Donadio, 1998) but this remains to be controversial due to its varying therapeutic properties in different populations thus requiring further investigations (Dillon, 1997).

1.2.4.8 Disease Association

Tonsillitis and upper respiratory infections such as bronchiolitis are associated with IgAN (Wardle, 2004) and transitory associations are found with pharyngitis and gastroenteritis (Olson, 2001). IgAN was reported to follow pulmonary tuberculosis (TB) which is a respiratory tract infection but disappeared after six months of treatment with first line TB drugs (De Siati *et. al.*, 1999).

Other diseases associated with IgAN are rheumatoid arthritis, ankylosing spondylitis, viral diseases such as hepatitis and HIV, celiac disease, Henoch-Schönlein purpura (HSP) (Floege and Feehally, 2000) as well as mastitis (Thomas *et. al.*, 1985).

Another reported case was that of an association between IgAN and *mycoplasma pneumoniae* infection (Suzuki *et. al.*, 2005) but no significant association has been found between IgAN and certain common viral infections such as cytomegalovirus (CMV), Herpes Simplex Virus (HSV), Varicella-Zorster Virus (VZV), Influenza Type A and Type B Viruses and Epstein-Barr Virus (EBV) (Hung *et. al.*, 1996).

Superimposed IgAN has been demonstrated associated with Type II diabetic patients by high IgA concentration in the sera of the patients (Kanauchi *et. al.*, 2000).

1.2.5 Focal Segmental Glomerular Sclerosis (FSGS)

Focal Segmental Glomerular Sclerosis is the sclerosis of certain glomeruli ('focal') with scarring in particular parts of the glomerular network ('segmental') (Rossini and Fogo, 2004; Schnaper, 1997). There are several types of glomerulosclerosis *viz.* primary FSGS whereby the cause is unknown hence it is referred to as being 'idiopathic', secondary FSGS which is associated with diseases or clinical conditions which are known (Schnaper, 1997), genetic (Khoshnoodi and Tryggvason, 2001; Rossini and Fogo, 2004) or familial FSGS (Mathis *et. al.*, 1998; Winn *et. al.*, 1999) which have been shown to be

linked to chromosomes 19 and 11 respectively and recurrent FSGS in transplant patients (Rossini and Fogo, 2004).

1.2.5 1 Aetiology

The aetiology of primary FSGS is not really known (Govan *et. al.*, 1995) but it appears to be immunologically related (Brady *et. al.*, 2005). A recent study demonstrated that FSGS may be induced via exposure to sirolimus at high concentrations (Letavernier *et. al.*, 2007).

1.2.5 2 Morphology

Histology reveals segmental glomerular scarring and no acute inflammation (Goddard *et. al.*, 2006). Primary FSGS discloses extensive podocyte effacement, the absence of electron dense deposits and the absence of immunoglobulins except IgM (Furness *et. al.*, 2007).

1.2.5.3 Clinical Features

During initial stages, clinical features are comparable to that of Minimal Change GN (MCGN) but lesions produced by FSGS are progressive (Govan *et. al.*, 1995). Other features that distinguish FSGS from MCGN are an increased frequency of haematuria; reduced GFR and hypertension; proteinuria is more frequently nonselective in FSGS;

FSGS responds to a prolonged course of high dose corticosteroid therapy; there is progression to chronic glomerulosclerosis; immunofluorescence microscopy may reveal indistinguishable IgM and C3 deposition in the sclerosed areas (Alpers, 2005) and accumulation of extracellular matrix within the glomerular capillaries with destruction of the capillary lumen (Reidy and Kaskel, 2007).

There are several variants of primary FSGS including collapsing glomerulopathy (Alpers, 2005), FSGS not otherwise specified, perihilar variant, cellular variant, and tip variant (Rossini and Fogo, 2004). Primary FSGS normally presents as idiopathic nephrotic syndrome in approximately 66% of the patients and approximately 33% as subnephrotic proteinuria (Brady *et. al.*, 2005) which often advances to ESRD (Gerbase *et. al.*, 1998). Typical presentation is often massive proteinuria, haematuria, hypertension and presence of blood and leukocytes in the urine (Brady *et. al.*, 2005; Yaqoob, 2005).

Recurrent FSGS occurs directly after the clamp has been detached from the renal artery or shortly afterwards (Lesavre and Grünfeld, 1996) and presents with significant proteinuria within 24 hours of the patient receiving the graft (Fine, 2007) but sclerosis may only become evident several weeks later (Rossini and Fogo, 2004) with clinical recurrence occurring more than a year later (Fine, 2007).

There are also several variants of secondary FSGS *viz.* FSGS secondary to immune complex or proliferative disease, secondary to C1q nephropathy, secondary to diabetes,

secondary to reflux nephropathy, secondary to HIV infection and FSGS associated with arterionephrosclerosis (Rossini and Fogo, 2004).

1.2.5.4 Diagnosis

Light microscopy reveals basement membrane collapse (Alpers, 2005) increased mesangial accumulation in certain parts of the glomerulus (Schnaper, 1997) and amorphous hyaline material (Brady *et. al.*, 2005) building up in the adjacent capillary loops (Schnaper, 1997). Light microscopy may fail to detect segmental lesions if the biopsy specimen does not contain sufficient glomeruli since these lesions are only present in certain glomeruli (Alpers, 2005) or if cortico-medullary tissue is not present for the diagnosis of early lesions.

Electron microscopy is an important tool in diagnosing FSGS as it detects other possible causes of glomerular scarring which could be assumed to be FSGS by simple light microscopy (Falk *et. al.*, 2000). Electron microscopy discloses visceral epithelial cell damage (Brady *et. al.*, 2005) and shows the eradication of the foot processes of the podocytes (Rossini and Fogo, 2004).

Immunofluorescent microscopy may reveal the accumulation of coarse, granular IgM with C3 at low levels (Falk *et. al.*, 2000) and fibrin in the sclerosed areas of the glomeruli (Wilson *et. al.*, 1987) or mesangium (Alpers, 2005). In addition hyalinosis and thickening of afferent arterioles may be evident (Alpers, 2005).

As the disease progresses more glomeruli become involved resulting in more global sclerosis within each glomerulus and expansion of the mesangial matrix (Alpers, 2005). Ultimately the entire glomerulus becomes sclerosed with evident tubular atrophy and interstitial fibrosis (Alpers, 2005).

1.2.5.5 Pathology

Complete sclerosis of the affected glomeruli occurs commencing at the juxtamedullary glomeruli and dispersing outwards to the outer cortical glomeruli resulting in atrophy of associated tubules (Govan *et. al.*, 2005). Podocytes are postulated to be the prime target in FSGS (Lesavre and Grünfeld, 1996).

1.2.5.6 Pathogenesis

A common pathogenic mechanism is proposed for classic and recurrent FSGS (Caridi *et. al.*, 2006).

In FSGS patients a significant decrease in the number of podocytes as well as decrease in expression of integrin $\alpha_1\beta_3$ by podocytes was noted (Chen *et. al.*, 2006). Patients with FSGS excrete urine with a high concentration of podocytes (Nakamura *et. al.*, 2000) leading Chen *et. al.* (2006) to believe that the decrease in the number of podocytes per glomerulus is due to detachment of podocytes from the GBM into urine as a result of the decrease in expression of integrin $\alpha_1\beta_3$ by podocytes.

Podocytes are unable to regenerate hence they cannot be replaced if lost resulting in areas of the glomerulus to be exposed and eradication of the barrier between the glomerular tuft and Bowman's capsule resulting in the adhesion of the parietal epithelial cells to the GBM (Kriz, 2003). This phenomenon is referred to as a tuft adhesion and it is through this lesion that segmental glomerulosclerosis stems (Kriz, 2003). The tuft adhesion provides a parietal epithelial fissure through which direct contact between the injured tuft and interstitium occurs (Kriz, 2003). Misdirected filtration occurs if the capillaries of the tuft adhesion are perfused resulting in the glomerular filtrate being directed into the interstitium rather than the Bowman's space thus triggering local interstitial proliferation resulting in the misdirected filtrate to be surrounded by fibroblasts (Kriz, 2003).

The sequence of events that follow misdirected filtration result in the ultimate deterioration of the whole nephron (Kriz, 2003). These events include the formation of paraglomerular spaces which are predisposed to expansion via the detachment of the parietal epithelial from the GBM due to continuous misdirected filtration (Kriz, 2003). In turn this results in an increase in the parietal fissure and advancing rupture of the sclerotic tuft into the periglomerular space (Kriz, 2003). The other loops of the tuft may become involved via the vascular pole eventually leading to total sclerosis of the glomerular tuft (Kriz, 2003).

Collapsing FSGS and HIV associated nephropathy (HIVAN) share a common pathogenic pathway whereby injury results in dysregulation of the podocyte phenotype which in turn arbitrates collapse of the glomerular tuft (Barisoni *et. al.*, 1999). This is important as the

change in podocyte phenotype and not the loss thereof plays an important role in the pathogenesis of FSGS (Barisoni *et. al.*, 1999).

Recurrent FSGS is due to a circulating factor in the serum which causes the glomeruli to become permeable to albumin and which may be the cause of proteinuria (Savin *et. al.*, 1996; Sharma *et. al.*, 1999).

1.2.5.7 Treatment

When treated with Prednisolone, 46.7% of patients with FSGS showed a decrease of urinary podocytes as well as urinary protein indicating a good response however treatment with cyclophosphamide as well as mizoribine proved to be ineffective (Nakamura *et. al.*, 2000).

Cyclosporine is also recommended as an effective drug against FSGS (Cattran, 2003). Patients with resistance to cyclosporine have been showed to be treated effectively with a combination of tacrolimus and steroids (Segarra *et. al.*, 2002) but mono-treatment with tacrolimus have been shown to be successful for only a short-term needing long term trials to prove its efficacy over long periods (Duncan *et. al.*, 2004). Day *et. al.* (2002) showed mycophenolate mofetil to be effective in treating patients with treatment-resistant, frequently relapsing NS as a result of FSGS.

Transplantation is the option of choice in FSGS with ESRD but is limited by the high recurrence rate which is between 30 to 50% for the first and up to 90% for the subsequent transplant (Valdiva *et. al.*, 2005).

Recurrent FSGS can be successfully treated by plasmapheresis (PP) (Valdiva *et. al.*, 2005) however not in all cases as reported by Singh *et. al.* (2006) where recurrent FSGS occurred in an elderly patient two days after receiving a cadaveric graft which had to be removed after three months (due to complications and infection) resulting in a rapid recovery. Plasma Exchange (PE) was shown to reduce the severity of proteinuria in a patient with FSGS but did not prevent disease progression resulting in graft loss (Akioka *et. al.*, 2004). A combination of plasmapheresis and cyclophosphamide is the preferred therapy for recurrent FSGS (Caridi *et. al.*, 2006).

Early corticosteroid withdrawal can also be employed posttransplant as it does not increase risk of recurrence but rather allows the patient to maintain the full benefits of the transplanted kidney (Boardman *et. al.*, 2005) but reduction in immunosuppressive drugs (tacrolimus or cyclosporine together with prednisolone and azathioprine or mycophenolate mofetil) resulted in the recurrence of FSGS (Akioka *et. al.*, 2006).

A vaccine is proposed to be effective if the circulating factor causing FSGS is identified (Altschuler, 2001). Since medication against the circulating factor might require ongoing administration, the patient may be faced with side-effects and possible toxicities. The author indicates that since a vaccine will not require ongoing administration it would not

present with side effects or toxicities. He also suggests that the vaccine may possibly bind to the circulating factor resulting in its eradication or may alter or block the structure thus causing proteinuria to come to an end.

1.2.5.8 Disease Association

FSGS has been reported to be associated with aplastic anemia (Park *et. al.*, 2004), strongly associated with HIV in London amongst those of Afro-Caribbean descent (Connolly *et. al.*, 1995) but rare in Venezuelan populations (Vargas-Arenas *et. al.*, 2002) as well as cyanotic congenital heart disease (CCHA) (Hida *et. al.*, 2002).

1.2.6 Membranous Glomerulonephritis (MGN)

Until recently MGN was known to be the most common cause of nephrotic syndrome in adults and is characterized into various stages with lesions consistent with disease progression (Gluck *et. al.*, 1973).

1.2.6.1 Aetiology

Most cases are idiopathic and have no underlying condition whereas the rest are associated with various factors such as drugs (e.g. captopril and penicillamine), malignant tumors, infections (Govan *et. al.*, 1995) systemic lupus erythematosus (SLE), as well as other autoimmune disorders and are referred to as secondary MGN (Alpers, 2005).

1.2.6.2 Morphology of MGN

Light microscopy reveals diffuse thickening of the GBM which is distinct upon staining with periodic acid-Schiff (PAS) (Brady *et. al.*, 2005) with no indication of inflammation or cellular proliferation (Brady *et. al.*, 2005; Goddard *et. al.*, 2006; Pollak *et. al.*, 1968). Electron microscopy shows dense immune deposition of irregular nature between the basement membrane and the epithelial cells (containing effaced foot processes) which cover it (Alpers, 2005). Basement membrane material protrude between the deposits and appear as spikes, best seen by silver staining, which gradually thickens forming a dome like structure that ultimately encloses the deposits (Alpers, 2005).

Immunofluorescence microscopy displays granular IgG and C3 deposition (Alpers, 2005) as well as the terminal components of complement (C5b-9) along the capillary wall of the glomerulus (Brady *et. al.*, 2005). As the disease progresses, the thickened membrane enroaches into the lumen of the capillaries which could possibly result in the mesangium being sclerosed and gradually total sclerosis of the glomeruli may occur (Alpers, 2005).

1.2.6.3 Clinical Features

Males have been shown to be more prone to MGN than females (Gluck *et. al.*, 1973). Majority of MGN patients present with nephrotic syndrome with unselective proteinuria (Brady *et. al.*, 2005). Almost 50% of patients present with microscopic haematuria and hypertension in approximately 20% of patients at the onset of disease (Brady *et. al.*, 2005).

Patients that present with recurrent MGN often display nephrotic-range proteinuria (McKay *et. al.*, 2000). Elderly males with hypertension, severe proteinuria, hyperlipidemia and impaired renal function are indicative of poor prognosis (Brady *et. al.*, 2005).

1.2.6.4 Pathogenesis

Not much is known about the pathogenesis of Idiopathic MGN but IgG and C3 containing electron deposits suggest an immune process (Brady *et. al.*, 2005).

1.2.6.5 Treatment

Prednisone was shown not to have an effect in reducing proteinuria (Pollak *et. al.*, 1968). High risk patients indicative of disease progression should be offered cytotoxic therapy (Muirhead, 1999). Cyclophosphamide and chlorambucil can be used with cyclosporine and could potentially be the treatment of choice if proved to be efficient by further studies (Muirhead, 1999).

1.2.6.6 Disease Association

MGN has been documented to be associated with benign kidney tumours (Bian *et. al.*, 2003; Gluck *et. al.*, 1973) as well as malignant tumors (Altiparmak *et. al.*, 2003; Evans *et. al.*, 2003; Fang *et. al.*, 2001; Şahiner, 2004) Cronkhite-Canada Syndrome (Takeuchi

et. al., 2003) inflammatory demyelinating polyradiculoneuropathy (Emsley and Molloy, 2002; Mobbs *et. al.*, 2000) IgAN (Koselj-Kajtna, 2002) and hepatitis (Bates *et. al.*, 1996; Takahashi *et. al.*, 2001).

A case of MGN was also documented in an arthritic patient receiving a gold containing oral preparation called auronafin (Plaza *et. al.*, 1982) as well as with a patient with hemiretinal vein occlusion (Jorge *et. al.*, 2002). MGN in the presence of neoplasms suggests the presence of circulating antigen-antibody complexes formed by host antibodies and tumour antigens.

CHAPTER 2

PATIENTS AND METHODS

2.1 Ethical Approval

Ethical Approval has been granted by the Biomedical Research Ethics Committee (Reference: H188/05) of the Nelson R Mandela School of Medicine, University of KwaZulu-Natal. The Postgraduate Education Committee has also approved this study (Reference: H188/05).

2.2 Patient and Control Recruitment

Patients were recruited from the provincial KwaZulu-Natal Kidney Transplant register and KwaZulu-Natal Kidney Transplant waiting list as well as the outpatient list from the Renal Clinic at Inkosi Albert Luthuli Central Hospital (IALCH). Inclusion criteria: all adults patients from the 1970's to the 2000's with appropriate diagnosis. All patients with unconfirmed diagnosis were excluded from the study. Patients were diagnosed by histology.

Patients were educated verbally about the study, an information document describing the study was issued and informed consent was obtained. Where applicable, a translator was utilized. Patients were not forced to participate in the study.

Controls were selected from the donor population on the kidney transplant list from the 1970's to present. These were cadaver (some of which death was due to gunshot wounds) as well as living (friends, relatives) donors. The control population was extensively studied. They present no evidence of renal disease. Biopsy was not done on the control population as it is not ethical to biopsy patients with no evidence of renal disease. The average age of the donor was 31 years. Males and females contributed equally to the donor pool.

2.3 Sample Size

2.3.1 Patient Sample Size

This study was divided into three parts *viz.* Part A, Part B and Part C.

Part A was a screening study and it involved finding associations between HLA and Hypertension in renal patients in the KZN setting.

Part B involved finding associations between HLA and three glomerular diseases *viz.* IgAN, FSGS and MGN. Certain patients in this study present with MGN secondary to autoimmune diseases such as SLE.

Part C aimed to investigate if the presence or absence of certain HLA loci are responsible for the African population's decreased susceptibility to IgAN.

2.3.1.1 Part A Sample Size

The hypertensive Indian population comprised 93 patients, the African hypertensive population comprised 71 patients and the White hypertensive population comprised 12 patients. Diagnosis of hypertension in these patients is presumptive and it cannot be excluded that the hypertension could be secondary to underlying renal diseases.

2.3.1.2 Part B Sample Size

Initially 30 patients from each disease were proposed to be recruited each with equal frequency from the African, Indian and White populations. Due to insufficient numbers, IgAN, MGN and FSGS were grouped as glomerulonephritides. All patients were diagnosed by biopsy.

The African population contained 0 IgAN patients and 24 patients with glomerulonephritides. Patients with glomerulonephritides included 9 FSGS, 6 MGN and 9 NS with no known aetiology.

Sixty-two Indian patients were recruited. Indian patients with glomerulonephritides comprised of 14 IgAN, 10 FSGS, 14 MGN, 5 MCGN, 1 immune complex GN and 18 NS with no known aetiology.

The White population encompassed 12 individuals with glomerulonephritides. White patients with glomerulonephritides included 3 IgAN, 3 MCGN, 3 MGN, 1 FSGS and 2 NS with unknown aetiology.

The total patient sample size is thus 98 encompassing 24 African patients, 62 Indian patients and 12 White patients.

2.3.1.3 Part C Sample Size

Part C comprised 201 African, 436 Indian and 290 White Individuals (Explained in Chapter 2.7).

2.3.2 Control Sample Size

The total control population comprised 484 apparently healthy individuals from the donor list. Of these individuals, 67 are African (See discussion); 187 are Indian and 230 are White.

2.4 Sample Collection

After obtaining the patients consent, venous blood was collected in vacutainers containing EDTA (purple caps) during their routine check up.

2.5 Part A Method

This is a retrospective study. The transplant waiting list and the transplant register with records dating back to the 1970's were studied. Ninety-three Indian, 71 African and 12 White hypertensive patients were selected. The HLA-A, -B and -DR loci were analyzed and statistics was performed by the method described in Chapter 2.8.

2.6 Part B Method

2.6.1 DNA Extraction

DNA was extracted by means of the Qiagen QIAmp® DNA Blood Midi Kit (Southern Cross Biotechnology, Cape Town) according to manufacturer's instructions. A volume of 1ml blood was used in the extraction.

2.6.2 DNA Quantification

DNA was quantified by the NanoDrop® ND 1000 spectrophotometer. The concentration of the quantified DNA was between 37.65-148.38 ng/µl.

2.6.3 DNA Amplification

2.6.3.1 Principle of Procedure

The Dynal RELI™ SSO HLA Typing Kits are based on the following three procedures. The First is DNA amplification by a Polymerase Chain Reaction (PCR) where the DNA

is multiplied exponentially by a chain reaction. This is explained in detail by Mullis and Faloona (1987).

The second step is hybridization of the PCR products onto a nylon strip containing a collection of fixed, Sequence Specific Oligonucleotide (SSO) probes which bind to denatured biotin-labelled amplicons derived from the first step. The final step is colorometric detection using streptavidin-horseradish peroxidase (SA-HRP). These principles are fully explained by Saiki *et. al.* (1989).

2.6.3.2 Procedure

The quantified DNA was thereafter diluted to a concentration of 15ng/µl (See Appendix) with Qiagen nuclease-free H_2O (Southern Cross Biotechnology, Cape Town).

For the HLA-A, -B and -DR loci, the sample DNA together with a positive control (provided in kit) as well as a negative control (nuclease-free water) was amplified using the Dynal RELI™ SSO HLA-A, -B and -DR Typing Kits (Southern Cross Biotechnology, Cape Town) according to manufacturer's instructions.

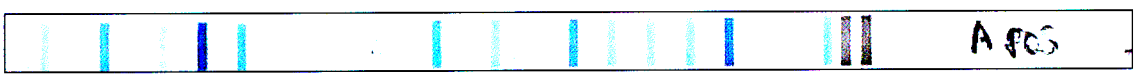

Figure 2.1 HLA-A positive control strip. Principle explained in text.

2.6.4 Probe Hybridization

Probes were hybridized onto strips with the Dynal RELI™ SSO Strip Detection Reagent Kit (Southern Cross Biotechnology, Cape Town) according to manufacturer's instructions. Time and temperature are pertinent during these steps. Positive probes were visualized as blue bands on strips (Figure 2.1).

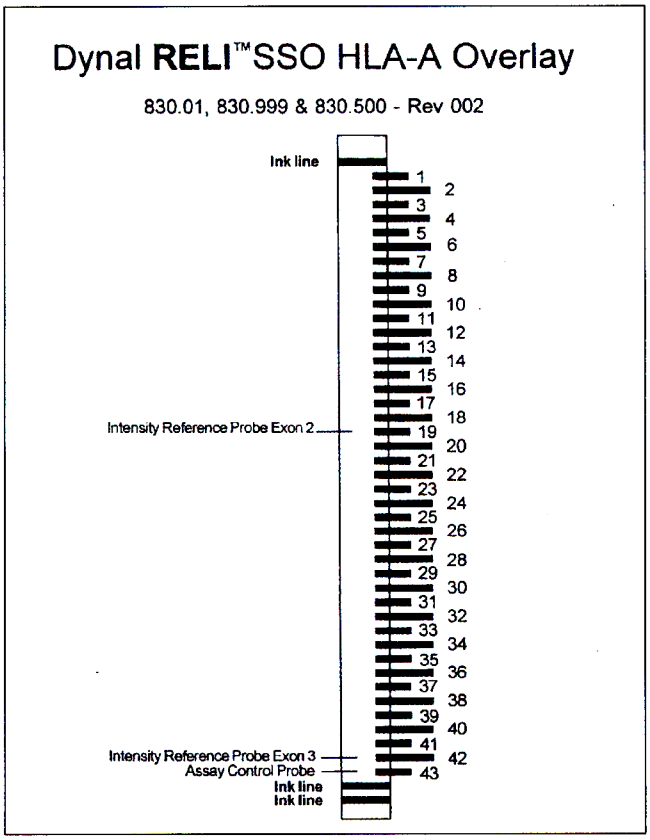

Figure 2.2 Transparent HLA-A locus template.

2.6.5 Allele Detection

After probe hybridization, strips (e.g. Figure 2.1) were aligned onto a transparent template which is provided with the kit (e.g. Figure 2.2) and analyzed according to manufacturer's instructions. Exon 2 and Exon 3 are examined to determine the alleles present. Bands 19, 42 and 43 are control probes on Figure 2.2 and appear as distinct bands. Bands appearing on the negative control strip indicate cross contamination. Each locus has a unique template with unique control probes.

Two methods of allele detection were employed to ensure accuracy. The Dynal Biotech Pattern Matching Program (*PMP*) 5.41 was used to interpret the results. The first is an automatic method where strips were placed on a metal template and scanned onto the computer using a Hewlett-Packard (*hp*) scanjet 3670 scanner. The alleles were automatically detected using the above-mentioned program. The manual method involved clicking the positive probes (blue bands) on a virtual strip present on the computer program. Once all the positive probes are clicked on the strip, the computer detects the allele/s present.

2.7 Part C Method

Part C involved the combination of both the diseased and donor populations from the transplant register and waiting list as well as the Part B patient population. The haplotypes of the individuals were analyzed and compared. Statistics was employed for the HLA-A30, HLA-A29, HLA-A34, HLA-B42, HLA-B70, HLA-B58 and HLA-DR11

loci between the African and Indian and African and White populations in attempt to investigate a possible reason underlying the protection of the African population from IgAN. These particular loci were selected as they are present in far greater amounts in the African population than the Indian and White populations. These alleles were therefore analyzed as it was presumed that they might contribute to the African population's "immunity" to IgAN.

2.8 Statistical Analysis

Statistics was performed using the Graphpad InStat2™ statistical program. Due to the small numbers for comparison, the frequency distribution of the HLA-A, -B and -DR loci in hypertensives, renal patients and normal apparently healthy controls were compared with a two-tailed Fisher's exact test with Yates' Continuity correction. The Relative risk was calculated. The *P* values were corrected (P_c) by the following equation:

$$P_c = 1-(1-P)^n$$

Where n is the number of alleles tested for at each locus. *P* values in text are uncorrected unless indicated by P_c. *P* values were corrected for 16 HLA-A, 15 HLA-B and 10 HLA-DR comparisons. Unless otherwise stated, significance associated with *P* and P_c, <0.05.

CHAPTER 3

RESULTS

3.1 PART A: Hypertension in Renal Disease in KwaZulu-Natal Indian Population

An association was noted between HLA-A28 and the Indian control population (P= 0.0330; RR=0.3; 95% CI: 0.1245-0.9820) (Table 3.1.1). A relative risk less than one indicated a decreased risk however when corrected for P, P_c= 0.99 hence there was no association between hypertension and HLA-A28.

Table 3.1.1 also showed an association between HLA-A33 and hypertension (P= 0.0146; RR=2.6; 95% CI: 1.029-5.416). When corrected, P_c= 0.99.

Table 3.1.2 showed associations between HLA-B38 (P= 0.0165; RR=10.054; 95% CI: 1.191-84.865), HLA-B40 (P<0.0001; RR=32.2; 95% CI: 4.330-239.01) and HLA-B52 (P= 0.0290; RR=0.4399; 95% CI: 0.2018-0.9588) however when corrected for P, only HLA-B40 (P_c<0.05) was significantly associated with hypertension. Therefore HLA-B40 is a possible susceptibility marker for hypertension in Indian patients carrying the allele.

Associations between Blank HLA-DR alleles (explained in Chapter1.1.2.2) and the control population (P= 0.0101; RR=0; 95% CI: $-\infty$-∞) as well as HLA-DR2 (P= 0.0020; RR=0.2873; 95% CI: 0.1164-0.7091) and the hypertensive population are indicated by Table 3.1.3. Again when corrected for P, both loci showed no significant association.

Table 3.1.1 HLA-A antigen frequencies in Indian hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 374	%	*n* = 186	%		*p* value
Blank	2	0.53	0	0.00	0.0	1.0000
A1	46	12.30	26	13.98	1.1	0.5635
A2	78	20.86	34	18.28	0.9	0.4389
A3	18	4.81	15	8.06	1.7	0.1193
A4	1	0.27	1	0.54	2.0	1.0000
A11	60	16.04	25	13.44	0.8	0.4096
A23	2	0.53	0	0.00	0.0	1.0000
A24	83	22.19	46	24.73	1.1	0.4468
A26	16	4.28	8	4.30	1.0	1.0000
A28	23	6.15	4	2.15	0.3	0.0330*
A29	3	0.80	0	0.00	0.0	0.5532
A30	6	1.60	3	1.61	1.0	1.0000
A31	15	4.01	5	2.69	0.7	0.4721
A32	8	2.14	4	2.15	1.0	1.0000
A33	11	2.94	14	7.53	2.6	0.0146*
A34	1	0.27	1	5.00	2.0	1.0000
A68	1	0.27	0	0.00	0.0	1.0000

* indicates statistical significance.

Table 3.1.2 HLA-B antigen frequencies in Indian hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 374	%	n = 186	%		p value
Blank	2	0.53	0	0.00	0.0	1.0000
B1	3	0.80	0	0.00	0.0	0.5532
B4	2	0.53	0	0.00	0.0	1.0000
B7	28	7.49	12	6.62	0.9	0.7191
B8	11	2.94	8	4.41	1.5	0.4512
B13	15	4.01	3	2.21	0.4	0.1945
B14	1	0.27	0	0.00	0.0	1.0000
B15	4	1.07	6	2.21	3.0	0.0879
B16	5	1.34	0	0.00	0.0	0.1737
B17	1	0.27	0	0.00	0.0	1.0000
B18	2	0.53	6	2.21	6.0	0.0179
B22	4	1.07	0	0.00	0.0	0.3053
B27	2	0.53	2	0.74	2.0	0.6021
B35	44	11.76	15	10.29	0.7	0.1648
B37	14	3.74	4	2.21	0.3	0.0152
B38	1	0.27	5	2.21	10.1	0.0165*
B39	4	1.07	0	0.00	0.0	0.3053
B40	1	0.27	16	6.62	32.2	< 0.0001*
B44	23	6.15	16	18.09	1.4	0.2757
B48	0	0.00	2	0.74	∞	0.1095
B49	4	1.07	5	2.21	2.5	0.1641
B50	0	0.00	2	0.74	∞	0.1095
B51	46	12.30	24	13.97	1.0	0.8837
B52	32	8.56	7	4.41	0.4	0.0290*
B53	2	0.53	1	0.74	1.0	1.0000
B55	4	1.07	0	0.00	0.0	0.3053
B57	29	7.75	19	10.29	1.3	0.3159
B58	8	2.14	5	2.94	1.3	0.7649
B60	17	4.55	6	3.68	0.7	0.4989
B61	42	11.23	18	10.29	0.9	0.6433
B62	15	4.01	3	1.47	0.4	0.1941
B63	1	0.27	0	0.00	0.0	1.0000
B65	1	0.27	0	0.00	0.0	1.0000
B70	6	1.60	1	0.74	0.3	0.4309

* indicates statistical significance.

Table 3.1.3 HLA-DR antigen frequencies in Indian hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 374	%	*n* =186	%		*p* value
Blank	12	3.21	0	0.00	0.0	0.0101*
DR1	8	2.14	6	3.68	1.5	0.5611
DR2	35	9.36	5	3.68	0.3	0.0020*
DR3	38	10.16	12	7.35	0.6	0.1390
DR4	50	13.37	25	13.97	1.0	1.0000
DR5	13	3.48	2	1.47	0.3	0.1559
DR6	6	1.60	1	0.74	0.3	0.4309
DR7	56	14.97	35	18.38	1.3	0.2293
DR8	7	1.87	6	2.94	1.7	0.3684
DR9	4	1.07	2	0.74	1.0	1.0000
DR10	19	5.08	12	5.88	1.3	0.5454
DR11	9	2.41	10	4.41	1.1	1.0000
DR12	10	2.67	6	2.94	1.2	0.7860
DR13	23	6.15	13	6.62	1.1	0.7072
DR14	33	8.82	16	7.35	1.0	1.0000
DR15	51	13.64	35	19.85	1.4	0.0985

* indicates statistical significance.

African Population

An association between HLA-A68 (P=0.0269; RR=3.8; 95% CI: 1.114-12.793) and HLA-A74 (P=0.0026; RR=6.6; 95% CI: 1.559-27.988) with hypertension in Africans is shown in Table 3.1.4. When corrected for P, no association was detected.

An association between HLA-B15 (P<0.0001; RR=9.4; 95% CI: 2.292-38.847) as well as HLA-B44 (P=0.0075; RR=2.6; 95% CI: 1.241-5.424) and hypertension is shown by Table 3.1.5. When corrected for P, only HLA-B15 maintained significance (P_c<0.02).

An association between HLA-DR13 (P=0.0193; RR=1.8; 95% CI: 1.106-3.023) and African hypertensive patients is noted before correction (Table 3.1.6). After correction, P_c= 0.99.

Table 3.1.4 HLA-A antigen frequencies in African hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 134	%	*n* = 142	%		*p* value
A1	4	2.99	6	4.23	1.4	0.7456
A2	26	19.40	25	17.61	0.9	0.7255
A3	8	5.97	10	7.04	1.2	0.8029
A11	1	0.75	1	0.70	0.9	1.0000
A23	14	10.45	18	12.68	1.2	0.5525
A24	4	2.99	3	2.11	0.7	0.7127
A26	5	3.73	1	0.70	0.2	0.1081
A28	12	8.96	11	7.75	0.9	0.8202
A29	13	9.70	6	4.23	0.4	0.0822
A30	25	18.66	19	13.38	0.7	0.2045
A31	1	0.75	1	0.70	0.9	1.0000
A32	2	1.49	1	0.70	1.9	1.0000
A34	11	8.21	11	7.75	0.9	1.0000
A43	2	1.49	1	0.70	1.9	1.0000
A66	0	0.00	2	1.41	∞	0.4968
A68	3	2.24	12	8.45	3.8	0.0269*
A74	2	1.49	14	9.86	6.6	0.0026*
A80	1	0.75	0	0.00	0.0	0.4855

* indicates statistical significance.

Table 3.1.5 HLA-B antigen frequencies in African hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 134	%	*n* = 142	%		*p* value
Bu	1	0.75	0	0.00	0.0	0.4855
B7	9	6.72	13	9.15	0.6	0.3357
B8	12	8.96	8	5.63	0.6	0.3357
B12	1	0.75	1	0.70	0.9	1.0000
B13	2	1.49	0	0.00	0.0	0.2339
B14	8	5.87	12	8.45	1.4	0.4733
B15	2	1.49	20	14.08	9.4	< 0.0001*
B16	0	0.00	1	0.70	∞	1.0000
B17	2	1.49	0	0.00	0.0	0.2339
B18	4	2.99	3	2.11	0.7	0.7127
B27	1	0.75	0	0.00	0.0	0.4855
B35	8	5.91	6	4.23	0.7	0.5790
B36	1	0.75	0	0.00	0.0	0.4855
B39	1	0.75	0	0.00	0.0	0.4855
B41	2	1.49	3	2.11	1.6	0.6711
B42	16	11.94	15	10.56	0.9	0.8386
B44	8	5.97	22	15.49	2.6	0.0075*
B45	0	0.00	2	1.41	∞	0.4968
B49	1	0.75	0	0.00	0.0	0.4855
B50	0	0.00	2	1.41	∞	0.4968
B51	4	2.99	1	0.70	0.2	0.1989
B57	13	9.70	9	6.34	0.7	0.3539
B58	18	13.43	9	6.34	0.5	0.0523
B61	0	0.00	1	0.70	∞	1.0000
B62	2	1.49	1	0.70	1.9	1.0000
B65	0	0.00	1	0.70	∞	1.0000
B70	17	12.69	10	7.04	0.6	0.1324
B81	1	0.75	2	1.41	1.9	1.0000

* indicates statistical significance.

Table 3.1.6 HLA-DR antigen frequencies in African hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 134	%	*n* = 142	%		*p* value
Blank	2	1.47	0	0.00	0.0	0.2339
DR1	1	0.74	3	2.11	2.8	0.6201
DR2	2	1.47	3	2.11	1.4	1.0000
DR3	24	18.38	26	18.31	1.0	1.0000
DR4	8	5.88	8	5.63	0.9	1.0000
DR5	5	3.68	1	0.70	0.2	0.1081
DR6	4	2.94	1	0.70	0.2	0.1989
DR7	16	11.76	13	9.15	0.8	0.5313
DR8	5	3.68	3	2.11	0.6	0.4842
DR9	2	1.47	1	0.70	1.9	1.0000
DR10	1	0.74	4	2.82	3.8	0.3669
DR11	24	18.38	24	16.90	0.9	0.8590
DR12	4	2.94	2	1.41	0.5	0.4313
DR13	16	11.76	31	21.83	1.8	0.0193*
DR14	1	0.74	3	2.11	2.8	0.6201
DR15	18	13.24	19	13.38	1.0	1.0000
DR16	1	0.74	0	0.00	0.0	0.4855

* indicates statistical significance.

White Population

There is an association between HLA-A2 and the control population (P=0.0317; RR=0.4; 95% CI: 0.1566-1.125) as shown by Table 3.1.7. An association was also noted between HLA-A31 and the hypertensive population (P=0.0413; RR=7.7; 95% CI: 1.653-35.330). When corrected, P_c= 0.99 for both loci. No significant conclusion can be made.

Table 3.1.8 showed an association between HLA-B42 and hypertension (P=0.0066; RR=38.3; 95% CI: 3.730-393.92) but P_c=0.95.

An association is noted between Blank HLA-DR alleles and the control population (P=0.0401; RR=0; 95% CI: $-\infty$-∞) (Table 3.1.9). An association is shown between HLA-DR3 and hypertension (P=0.0032; RR=2.7; 95% CI: 1.728-4.340). Corrected P values indicated no association. P_c=0.86 and 0.79 respectively.

Table 3.1.7 HLA-A antigen frequencies in White hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 460	%	*n* = 24	%		*p* value
Blank	2	0.43	0	0.00	0.0	1.0000
A1	72	15.65	6	25.00	1.6	0.2090
A2	137	29.78	3	13.00	0.4	0.0317*
A3	68	14.78	3	13.00	0.8	1.0000
A11	24	5.22	1	4.20	0.8	1.0000
A23	13	2.83	0	0.00	0.0	1.0000
A24	39	8.48	1	4.20	0.5	0.6963
A25	6	1.30	2	8.00	6.4	0.0535
A26	21	4.57	1	4.20	0.0	0.6069
A28	20	4.35	0	0.00	0.0	0.6061
A29	15	3.26	2	8.00	2.6	0.2022
A30	9	1.96	2	8.00	4.3	0.0967
A31	5	1.09	2	8.00	7.7	0.0413*
A32	21	4.57	1	4.20	0.9	1.0000
A33	4	0.87	0	0.00	0.0	1.0000
A34	1	0.22	0	0.00	0.0	1.0000
A36	1	0.22	0	0.00	0.0	1.0000
A68	1	0.22	0	0.00	0.0	1.0000
A82	1	0.22	0	0.00	0.0	1.0000

* indicates statistical significance.

Table 3.1.8 HLA-B antigen frequencies in White hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 460	%	*n* = 24	%		*p* value
B3	1	0.22	1	4.00	19.2	0.0969
B5	1	0.22	0	0.00	0.0	1.0000
B7	82	17.83	3	12.50	0.7	0.5485
B8	59	12.83	3	12.50	1.0	1.0000
B11	1	0.22	0	0.00	0.0	1.0000
B12	3	0.65	0	0.00	0.0	1.0000
B13	6	1.30	1	4.00	3.2	0.3028
B14	24	5.22	0	0.00	0.0	0.6149
B15	15	3.26	1	4.00	1.3	0.5686
B16	2	0.43	0	0.00	0.0	1.0000
B17	3	0.65	0	0.00	0.0	1.0000
B18	13	2.83	1	4.00	1.5	0.5193
B22	1	0.22	0	0.00	0.0	1.0000
B27	15	3.26	0	0.00	0.0	1.0000
B29	1	0.22	0	0.00	0.0	1.0000
B35	34	7.39	2	8.00	1.1	0.6948
B37	4	0.87	0	0.00	0.0	1.0000
B38	7	1.52	1	4.00	2.7	0.3384
B39	9	1.96	0	0.00	0.0	1.0000
B40	7	1.52	1	4.00	2.7	0.3384
B41	2	0.43	0	0.00	0.0	1.0000
B42	1	0.22	2	8.00	38.3	0.0066*
B44	46	10.00	1	4.00	0.4	0.4700
B45	1	0.22	0	0.00	0.0	1.0000
B47	2	0.43	0	0.00	0.0	1.0000
B48	1	0.22	0	0.00	0.0	1.0000
B49	2	0.43	0	0.00	0.0	1.0000
B50	4	0.87	0	0.00	0.0	1.0000
B51	15	3.26	1	4.00	1.3	0.5686
B52	3	0.65	0	0.00	0.0	1.0000
B55	3	0.65	0	0.00	0.0	1.0000
B56	4	0.87	0	0.00	0.0	1.0000
B57	11	2.39	1	4.00	1.7	0.4647
B58	10	2.17	0	0.00	0.0	1.0000
B60	20	4.35	1	4.00	1.0	1.0000
B61	10	2.17	1	4.00	1.9	0.4354
B62	28	6.09	2	8.00	1.4	0.6484
B63	3	0.65	0	0.00	0.0	1.0000
B65	1	0.22	0	0.00	0.0	1.0000
B70	3	0.65	1	4.00	6.4	0.1851
B71	1	0.22	0	0.00	0.0	1.0000
B75	1	0.22	0	0.00	0.0	1.0000

* indicates statistical significance.

Table 3.1.9 HLA-DR antigen frequencies in White hypertensive patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 460	%	*n* = 24	%		*p* value
Blank	62	13.48	0	0.00	0.0	0.0401*
DR1	36	7.83	0	0.00	0.0	0.2225
DR2	16	3.48	0	0.00	0.0	1.0000
DR3	56	12.17	8	33.00	2.7	0.0032*
DR4	69	15.00	5	20.00	1.4	0.5206
DR5	6	1.30	0	0.00	0.0	1.0000
DR6	10	2.17	0	0.00	0.0	1.0000
DR7	44	9.57	1	4.00	0.4	0.7017
DR8	13	2.83	0	0.00	0.0	1.0000
DR9	9	1.96	0	0.00	0.0	1.0000
DR10	7	1.52	1	4.00	2.7	0.3384
DR11	24	5.22	3	12.50	0.2	0.1374
DR12	10	2.17	1	4.00	1.9	0.4354
DR13	35	7.61	1	4.00	0.5	1.0000
DR14	9	1.95	0	0.00	0.0	1.0000
DR15	49	10.65	4	17.00	1.6	0.3020
DR16	5	1.09	0	0.00	0.0	1.0000

* indicates statistical significance.

3.2 PART B: HLA allele frequencies in Renal Disease in KwaZulu-Natal

3.2.1 Indian Population

An association is shown between HLA-A33 (P<0.0001; RR=4.4; 95% CI: 2.152-8.942) as well as HLA-A68 (P=0.0003; RR=21.1; 95% CI: 2.648-168.33) with glomerulonephritides in the Indian A locus (Table 3.2.1.1). When corrected, P_c<0.049 for HLA-A33 and 0.14 for HLA-A68.

The distribution of HLA-DR alleles in glomerulonephritides patients vs. controls is shown in Table 3.2.1.2. An association is noted between Blank HLA-DR alleles and the control population (P=0.0413; RR=0; 95% CI: $-\infty$-∞) but P_c=0.99 indicating no significance.

Table 3.2.1.1 HLA-A antigen frequencies in Indian glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 374	%	*n* =124	%		*p* value
Blank	2	0.53	0	0.00	0.0	1.0000
A1	46	12.30	13	13.54	0.9	0.6092
A2	78	20.86	21	21.88	0.8	0.2977
A3	18	4.81	6	6.25	1.0	1.0000
A4	1	0.27	0	0.00	0.0	1.0000
A11	60	16.04	20	20.83	1.0	1.0000
A23	2	0.53	0	0.00	0.0	1.0000
A24	83	22.19	22	22.92	0.8	0.2380
A26	16	4.28	3	3.13	0.6	0.4197
A28	23	6.15	8	8.33	1.0	1.0000
A29	3	0.80	1	1.04	1.0	1.0000
A30	6	1.60	0	0.00	0.0	0.3412
A31	15	4.01	4	4.17	0.8	0.7896
A32	8	2.14	3	3.13	1.1	1.0000
A33	11	2.94	16	16.67	4.4	< 0.0001*
A34	1	0.27	0	0.00	0.0	1.0000
A68	1	0.27	7	7.29	21.1	0.0003*

* indicates statistical significance.

Table 3.2.1.2 HLA-DR antigen frequencies in Indian glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 374	%	*n* =124	%		*p* value
Blank	12	3.21	0	0.00	0.0	0.0413*
DR1	8	2.14	5	4.03	1.9	0.3199
DR2	35	9.36	8	6.45	0.7	0.3378
DR3	38	10.16	11	8.87	0.9	0.7162
DR4	50	13.37	18	14.52	1.1	0.7437
DR5	13	3.48	6	4.84	1.4	0.5805
DR6	6	1.60	5	4.03	2.5	0.1478
DR7	56	14.97	24	19.35	1.3	0.2121
DR8	7	1.87	3	2.42	1.3	0.7134
DR9	4	1.07	2	1.61	1.5	0.6407
DR10	19	5.08	7	5.65	1.1	0.8122
DR11	9	2.41	6	4.84	2.0	0.2144
DR12	10	2.67	1	0.81	0.3	0.3009
DR13	23	6.15	4	3.23	0.5	0.1713
DR14	33	8.82	7	5.65	0.6	0.3185
DR15	51	13.64	17	13.71	1.0	1.0000

* indicates statistical significance.

3.2.2 African Population

The distribution of the various HLA-A locus alleles is shown between the African glomerulonephritides and control populations (Table 3.2.2.1). An association between HLA-A2 (P=0.0010; RR=9.8; 95% CI: 2.178-43.837) as well as HLA-A68 (P=0.0448; RR=3.4; 95% CI: 1.070-12.559) is noted in the glomerulonephritides patient population but when corrected P_c=0.17 and 0.99 respectively.

The African HLA-DR locus showed no associations with the glomerulonephritides patient and control populations (Table 3.2.2.2).

Table 3.2.2.1 HLA-A antigen frequencies in African glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 134	%	n = 48	%		p value
A1	4	2.99	2	4.17	1.4	0.6523
A2	26	19.40	7	14.58	9.8	0.0010*
A3	8	5.97	1	2.08	0.3	0.4360
A11	1	0.75	0	0.00	0.0	1.0000
A23	14	10.45	5	10.42	1.0	1.0000
A24	4	2.99	1	2.08	0.7	1.0000
A26	5	3.73	0	0.00	0.0	0.3213
A28	12	8.96	2	4.17	0.5	0.3397
A29	13	9.70	5	10.42	1.1	1.0000
A30	25	18.66	5	10.42	0.6	0.2059
A31	1	0.75	1	2.08	0.5	2.7920
A32	2	1.49	0	0.00	0.0	1.0000
A34	11	8.21	2	4.17	0.5	0.5012
A36	0	0.00	1	2.08	∞	0.2637
A43	2	1.49	2	4.17	2.8	0.2830
A68	3	2.24	11	22.92	3.7	0.0448*
A74	2	1.49	3	6.25	2.1	0.3748
A80	1	0.75	0	0.00	0.0	1.0000

* indicates statistical significance.

Table 3.2.2.2 HLA-DR antigen frequencies in African glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 134	%	n = 48	%		p value
Blank	2	1.47	0	0.00	0.0	1.0000
DR1	1	0.74	0	0.00	0.0	1.0000
DR2	2	1.47	1	2.08	1.4	1.0000
DR3	24	18.38	11	22.92	1.3	0.4655
DR4	8	5.88	3	6.25	1.0	1.0000
DR5	5	3.68	0	0.00	0.0	0.5698
DR6	4	2.94	0	0.00	0.0	0.3201
DR7	16	11.76	5	10.42	0.9	1.0000
DR8	5	3.68	1	2.08	0.6	1.0000
DR9	2	1.47	0	0.00	0.0	1.0000
DR10	1	0.74	0	0.00	0.0	1.0000
DR11	24	18.38	12	25.00	1.4	0.2353
DR12	4	2.94	0	0.00	0.0	0.3201
DR13	16	11.76	7	14.58	1.2	0.5691
DR14	1	0.74	2	4.17	5.6	0.1689
DR15	18	13.24	6	12.50	0.9	1.0000
DR16	1	0.74	0	0.00	0.0	1.0000

3.2.3 White Population (glomerulonephritides)

A similar distribution between the HLA-A locus alleles of White glomerulonephritides patients and controls is shown by Table 3.2.3.1. No increased frequencies and no associations were noted.

HLA-B locus alleles of White glomerulonephritides patients and controls are presented in Table 3.2.3.2. HLA-B18 (P=0.0363; RR=4.4; 95% CI: 1.550-14.687); HLA-B27 (P=0.0499; RR=3.8; 95% CI: 1.282-11.465) and HLA-B40 (P=0.0093; RR=8.2; 95% CI: 2.420-27.878) are show associations in the patient population but when corrected for P, no significance was noted.

An association is noted between Blank HLA-DR alleles in the control population (P=0.0401; RR=0.0; 95% CI: $-\infty$-∞) as shown by Table 3.2.3.3. HLA-DR3 (P=0.0154; RR=2.4; 95% CI: 1.410-4.070) and HLA-DR16 (P=0.0413; RR=7.7; 95% CI: 1.653-35.330) are associated with glomerulonephritides but when corrected for P, P_c=0.99 for all three loci hence no significant association was observed.

Table 3.2.3.1 HLA-A antigen frequencies in White glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 460	%	*n* = 24	%		*p* value
Blank	2	0.43	0	0.00	0.0	1.0000
A1	72	15.65	7	29.20	1.9	0.0627
A2	137	29.78	5	20.80	0.7	0.2420
A3	68	14.78	4	16.60	1.1	0.7539
A11	24	5.22	1	4.20	0.8	1.0000
A23	13	2.83	2	8.30	2.9	0.1650
A24	39	8.48	3	12.50	1.5	0.4421
A25	6	1.30	1	4.20	3.2	0.3028
A26	21	4.57	0	0.00	0.0	0.6069
A28	20	4.35	0	0.00	0.0	0.6061
A29	15	3.26	0	0.00	0.0	1.0000
A30	9	1.96	0	0.00	0.0	1.0000
A31	5	1.09	1	4.20	3.8	0.2654
A32	21	4.57	0	0.00	0.0	0.6069
A33	4	0.87	0	0.00	0.0	1.0000
A34	1	0.22	0	0.00	0.0	1.0000
A36	1	0.22	0	0.00	0.0	1.0000
A68	1	0.22	0	0.00	0.0	1.0000
A82	1	0.22	0	0.00	0.0	1.0000

* indicates statistical significance.

Table 3.2.3.2 HLA-B antigen frequencies in White glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 460	%	*n* = 24	%		*p* value
B3	1	0.22	0	0.00	0.0	1.0000
B5	1	0.22	0	0.00	0.0	1.0000
B7	82	17.83	3	12.50	0.7	0.5485
B8	59	12.83	5	20.80	1.6	0.3104
B11	1	0.22	0	0.00	0.0	1.0000
B12	3	0.65	0	0.00	0.0	1.0000
B13	6	1.30	0	0.00	0.0	1.0000
B14	24	5.22	0	0.00	0.0	0.6149
B15	15	3.26	1	4.20	1.3	0.5686
B16	2	0.43	1	4.20	9.6	0.1421
B17	3	0.65	0	0.00	0.0	1.0000
B18	13	2.83	3	12.50	4.4	0.0363*
B22	1	0.22	0	0.00	0.0	1.0000
B27	15	3.26	3	12.50	3.8	0.0499*
B29	1	0.22	0	0.00	0.0	1.0000
B35	34	7.39	1	4.20	0.6	1.0000
B37	4	0.87	1	4.20	4.8	0.2262
B38	7	1.52	0	0.00	0.0	1.0000
B39	9	1.96	2	8.30	4.3	0.0967
B40	7	1.52	3	12.50	8.2	0.0093*
B41	2	0.43	0	0.00	0.0	1.0000
B42	1	0.22	0	0.00	0.0	1.0000
B44	46	10.00	0	0.00	0.0	0.1299
B45	1	0.22	0	0.00	0.0	1.0000
B47	2	0.43	0	0.00	0.0	1.0000
B48	1	0.22	0	0.00	0.0	1.0000
B49	2	0.43	0	0.00	0.0	1.0000
B50	4	0.87	0	0.00	0.0	1.0000
B51	15	3.26	0	0.00	0.0	1.0000
B52	3	0.65	0	0.00	0.0	1.0000
B55	3	0.65	0	0.00	0.0	1.0000
B56	4	0.87	0	0.00	0.0	1.0000
B57	11	2.39	0	0.00	0.0	1.0000
B58	10	2.17	0	0.00	0.0	1.0000
B60	20	4.35	0	0.00	0.0	0.6061
B61	10	2.17	0	0.00	0.0	1.0000
B62	28	6.09	0	0.00	0.0	0.3698
B63	3	0.65	1	4.20	6.4	0.1851
B65	1	0.22	0	0.00	0.0	1.0000
B70	3	0.65	0	0.00	0.0	1.0000
B71	1	0.22	0	0.00	0.0	1.0000
B75	1	0.22	0	0.00	0.0	1.0000

* indicates statistical significance.

Table 3.2.3.3 HLA-DR antigen frequencies in White glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 460	%	*n* = 24	%		*p* value
Blank	62	13.48	0	0.00	0.0	0.0401*
DR1	36	7.83	3	12.50	0.2	0.4163
DR2	16	3.48	1	4.20	1.2	0.5915
DR3	56	12.17	7	29.00	2.4	0.0154*
DR4	69	15.00	4	16.70	1.1	0.7569
DR5	6	1.30	0	0.00	0.0	1.0000
DR6	10	2.17	0	0.00	0.0	1.0000
DR7	44	9.57	1	4.20	0.4	0.7017
DR8	13	2.83	1	4.20	1.5	0.5193
DR9	9	1.96	0	0.00	0.0	1.0000
DR10	7	1.52	0	0.00	0.0	1.0000
DR11	24	5.22	3	12.50	2.4	0.1374
DR12	10	2.17	0	0.00	0.0	1.0000
DR13	35	7.61	1	4.20	0.5	1.0000
DR14	9	1.95	0	0.00	0.0	1.0000
DR15	49	10.65	1	4.20	0.4	0.4683
DR16	5	1.09	2	8.30	7.7	0.0413*

* indicates statistical significance.

3.2.4 Combined Race Groups

Race groups were combined to determine if race was independent of GD.

The HLA-A locus of combined Race groups (Table 3.2.4.1) show an association between HLA-A2 and the control population (P= 0.0052; RR=0.68; 95% CI: 0.5106-0.9143) but when corrected for P, P_c=0.99. Another association was found between HLA-A74 and Race combined glomerulonephritides patients (P= 0.0350; RR=7.5; 95% CI: 0.7186-35.372). No significance was maintained when corrected for P (P_c=1). Two associations were noted between HLA-A33 (P<0.0001; RR=5.3; 95% CI: 2.724-10.401) and HLA-A68 (P<0.0001; RR=17.96; 95% CI: 6.832-47.232) and the Race combined glomerulonephritides patients. When corrected for P, both loci showed no significance (P_c=0.11)

The Race combined HLA-DR locus showed an association between Blank HLA-DR alleles and the Race combined control population (P<0.0001; RR=0; 95% CI: $-\infty$-∞) but P_c=0.11. Another association was found between HLA-DR11 (P=0.0137; RR=1.8; 95% CI: 1.172-2.884) and the Race combined glomerulonephritides patients. When corrected for P however, no association was maintained (P_c=0.99).

Table 3.2.4.1 HLA-A antigen frequencies of combined Race group glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 968	%	*n* =194	%		*p* value
Blank	4	0.41	0	0.00	0.00	1.0000
A1	122	12.60	22	11.34	0.90	0.6993
A2	241	24.90	33	17.01	0.68	0.0052*
A3	94	9.71	11	5.67	0.58	0.0611
A4	1	0.10	1	0.52	4.99	0.3063
A11	85	8.78	22	11.34	1.29	0.2513
A23	29	3.00	8	4.12	1.38	0.3698
A24	126	13.02	24	12.37	0.95	0.8989
A25	6	0.62	0	0.00	0.00	0.5960
A26	42	4.34	3	1.55	0.36	0.0623
A28	55	5.68	10	5.15	0.91	0.8612
A29	31	3.20	6	3.09	0.97	1.0000
A30	40	4.13	6	3.09	0.75	0.6797
A31	21	2.17	5	2.58	1.19	0.7868
A32	31	3.20	3	1.55	0.46	0.2430
A33	15	1.55	16	8.25	5.32	<0.0001*
A34	13	1.34	2	1.03	0.77	1.0000
A36	1	0.10	1	0.52	4.99	0.3063
A43	2	0.21	0	0.00	0.00	1.0000
A68	5	0.52	18	9.28	17.96	<0.0001*
A74	2	0.21	3	1.55	7.49	0.0350*
A80	1	0.10	0	0.00	0.00	1.0000
A82	1	0.10	0	0.00	0.00	1.0000

* indicates statistical significance.

Table 3.2.4.2 HLA-DR antigen frequencies of combined Race group glomerulonephritides patients and controls in KwaZulu-Natal.

	Controls		Patients		Relative Risk	
	n = 968	%	*n* =194	%		*p* value
Blank	76	7.85	0	0.00	0.00	<0.0001*
DR1	45	4.65	8	4.12	0.89	0.8485
DR2	53	5.48	9	4.64	0.85	0.7210
DR3	118	12.19	29	14.95	1.23	0.2522
DR4	127	13.12	25	12.89	0.98	1.0000
DR5	24	2.48	6	3.09	1.25	0.6155
DR6	20	2.07	5	2.58	1.25	0.5894
DR7	116	11.98	30	15.46	1.29	0.1591
DR8	25	2.58	5	2.58	1.00	1.0000
DR9	15	1.55	2	1.03	0.67	0.7509
DR10	27	2.79	7	3.61	1.29	0.4836
DR11	57	5.89	21	10.82	1.84	0.0137*
DR12	24	2.48	1	0.52	0.21	0.1001
DR13	74	7.64	12	6.19	0.81	0.5329
DR14	43	4.44	9	4.64	1.04	0.8471
DR15	118	12.19	24	12.37	1.02	1.0000
DR16	6	0.62	1	0.52	0.83	1.0000

* indicates statistical significance.

3.3 Part C: The Rarity of IgAN In Africans

This section involved the comparison of the combination of alleles from both the diseased and control populations of all race groups *viz.* African, Indian and White. Our KZN setting, concurrent with other settings, has shown that IgAN is very rare or non-existent in the African population. This is shown by Figure 3.3.1

The aim of this section was to investigate a possible underlying reason for the African race group to be protective against IgAN.

In comparing the A locus alleles (Figure 3.3.2) of the three race groups, the following was observed. Allele HLA-A30 was extremely significantly higher in the African population when compared to the Indian and White populations. African vs. Indian ($P<0.0001$, RR=9.1, 95% CI: 5.230-15.977) and African vs. White ($P<0.0001$, RR=5.7, 95% CI: 3.315-9.715). HLA-A29 was also significantly increased in the African vs. Indian ($P<0.0001$, RR=9.6, 95% CI: 4.770-19.486) and African vs. White ($P<0.0001$, RR=2.9, 95% CI: 1.740-4.785) populations. Another A locus allele, HLA-A34 showed to be extremely significant in Africans when compared to Indians ($P<0.0001$, RR=30.4, 95% CI: 7.303-126.28) and Whites ($P<0.0001$, RR=20.2, 95% CI: 4.865-83.865).

The B locus (Figure 3.3.3) showed extremely significant increases in HLA-B42, HLA-B58 and HLA-B70 in the African population. HLA-B42, Africans vs. Indians ($P<0.0001$, RR=∞, 95% CI: $-\infty$-∞) and Africans vs. Whites ($P<0.0001$, RR=10.1, 95% CI: 4.375-23.312). HLA-B58, Africans vs. Indians ($P<0.0001$, RR=4.7, 95% CI: 2.819-7.717) and Africans vs. Whites ($P<0.0001$, RR=4.4, 95% CI: 2.482-7.881). HLA-B70, Africans vs. Indians

(P<0.0001, RR=9.3, 95% CI: 4.784-18.185) and Africans vs. White (P<0.0001, RR=15.5, 95% CI: 5.655-42.537).

The DR locus (Figure 3.3.4) showed an extremely significant increase in HLA-DR11 in the African population when compared to Indians (P<0.0001, RR=5.4, 95% CI: 3.623-7.903) and Whites (P<0.0001, RR=6.1, 95% CI: 4.219-8.822).

P was not corrected intentionally. This was done because HLA loci were not compared to a particular disease rather the aim of this part of the study was to observe whether the African Race as a whole (diseased and normal) carried specific alleles that contributed to their protection from IgAN. A different form of analysis was carried out therefore P was not corrected as I was not aiming to find an association.

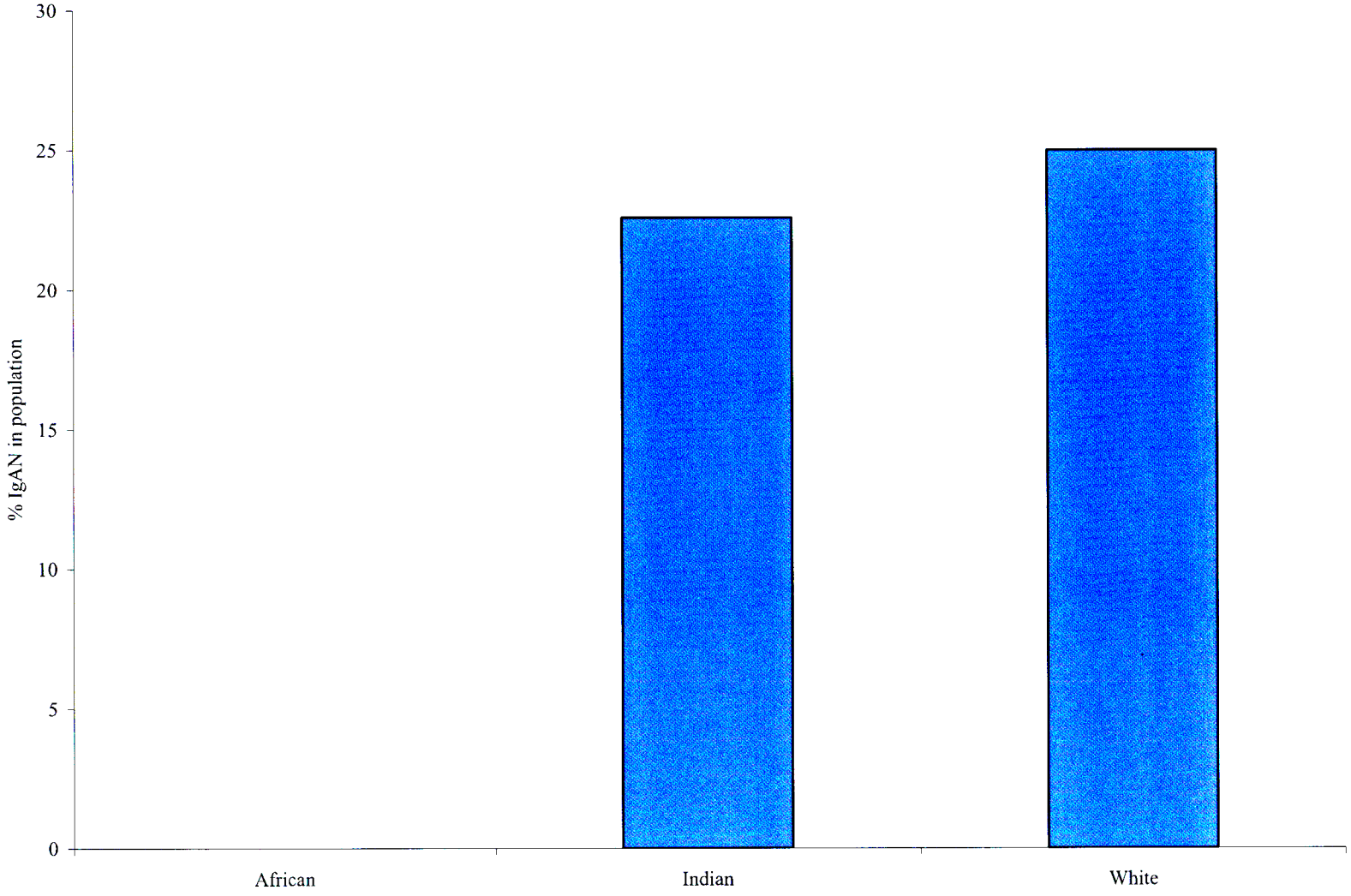

Figure 3.3.1 The prevalence of IgAN in three KZN populations *viz*. African, Indian and White.

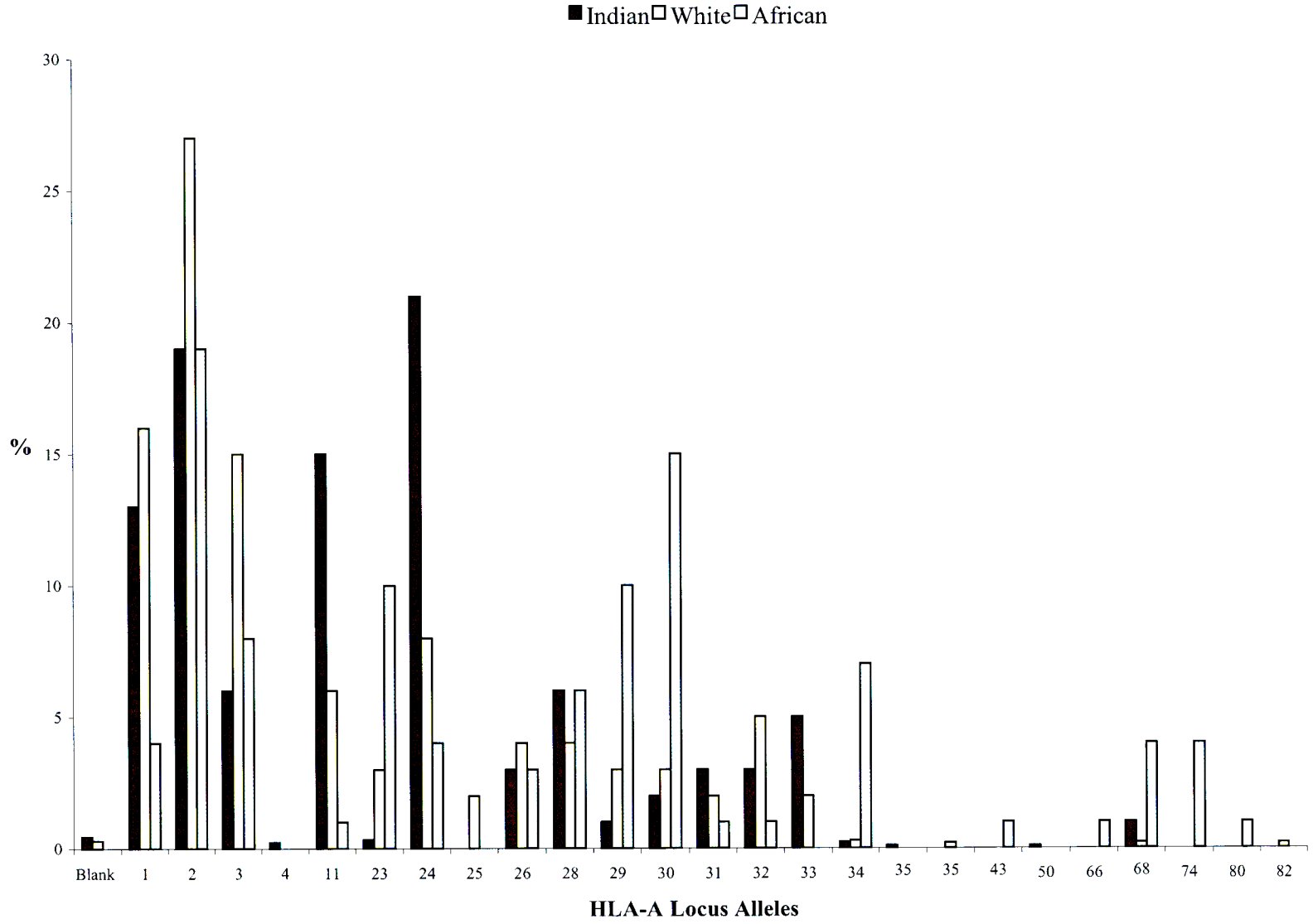

Figure 3.3.2 HLA-A combined (renal patients and normal controls) antigen frequencies of Indians, Whites and Africans in KwaZulu-Natal.

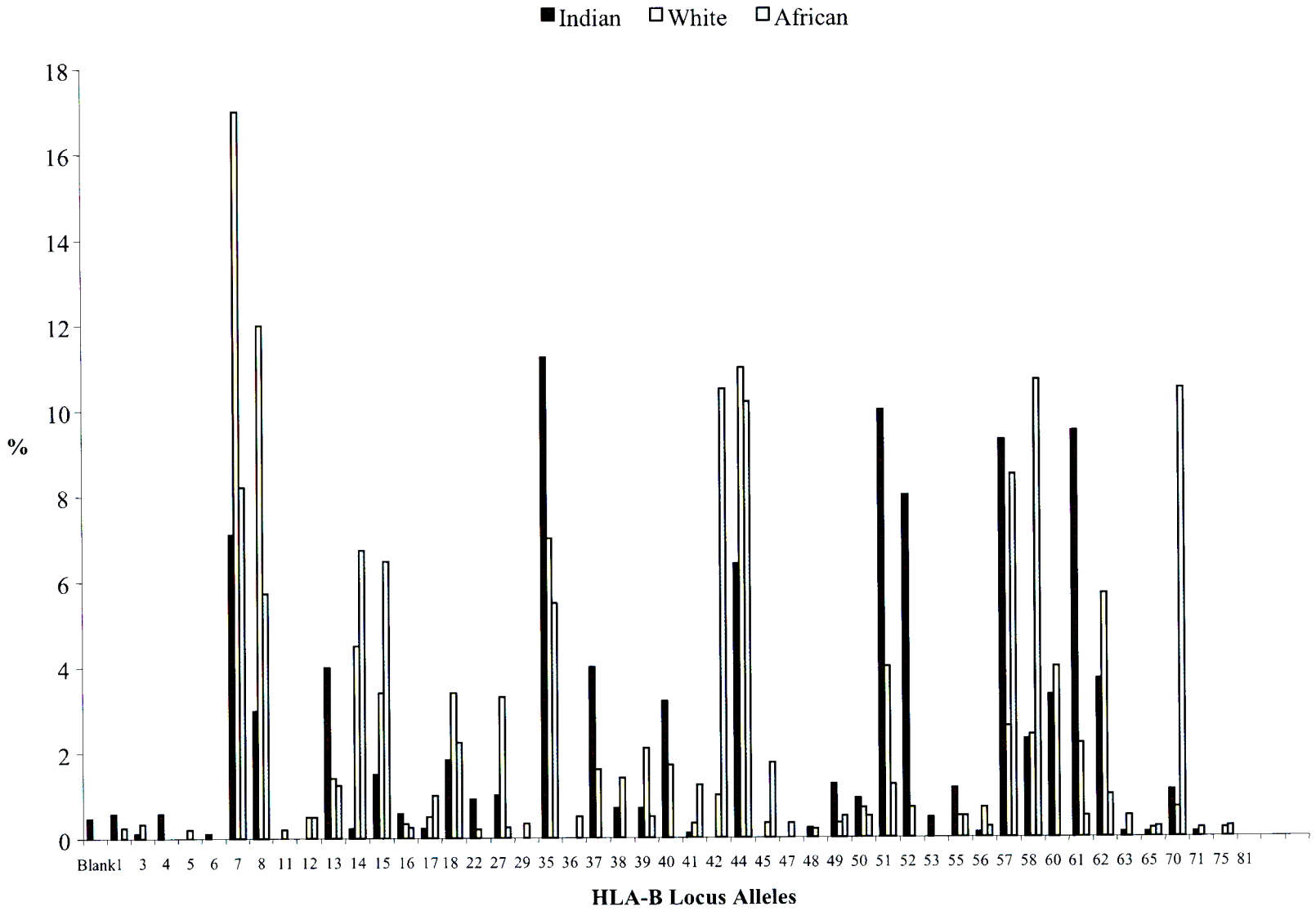

Figure 3.3.3 HLA-B combined (renal patients and normal controls) antigen frequencies of Indians, Whites and Africans in KwaZulu-Natal.

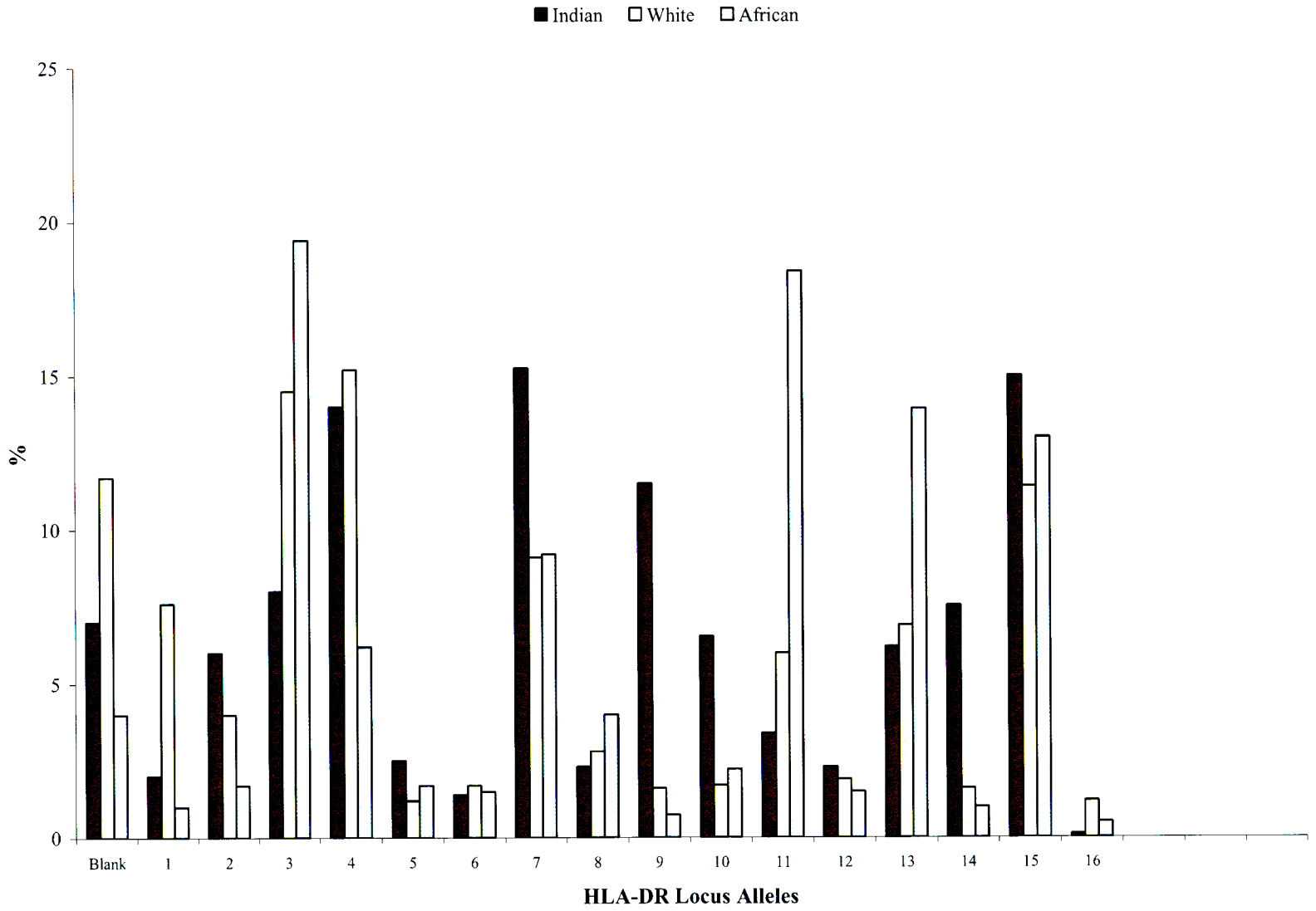

Figure 3.3.4 HLA-DR combined (renal patients and normal controls) antigen frequencies of Indians, Whites and Africans in KwaZulu-Natal.

DISCUSSION

This study is divided into three parts *viz* Part A, Part B and Part C.

The first part of this study deals with the association of the HLA antigens with hypertension in three different KZN populations *viz*. African, Indian and White.

The clinical data of this study (not published) indicates a high prevalence of hypertension in the KZN Indian population. In addition hypertension has been shown to be huge problem in India (Gupta *et. al*., 200) wherefrom South African Indians descend. When compared between two different geographically separated Indian populations *viz*. North Indian and South Indian, hypertension was shown to be more prevalent in the South Indian population despite a lower fat and salt intake (Malhotra, 1970). Diet therefore seemed to be independent of hypertension although the author suggested the influence of dietary factors on blood physiology.

Although two different Indian populations were studied, both responded differently to hypertension in spite of diet. This suggests that the geographic location might have an influence due to environmental factors. This is concurrent with the HLA principle where different populations in terms of race and geographic location respond differently to diseases.

This study showed a significant association between HLA-B40 and hypertension in the Indian population suggesting a possible disease susceptibility marker. A relative risk of

32.2 indicates that an Indian individual possessing the HLA-B40 allele will be 32.2 times more likely to develop hypertension than a person lacking the HLA-B40 allele.

During the apartheid era, natives (as Africans were known) were forced into small areas and the population increased by a ratio of 0.76 in a period of twenty years (Kuper, 1950). This increase in population was due to interbreeding, which resulted in overcrowding, poor socio-economic status and limited genetic diversity. Rural African men were therefore forced to leave their families to tend to the farms and move to urban areas, a process known as urbanization (Kothari and Mehta, 1984) in search of a 'better life' where they had to tend to their own nutritional needs. This is a drastic change from their healthy, low fat, low salt rural diets which did not comprise unhealthy processed foods. An unhealthy diet gradually leads to obesity which is associated with hypertension. Although a rural diet seems to be healthier, a stigma attached to African men in terms of their ability to provide for their families results in rural African women having a greater waist size (Puoane *et. al.*, 2002) hence being more susceptible to hypertension.

Unlike the inconsistency in the Indian population, hypertension seems to be constantly prominent among the African race group in various populations (McClellan *et. al.*, 1988; Tierny *et. al.*, 1989) and in spite of blood pressure control kidney function seems to decline (Rostand *et. al.*, 1989). In South Africa urbanization is said to increase the prevalence of hypertension in the African population by 25% (Steyn *et. al.*, 2001).

This study shows an association between HLA-B15 and hypertension in the KZN African population suggesting a possible disease susceptibility allele. Although different ethnicities these results correlate with a Swedish (Hilme *et. al.*, 1993) as well as an English population (Gudbrandsson *et. al.*, 1980). A relative risk of 9.4 indicates that KZN Africans with the HLA-B15 allele are 9.4 times more likely to develop hypertension than Africans without the allele.

No significant association was found between HLA and hypertension in the White population. This is due to a small sample size which makes it difficult to perform a statistical test or reach significance. There are two possible factors contributing to the small sample size.

The first could be due to diet. During the period of apartheid White South Africans had the privilege of education and private health care. This posed as an advantage as the White population were aware of what a healthy diet entailed although recently (post-apartheid) educated White women were still shown to have smaller waists than rural African women (Puoane *et. al.*, 2002). Waist size is a determinant of abdominal obesity which predisposes an individual to hypertension hence the smaller the waist size, the less likely to develop hypertension. South African Whites were shown to ingest a greater amount of sodium than Africans and still maintain a lower blood pressure (Charlton *et. al.*, 2005). This suggests that a healthier diet (White vs. urban Africans) can withstand a greater intake of salt. The South Indian population inversely correlates to the South African White population, i.e. an excessive salt intake in Whites results in a lower

prevalence of hypertension as compared to the South Indians where a reduced salt intake results in a higher prevalence of hypertension.

The next reason for a small White sample size could be socio-economic. The supremacy stigma attached to the White population during apartheid still lingers. Whites were said to be more superior hence high up on the hierarchy. Due to their superiority, they are accustomed to better living conditions which attributes to a stronger immune system due to proper sanitation and good nutrition. The wealth attached to supremacy allows private health care (Kothari and Mehta, 1984) where education about proper diet is available (Charlton *et. al.*, 2005). White patients are therefore rarely found in public health care facilities and as a consequence little access is made to them.

Although an association with hypertension was not detected in this White population, HLA-DR4 was shown to be associated with hypertensive Brazilian Caucasians (Gerbase-DeLima *et. al.*, 1992).

Part B of this study aimed to find associations between HLA and glomerulonephritides. The initial glomerular diseases selected were IgAN, MGN and FSGS but due to inadequate numbers, IgAN, MGN and FSGS had to be grouped as glomerulonephritides together with other NS diseases. This may present a bias as there is a vast array of glomerular diseases hence patients with specific diseases may present with specific HLA antigens.

HLA profiles differ between race and geographic location but studies have also aimed to prove associations between the severity of renal disease and the race of a population. FSGS has been on the increase globally (Balakrishnan *et al.*, 2003; Bhimma *et. al.*, 2006; Sorof *et. al.*, 1998) and has also replaced MGN as being the most common cause of NS (Pontier and Patel, 1994; Sorof *et. al.*, 1998; Swaminathan *et. al.*, 2006).

FSGS has been shown to be more common in Black populations compared to White and Hispanic populations (Bonilla-Felix *et. al.*, 1990) although Dragovic *et. al.* (2005) showed no difference between the increase of hypertension in Blacks, Whites and Hispanics. In addition FSGS is more common in males than females (Korbet *et. al.*, 1996). This setting showed a similar increase of FSGS in Black African and Indian children with a trend towards the male sex (Bhimma *et. al.*, 2006). Similarly, clinical data from this study shows a comparable prevalence of FSGS between Indians and Africans. Authors have suggested that the increase of FSGS is due to factors other than race (Swaminathan *et. al.*, 2006). Although a study failed to show any HLA associations with FSGS (Freedman *et. al.*, 1994b) there has been a confirmed association with HLA antigens (Gerbase-DeLima *et. al.*, 1998).

The present study showed a significant association with HLA-A33 and glomerulonephritides in the Indian adult population with a relative risk of 4.4. This denotes that Indians carrying the HLA-A33 antigen are 4.4 more likely to develop glomerulonephritides than those not bearing the allele. The HLA-A locus alleles are

Class I alleles. These results challenge other studies where Class II HLA associations were identified.

HLA-DR3 was shown to be associated with NS in German Caucasian children (Ruder *et. al.*, 1990) and with Idiopathic MN in an English (Klouda *et. al.*, 1979) as well as an American population together with HLA DR5 (Freedman *et. al.*, 1994a).

Alfiler *et. al.* (1980) showed an association between HLA-DR*w*7 and Steroid-responsive NS in Australian Caucasian children. The Class II HLA-DR4 allele has been shown to be associated with Idiopathic FSGS patients from a Brazilian White population (Gerbase-DeLima *et. al.*, 1998) as well as a Polish population (Krasowska-Kwiecien *et. al.*, 2001).

In this setting the Class II HLA DQB1*0603 has been shown to be associated with HBVMN in the African pediatric population (Bhimma *et. al.*, 2002). Interestingly Class I associations were found in this setting. HLA-B*w*44 was shown to be associated with MCNS in the Indian population and HLA-B*w*21 with MN in the African pediatric population (Adhikari *et. al.*, 1985).

A Race combined analysis has been carried out in an American setting with significant associations (Freedman *et. al.*, 1994b). This study showed positive associations between HLA-A74, HLA-A33, HLA-A68 and HLA-DR11 and Race-combined glomerulonephritides patients however when corrected for *P*, no significance was

maintained. This suggests that Race plays a role in determining susceptibility to glomerulonephritides in KZN.

Although IgAN is the leading cause of GN (Swaminathan *et. al.*, 2006) it is a disease that is uncommon in the African race although GN accounts for 25% of the African renal population (Seedat *et. al.*, 1984). Seedat *et. al.* (1988) found 2 cases of IgAN in an African population of 252 patients in KZN and Jennette and colleagues (1985) reported 6 cases out of a total of 461 African American patients. A study with the greatest recorded sample size of African IgAN patients (Crowley-Nowick *et. al.*, 1991) revealed the following: 1) African IgAN patients progress to ESRD at the same pace as White patients with a similar clinical course and 2) The IgA2 (A2m(1) and A2m(2)) allotypes do not serve as markers for or protect the African race from IgAN.

IgAN is also uncommon in this African population. The clinical data reveal only 3 patients with IgAN from an excess of 500 patients. Unfortunately due to lack of follow up samples were not collected from these patients. Follow up is very important as IgAN patients are suggested to be a high risk group (Freedman *et. al.*, 1994b). Since the IgA immune system was unable to clarify reasons for the African population being protected from IgAN (Crowley-Nowick *et. al.*, 1991), I decided to explore the HLA complex genes for a possible reason.

HLA-A30, HLA-A29, HLA-A34, HLA-B70, HLA-B58, HLA-B42 and HLA-DR11 were shown to be extremely significantly higher in the African population when compared to

the Indian and White populations. These results correspond to Assounga *et. al.* (2007, Congress Abstract) who also showed a marked increase of HLA-A30 and HLA-B58 in African patients when compared to White and Indian populations. In White Americans HLA-B27 and HLA-DR1 occur in linkage disequilibrium. The susceptibility of Whites to IgAN is suggested to stem directly or from extended haplotypes of HLA-B27 and HLA-DR1 (Freedman *et. al.*, 1994b). This triggers the converse question as to whether HLA-A30 and HLA-B58 in linkage in this environment serve as protective loci for Africans against IgAN but further in depth studies are required to aim to prove that phenomenon. Also HLA-A33 was shown to be represented by the White and Indian populations but absent in the African population. This deficiency may suggest that HLA-A33 is a possible protective allele.

It is also interesting to note that although IgAN is common in the White population (Swaminathan *et. al.*, 2006), only three cases were found in this patient population but this could be due to White patients attending private health care facilities. Seedat *et. al.* (1984) showed analgesic nephropathy to be the most common cause of ESRD in KZN White patients compared to GN and hypertension. Due to the small sample size of White patients, this study was intended to expand into the Pietermaritzburg region where White patients are said to be common. However attempts made to approach the hospital (Grey's) were unfruitful.

HLA association studies with IgAN have been performed in various settings. A study by Li *et. al.* (1991) showed an association between HLA-DQ*w*7 and IgAN in Caucasians.

Doxiadis *et. al.* (2001) showed HLA-B35 to be increased in IgAN patients on the transplant list. It is interesting to note that race was not mentioned so it cannot be concluded if this association was race determined. Freedman *et. al.* (1994b) reported positive (HLA-B27 and HLA-DR1) as well as negative (HLA-DR2) HLA associations with IgAN proposing that HLA-B27 and HLA-DR1 are disease susceptible alleles and HLA-DR2 is a protective allele. Alternatively, Fennessy *et. al.* (1996) and Huang *et. al.* (1989) noted no association with HLA.

HLA differs between race and geographic locations. In the Japanese population, IgA nephropathy has been shown to be associated with HLA-DQ*w*4 (Hiki *et. al.*, 1991) and has more than once been shown to be associated with HLA-DR4 (Hiki *et. al.*, 1982; Hiki *et. al.*, 1990; Kashiwabara *et. al.*, 1982; Kohara *et. al.*, 1985; Nomoto *et. al.*, 1984) directly or otherwise. Interestingly Fauchet *et. al.* (1980) showed an association of HLA-DR4 with IgAN.

Furthermore alleles of the central MHC (D6S273*133 and D6S273*131) have been shown to be strongly associated with the levels of IgA suggesting a possible predisposition to IgAN as IgA deficiency (IgAD) patients lacked central MHC alleles present in patients with IgAN (Matthews *et. al.*, 2002).

Unfortunately this study could not provide any associations with IgAN due to the small sample size. IgAN is common in KZN Indians and by increasing the sample size in future studies, significance might be obtained.

Although members of the African race group are said to reach ESRD more rapidly than any other race group, they rarely tend to donate kidneys. This is postulated to be associated with traditional beliefs. The donor list comprised of 67 African donors compared to 187 Indian and 230 White donors. This presents a great difficulty as HLA profiles are unique to populations and HLA matching is important in transplantation due to graft rejection. Assounga *et al.* (2007, Congress Abstract) suggests each population group should contribute equally to the donor pool to improve the donor/recipient matching.

The following limitations were presented by the study.

1) HLA compatibility is of utmost importance in organ transplant as it determines graft rejection. The donor and recipient therefore need to have a close HLA match. Since the donor population on the transplant register was used as the control population a bias might be presented. This study therefore needs to be extended to a wider control population.

2) This study may not reflect the total distribution of HLA types in the KZN populations as the transplant registry is not complete. According to the South African Department of Health website (http://www.doh.gov.za), this is due to lack of funds.

3) Due to the presumptive diagnosis of hypertension in patients, this study needs to be conducted in a population where the diagnosis of hypertension is known to be due to renal diseases.

4) This study involved HLA associations with glomerulonephritdes. Due to the extensive classification of glomerular diseases, HLA molecules in patients with these diseases may present different antigens. Therefore future studies should involve specific classifications (i.e. groups of patients with identical biopsy proven diseases).

4) This study presented challenges in the face of statistical analysis as a very small sample size has a few HLA antigens as opposed to the extensive array of alleles from a great population. It therefore becomes difficult to compare individual alleles with a large control population. An increase in sample size is therefore warranted.

5) HLA typing is very expensive therefore using it as a method of screening would be impractical in a poor setting. Therefore funding should be allocated for screening tests in provincial hospitals. The way forward would be to encourage the government to prioritize and provide resources for renal disease for although the kidney is the most precious vital organ it is often taken for granted.

CONCLUSION

In summary, HLA-B40 is a possible disease susceptibility marker for Hypertension in KZN Indian renal patients. Consistent with other studies HLA-B15 is shown to be associated with Hypertension in African KZN renal patients. White hypertensives are few hence no association was found with HLA. IgAN has been shown to be very rare in Africans in KZN. This is in accordance with other investigations. A possible linkage between HLA-A30 and HLA-B58 in this setting could be responsible for IgAN being uncommon among Africans but further studies are required. Glomerulonephritides is associated with HLA-A33 in the KZN Indian population with no associations among the African and White populations.

REFERENCES

ABBAS, A.K. AND LICHTMAN, A.H. 2003. The Major Histocompatability Complex, p.65-80. *In*: J. Malley and H. Krehling (eds.), Cellular and Molecular Immunology, 5th Edition. Elsevier Science. USA.

ADAMS, S.D., BARRACCHINI, K.C., CHEN, D., ROBBINS, F., WANG, L., LARSEN, P., LUHM, R. AND STRONECK, D.F. 2004. Ambiguous Allele Combinations In HLA Class I and Class II Sequence-Based Typing: When Precise Nucleotide Sequencing Leads To Imprecise Allele Identification. *Journal of Translational Medicine*. **2:**30-35.

ADHIKARI, M., COOVADIA, H.M. AND HAMMOND, M.G. 1985. Associations Between HLA Antigens and Nephrotic Syndrome In African and Indian Children In South Africa. *Nephron*. **41:**289-292.

AGNANI, S., VACHHARAJANI, V.T., GUPTA, R., ATRAY, N.K. AND VACHHARAJANI, T.J. 2005. Does Treating Obesity Stabilize Chronic Kidney Disease? *Biomed Central Nephrology*. **6:**7-10.

AKAGI, H., FUKUSHIMA, K., KOSAKA, M., DOI, A., OKANO, M., KARIYA, S., NISHIZAKI, K. AND MASUDA, Y. 2003. A 10 Year Retrospective Case-Control Study For IgA Nephropathy After Tonsillectomy. *International Congress Series*. **1257:**147-150.

AKIOKA, K., OKAMOTO, M., USHIGOME, H., KADOTANI, Y., OGINO, S., HIGUCHI, A., WAKABAYASHI, Y., KAIHARA, S. AND YOSHIMURA, M. 2004. Recurrence of Focal Glomerulosclerosis In Post-Renal Transplant Recipients: Report of Two Cases. *Transplantation Proceedings*. **36:**2167-2168.

AKIOKA, K., OKAMOTO, M., WAKABAYASHI, Y., NOBORI, S., OGINO, S., USHIGOME, H., URYUHARA, K., KAIHARA, S. AND YOSHIMURA, N. 2006. Long-Term Outcome of Renal Transplantation In Focal Glomerulosclerosis. *Transplantation Proceedings*. **38:**2819-2822.

ALFILER, C.A., ROY, L.P., DORAN, T., SHELDON, A. AND BASHIR, H. 1980. HLA-DRw7 and Steroid-Responsive Nephrotic Syndrome of Childhood. *Clinical Nephrology*. **14:**72-74.

ALPER, C.A., LARSEN, C.E., DUBEY, D.P., AWDEH, Z.L., FICI, D.A., AND YUNIS, E.J. 2006. The Haplotype Structure of The Human Major Histocompatibility Complex. *Human Immunology*. **67:**73-84.

ALPERS, C.E. 2005. The Kidney, p. 955-1021. *In*: V. Kumar, A.K. Abbas and N. Fausto (eds.), Robbins and Cotran, Pathologic Basis of Disease 7th Edition. Elsevier Saunders. Philadelphia.

ALTIPARMAK, R.M., PAMUK, Ö.N., PAMUK, G.E. AND ÖZBAY, G. 2003. Membranous Glomerulonephritis In a Patient With Choriocarcinoma: A Case Report. *Southern Medical Journal.* **96:**198-200.

ALTSCHULER, E.L. 2001. Consideration of Vaccination Against The FSGS Factor. *Medical Hypotheses.* **57:**703-704.

ALVARADO-GUERRI, R., CABRERA, C.M., GARRIDO, F. AND LÓPEZ-NEVOT. 2005. TAP1 and TAP2 Polymorphisms and Their Linkage Disequilibrium With HLA-DR, -DP and –DQ In An Eastern Andalusian Population. *Human Immunology.* **66:**921-930.

ANONYMOUS. 2007a. IMGT/HLA Database, Statistics. *In*: http://www.ebi.ac.uk/imgt/hla/stats.html. Date accessed: 6 August 2007.

ANONYMOUS. 2007b. Soluble HLA Revisited. *Leukemia Research.* **31:**121-125.

ASSOUNGA, A., SCHREIBER, V., ALLEN, V. AND ANLEY, D. 2007. Diversity In HLA Class I and Class II Kidney Donors and Recipients According to Race In KwaZulu-Natal – Implications For Transplantation. [Congress Abstract]. Nelson R. Mandela School of Medicine AstraZeneca Faculty Research Day. 11-12th September 2007.

AUSTYN, J.M. AND WOOD, K.J. 1993. The Major Histocompatibility Complex, p.63-114. *In*: Principles of Cellular and Molecular Immunology. Oxford University Press Inc. USA.

BALAKRISHNAN, N., KORULA, J.A., VISALAKSHI, A., TALAULIKAR, G.S., THOMAS, P.P. AND JACOB, C.K. 2003. Spectrum of Biopsy Proven Renal Disease and Changing Trends At a Tropical Tertiary Care Centre. *Indian Journal of Nephrology.* **13:**29-35.

BARISONI, L., KRIZ, W., MUNDEL, P. AND D'AGATI, V. 1999. The Dysregulated Podocyte Phenotype: A Novel Concept In The Pathogenesis of Collapsing Idiopathic Focal Segmental Glomerulosclerosis and HIV-Associated Nephropathy. *Journal of The American Society of Nephrology.* **10:**51-61.

BARRETT, J.T. 1988. The Major Histocompatibility Complex, p.76-83. *In*: S. Bircher (ed.), Textbook of Immunology, Fifth Edition. The C.V Mosby Company. USA.

BASSETT, M.L., HALLIDAY, J.W., POWELL, L.W., DORAN, T. AND BASHIR, H. 1979. Early Detection of Idiopathic Haemochromatosis: Relative Value of Serum-Ferritin and HLA Typing. *The Lancet*. 4-7.

BATES, W.D., MULLER, N., van BUUREN, A.J. AND STEYN, D.W. 1996. Pregnancy In Partially Remitted Hepatitis B-associated Membranous Glomerulonephritis. *International Journal of Gynecology and Obstetrics*. **52:**163-165.

BATTAGLIA, M. AND GORSKI, J. 2002. Overlap of Direct and Indirect Alloreactive T-Cell Repertoires When MHC Polymorphism Is Limited To The Peptide Binding Groove. *Human Immunology*. **63:**91-100.

BECK, S., GERAGHTY, D., INOKO, H. AND ROWEN, L. 1999. Complete Sequence and Gene Map of a Human Major Histocompatibility Complex. *Nature*. **401:**921-923.

BENNETTS, B.H., TEUTSCH, S.M., McBUHLER, M., HEARD, R.N.S. AND STEWART, G.J. 1999. HLA-DMB Gene and HLA-DRA Promoter Region Polymorphisms In Australian Multiple Sclerosis Patients. *Human Immunology*. **60:**886-893.

BERGER, J. AND HINGLAIS, N. 1968. Les Depots Intercapillaires d'IgA-IgG. *Journal of Urology and Nephrology*. **74:**694-695.

BETTINOTTI, M.P., HADZIKADIC, L., RUPPE, E., DHILLON, G., STRONCEK, D.S. AND MARINCOLA, F.M. 2003. New HLA-A, -B, and -C Locus-Specific Primers For PCR Amplification From cDNA: Application In Clinical Immunology. *Journal of Immunological Methods*. **279:**143-148.

BHIMMA, R., ADHIKARI, M. AND ASHARAM, K. 2006. Steroid-Resistant Nephrotic Syndrome: The Influence of Race on Cyclophosphamide Sensitivity. *Pediatric Nephrology*. **21:**1847-1853.

BHIMMA, R., HAMMOND, M.G., COOVADIA, H.M., ADHIKARI, M. AND CONNOLLY, C.A. 2002. HLA Class I and II In Black Children With Hepatitis B Virus-Associated Membranous Nephropathy. *Kidney International*. **61:**1510-1515.

BIAN, S., TAN, P.H., CHUAH, K.L., CHENG, C. AND TAN, J. 2003. A Case of Juxtaglomerular Cell Tumor Associated With Membranous Glomerulonephritis. *Annals of Diagnostic Pathology*. **7:**314-320.

BJORKMAN, P.J., SAPER, M.A., SAMRAOUI, B., BENNETT, W.S., STROMINGER, J.L. AND WILEY, D.C. 1987. Structure of The Human Class I Histocompatibility Antigen, HLA-A2. *Nature.* **329:**507-512.

BOARDMAN, R., TROFE, J., ALLOWAY, R., ROGERS, C., ROY-CHAUDHURY, P., CARDI, M., SAFDAR, S., GROENE, B., BUELL, J., HANAWAY, M., THOMAS, M., ALEXANDER, W., MUNDA, R. AND WOODLE, E.S. 2005. Early Steroid Withdrawal Does Not Increase Risk For Recurrent Focal Segmental Glomerulosclerosis. *Transplantation Proceedings.* **37:**817-818.

BONTROP, R.E. 2006. Comparative Genetics of MHC Polymorphisms In Different Primate Species: Duplications and Deletions. *Human Immunology.* **67:**388-397.

BONTROP, R.E. AND WATKINS, D.I. 2005. MHC Polymorphism: AIDS Susceptibility In Non-Human Primates. *Trends In Immunology.* **26:**227-233.

BORREGO, F., ULBRECHT, M., WEISS, E.H., COLIGAN, J.E. AND BROOKS, A.G. 1998. Recognition of Human Histocompatibility Leukocyte Antigen (HLA)-E Complexed with HLA Class I Signal Sequence-Derived Peptides by CD94/NKG2 Confers Protection From Natural Killer Cell-Mediated Lysis. *Journal of Experimental Medicine.* **187:**813-818.

BRADY, H.R., O'MEARA, Y.M. AND BRENNER, B.M. 2005. Glomerular Diseases, p. 1674-1694. *In*: D.L. Kasper, E. Braunwald, A.S. Fauci, S.L. Hauser, D.L. Longo and J.L. Jameson (eds.), Harrison's Principles of Internal Medicine, 16th Edition Volume II. McGraw-Hill Companies Inc. USA.

BRAKE, M. AND SOMERS, D. 2006. IgA Nephropathy. *In*: http://www.emedicine.com/med/TOPIC886.HTM. Date accessed: 26 March 2007.

BRAUD, V.M., ALLAN, D.S.J., O'CALLAGHAN, C.A., SÖDERSTRÖM, K., D'ANDREA, A., OGG, G.S., LAZETIC, S., YOUNG, N.T., BELL, J.I., PHILLIPS, J.H., LANIER, L.L. AND McMICHAEL, A.J. 1998. HLA-E Binds To Natural Killer Cell Receptors. *Nature.* **391:**795-798.

BRAUN, W.E. 1979. Introduction to The HLA Antigen System, p.1-11. *In*: HLA and Disease: A Comprehensive Review. CRC Press, Inc., USA.

BRODSKY, F.M. 2001. Antigen Presentation and The Major Histocompatibility Complex, p.82-94. *In*: T.G. Parslow, D.P. Sites, A.I. Terr and J.B. Imboden (eds.), Medical Immunology. The McGraw-Hill Companies, Inc. USA.

BROOKS, A.G., BORREGO, F., POSCH, P.E., PATAMAWENU, A., SCORZELLI, C.J., ULBRECHT, M., WEISS. E.H. AND COLIGAN, J.E. 1999. Specific Recognition of HLA-E, But Not Classical, HLA Class I Molecules By Soluble CD94/NKG2A and NK Cells. *Journal of Immunology.* **162:**305-313.

BROWN, J.H., JARDETZKY, T.S., GORGA, J.C., STERN, L.J., URBAN, R.G., STROMINGER, J.L. AND WILEY, D.C. 1993. Three-Dimensional Structure of The Human Class II Histocompatibility Antigen HLA-DR1. *Nature.* **364:**33-39.

BUCKLEY, R.H. 2003. Transplantation Immunology: Organ and Bone Marrow. *Journal of Allergy and Clinical Immunology.* **111:**S733-744.

CARIDI, G., DAGNINO, M., SANNA-CHERCHI, S. PERFUMO, F. AND GHIGGERI, G.M. 2006. Podocin Related Mechanisms In Posttransplantation Recurrence of Focal Segmental Glomerulosclerosis. *Transplantation Proceedings.* **38:**3486-3490.

CARUSO, C., CANDORE, G., ROMANO, G. C., LIO, D., BONAFÈ, M., VALENSIN, S. AND FRANCESCHI, C. 2001. Immunogenetics of Longevity. Is Major Histocompatibility Complex Polymorphism Relevant To The Control of Human Longevity? A Review of Literature Data. *Mechanisms of Ageing and Development.* **122:**445-462.

CATTRAN, D.C. 2003. Cyclosporine In The Treatment of Idiopathic Focal Segmental Glomerulosclerosis. *Seminars In Nephrology.* **23:**234-241.

CHARLTON, K.E., PHIL, M., STEYN, K., LEVITT, N.S., ZULU, J.V., CUR, B., JONATHAN, D., R.N., FREDERICK, J.V. AND NEL, J.H. 2005. Diet and Blood Pressure In South Africa: Intake of Foods Containing Sodium, Potassium, Calcium and Magnesium In Three Ethnic Groups. *Nutrition.* **21:**39-50.

CHEN, A-N., HWANG, J-C., GUH, J-Y., CHANG, J-M., LAI, Y-H. AND CHEN, H-C. 2006. Reduced Podocyte Expression of $\alpha_1\beta_3$ Integrins and Podocyte Depletion In Patients With Primary Focal Segmental Glomerulosclerosis and Chronic PAN-Treated Rats. *Journal of Laboratory and Clinical Medicine.* **147:**74-82.

CHEVRIER, D., GIRAL, M., MULLER, J.Y., BIGNON, J.D AND SOULILLOU, J.P. 1998. Impact of The MHC-Encoded HLA-DMA, DMB and LMP2 Gene Polymorphisms on Kidney Graft Outcome. *Human Immunology.* **59:**650-655.

CLARKSON, A.R., ELIAS, T.J., FAULL, R.J. AND BANNISTER, K.M. 1999. Immunoglobulin A Nephropathy and Renal Transplantation. *Transplantation Reviews*. **13:**174-182.

CONNOLLY, J.O., WESTON, C.E. AND HENDRY, B.M. 1995. HIV-Associated Renal Disease In London Hospitals. *Quarterly Journal of Medicine*. **88:**627-634.

COUSER, W.G. 1999. Glomerulonephritis. *The Lancet*. **353:**1509-1515.

CROWLEY-NOWICK, P.A., JULIAN, B.A., WYATT, R.J., GALLA, J.H., WALL, B.M., WARNOCK, D.G., MESTECKY, J. AND JACKSON, S. 1991. IgA Nephropathy In Blacks: Studies of IgA2 Allotypes and Clinical Course. *Kidney International*. **39:**1218-1224.

DAY, C.J, COCKWELL, P., LIPKIN, G.W., SAVAGE, C.O.S., HOWIE, A.J. AND ADU, D. 2002. Mycophenolate Mofetil In the Treatment of Resistant Idiopathic Nephrotic Syndrome. *Nephrology Dialysis Transplantation*. **17:**2011-2013.

De SIATI, L., PAROLI, M., FERRI, C., MUDA, A.O., BRUNO, G. AND BARNABA, V. 1999. Immunoglobulin A Nephropathy Complicating Pulmonary Tuberculosis. *Annals of Diagnostic Pathology*. **3:**300-303.

De WARDENER, H.E. 1981. Structure of The Kidney, p. 1-11. *In*: The Kidney, An Outline of Normal and Abnormal Function, Fifth Edition. Churchill Livingstone. USA.

DICK, T.P., BANGIA, N., PEAPER, D.R. AND CRESSWELL, P. 2002. Disulfide Bond Isomerization and The Assembly of MHC Class I-Peptide Complexes. *Immunity*. **16:**87-98.

DILLON, J.J. 1997. Fish Oil Therapy For IgA Nephropathy: Efficacy and Interstudy Variability. *Journal of The American Society of Nephrology*. **8:**1739-1744.

DONADIO, J.V., BERGSTRALH, E.J., OFFORD, K.P., SPENCER, D.C. AND HOLLEY, K.E. 1994. A Controlled Trial of Fish Oil In IgA Nephropathy. *New England Journal of Medicine*. **331:**1194-1199.

DONADIO, J.V. AND GRANDE, J.P. 1997. Immunoglobulin A Nephropathy: A Clinical Perspective. *Journal of The American Society of Nephrology*. 1324-1332.

DONADIO, J.V. AND GRANDE, J.P. 2002. IgA Nephropathy. *New England Journal of Medicine.* **347:**738-748.

DOXIADIS, I.I.N., De LANGE, P., De VRIES, E., PERSIJN, G.G. AND CLAAS, F.H.J. 2001. Protective and Susceptible HLA Polymorphisms In IgA Nephropathy Patients With End-Stage Renal Failure. *Tissue Antigens.* **57:**344-347.

DRAKE, R.L., VOGL, W. AND MITCHELL A.W.M. 2005. Abdomen, p. 220-362. *In*: B. Schmitt and L. van den Berghe (eds.), Gray's Anatomy For Students. Elsevier. Spain.

DUNCAN, N., DHAYGUDE, A., OWEN, J., CAIRNS, T.D.H., GRIFFITH, M., McLEAN, A.G., PALMER, A. AND TAUBE, D. 2004. Treatment of Focal and Segmental Glomerulosclerosis In Adults with Tacrolimus Monotherapy. *Nephrology Dialysis Transplantation.* **19:**3062-3067.

EDWARDS, S.V. AND HEDRICK, P.W. 1988. Evolution and Ecology of MHC Molecules: From Genomics to Sexual Selection. *TREE.* **13:**305-311.

ELLIS, P.A., REDDY, V., BARI, N. AND CAIRNS, H.S. 1998. Late Referral of End Stage Renal Failure. *Quarterly Journal of Medicine.* **91:**727-732.

EMSLEY, H.C.A. AND MOLLOY, J. 2002. Inflammatory Demyelinating Polyradiculoneuropathy Associated With Membranous Glomerulonephritis and Thrombocytopaenia. *Clinical Neurology and Neurosurgery.* **105:**23-26.

EVANS, D.J., MACANOVIC, M., DUNN, M.J. AND PUSEY, C.D. 2003. Membranous Glomerulonephritis Associated With Follicular B-Cell Lymphoma and Subepithelial Deposition of IgG1-κ Paraprotein. *Nephron Clinical Practice.* **93:**c112-c118.

FALK, R.J., JENNETTE, J.C. AND NACHMAN. P.H. 2000. Primary Glomerular Disease, p. 1263-1349. *In*: B.M. Brenner (ed.), Brenner and Rectors: The Kidney, 6th Edition. W.B. Saunders Company. Philadelphia.

FANG, J., CHEN, Y. AND HSUEH, S. 2001. Lymphatic Tumor Emboli of Perirenal Fat In Patient With Nephrotic Syndrome Receiving Renal Biopsy, Ultimately Received Gastric Adenocarcinoma With Membranous Glomerulonephritis. *Renal Failure.* **23:**743-748.

FAUCHET, R., Le POGAMP, P., GENETET, B., CHEVET, D., GUEGUEN, M., SIMON, P., RAMEE, M.P. AND CARTIER, F. 1980. HLA-DR4 Antigen and IgA Nephropathy. *Tissue Antigens.* **16:**405-410. [Abstract]

FAULKNER, L., BORYSIEWICZ, L.K AND MAN, S. 1998. The Use of Human Leukocyte Antigen Class I Transgenic Mice To Investigate Human Immune Function. *Journal of Immunological Methods.* **221:**1-16.

FENNESSY, M., HITMAN, G.A., MOORE, R.H., METCALFE, K., MEDCRAFT, J., SINICO, R.A., MUSTONEN, J.T AND D'AMICO, G. 1996. HLA-DQ Gene Polymorphism In Primary IgA Nephropathy In Three European Populations. *Kidney International.* **49:**477-480.

FINE, R.N. 2007. Recurrence of Nephrotic Syndrome/Focal Segmental Glomerulosclerosis Following Renal Transplantation In Children. *Pediatric Nephrology.* **22:**496-502.

FLOEGE, J., BURG, M. AND KLIEM, V. 1998. Recurrent IgA Nephropathy After Kidney Transplantation: Not a Benign Condition. *Nephrology Dialysis Transplantation.* **13:**1933-1935.

FLOEGE, J. AND FEEHALLY, J. 2000. IgA Nephropathy: Recent Developments. *Journal of The American Society of Nephrology.* **11:**2395-2403.

FREEDMAN, B.I., SPRAY, B.J., DUNSTON, G.M. AND HEISE, E.R. 1994a. HLA Associations In End-Stage Renal Disease Due To Membranous Glomerulonephritis: HLA-DR3 Associations With Progressive Renal Injury. *American Journal of Kidney Diseases.* **23:**797-802.

FREEDMAN, B.I., SPRAY, B.J. AND HEISE, E.R. 1994b. HLA Associations In IgA Nephropathy and Focal and Segmental Glomerulosclerosis. *American Journal of Kidney Diseases.* **23:**352-357.

FRIESE, M.A., JONES, E.Y. AND FUGGER, L. 2005. MHC II Molecules In Inflammatory Diseases: Interplay of Qualities and Quantities. *Trends In Immunology.* **26:**559-561.

FURNESS, P.N., STITSON, R.N.M. AND ROBERTS, I.S.D. 2007. Difficult Diagnoses In Renal Pathology: Evidence From EQA Schemes. *Current Diagnostic Pathology.* **13:**1-14.

GANONG, W.F. 2005. Formation and Excretion of Urine: Renal Function and Micturition, p.699-728. *In*: J. Foltin, H. Lebowitz and R.Y. Brown (eds.), Review of Medical Physiology, Twenty Second Edition. McGraw-Hill Companies. USA.

GOVAN, A.D.T., MACFARLANE, P.S. AND CALLANDER, R. 1995. Genitourinary System, p. 598-692. *In*: Pathology Illustrated, Fourth Edition. Churchill Livingstone. New York.

GERMAIN, R.N. 1994. MHC-Dependent Antigen Processing and Peptide Presentation: Providing Ligands for T Lymphocyte Activation. *Cell*. **76:**287-299.

GERBASE-DeLIMA, M., LADALARDO, M.A., DeLIMA, J.J.G., SILVA, H.B., BELLOTTI, G. AND PILEGGI, F. 1992. Essential Hypertension and Histocompatibility Antigens: An Association Study. *Hypertension*. **19:**400-402.

GERBASE-DeLIMA, M., PEREIRA-SANTOS, A., SESSO, R., TEMIN, J., ARAGÃO, E.S. AND AJZEN, H. 1998. Idiopathic Focal Segmental Glomerulosclerosis and HLA Antigens. *Brazilian Journal of Medical and Biological Research*. **31:**387-389.

GLUCK, M.C., GALLO, G., LOWENSTEIN, J. AND BALDWIN, D.S. 1973. Membranous Glomerulonephritis: Evolution of Clinical and Pathological Features. *Annals of Internal Medicine*. **78:**1-12.

GODDARD, J., TURNER, A.N., CUMMING, A.D. AND STEWART, L.H. 2006. Kidney and Urinary Tract Disease, p. 455-518. *In*: N.A. Boon, N.R. Colledge, B.R. Walker and J.A.A. Hunter (eds.), Davidson's Principles and Practice of Medicine, 20th Edition. Churchill Livingstone Elsevier. Philadelphia, USA.

GORER, P.A. 1936a. The Detection of A Hereditary Antigenic Difference In The Blood of Mice By Means of Human Group A Serum. *Journal of Genetics*. **32:**17-31.

GORER, P.A. 1936b. The Detection of Antigenic Differences in Mouse Erythrocytes by The Employment of Immune Sera. *British Journal of Experimental Pathology*. **17:**42-50.

GÖTZE, D. 1981. The Major Histocompatability Complex, p. 131-168. *In*: O.G Bier, W.D Da Silva, D. Götze and I. Mota (eds.), Fundamentals of Immunology. Springer-Verlag. NY.

GÖTZE, D. AND BURGER, R. 1986. The Major Histocompatability Complex, p. 139-178. *In*: O.G Bier, W.D Da Silva, D. Götze and I. Mota (eds.), Fundamentals of Immunology, Completely Revised 2nd Edition. Springer-Verlag. Berlin, Heidelberg.

GRANDE, J.P. AND DONADIO, J.V. 1998. Dietary Fish Oil Supplementation In IgA Nephropathy: A Therapy In search For a Mechanism. *Nutrition*: **14:**240-242.

GREEN, J.H. AND SILVER, P.H.S. 1981. The Abdominal Contents, p. 201-223. *In*: An Introduction To Human Anatomy. Oxford University Press. Great Britain.

GRUEN, J.R AND WEISSMAN, S.M. 1997. Evolving Views of The Major Histocompatibility Complex. *Blood.* **90:** 4552-4265.

GUDBRANSSON, T., HERLITZ, H., HANSSON, L. AND RYDBERG, L. 1980. Human Leukocyte Antigens In Patients With Previous Essential Malignant Hypertension. *Clinical Science (London).* **59 [Suppl. 6]:**431s-434s. [Abstract]

GUR, H., GEPPERT, T.D., WACHOLTZ, M.C. AND LIPSKY, P.E. 1999. The Cytoplasmic and Transmembrane Domains Are Not Sufficient For Class I MHC Signal Transduction. *Cellular Immunology.* **191:**105-116.

GUYTON, A.C. AND HALL, J.E. 2006. Urine Formation By The Kidneys: I. Glomerular Filtration, Renal Blood Flow, and Their Control, p. 307-326. *In*: W. Schmitt and R. Gruliow (eds.), Textbook of Medical Physiology, Eleventh Edition. Elsevier Inc. Philadelphia, Pennsylvania.

HARPER, L. AND SAVAGE, C.O.S. 1999. Treatment of IgA Nephropathy. *The Lancet.* **353:**860-862.

HARRISON, R.G. 1981. The Urogenital System, p. 531-585. *In*: G.J. Romanes (ed.), Cunningham's Textbook of Anatomy, Twelfth Edition. Oxford University Press. US.

HAUBITZ, M., WITTKE, S., WEISSINGER, E.M., WALDEN, M., RUPPRECHT, H.D., FLOEGE, J., HALLER, H. AND MISCHAK, H. 2005. Urine Protein Patterns Can Serve as Diagnostic Tools In Patients With IgA Nephropathy. *Kidney International.* **67:**2313-2320.

HAUPTMANN, G. AND BAHRAM, S. 2004. Genetics of The Central MHC. *Current Opinion in Immunology.* **16:**668-672.

HEISE, E.R. 1984. Transplantation Immunology, p. 319-335. *In*: Q.N Myrvik and R.S Weiser (eds.). Fundamentals of Immunology, 2nd Edition. Lea and Febiger. USA.

HIDA, K., WADA, J., YAMASAKI, H., NAGAKE, Y., ZHANG, H., SUGIYAMA, H., SHIKATA, K. AND MAKINO, H. 2002. Cyanotic Congenital Heart Disease Associated With Glomerulomegaly and Focal Segmental Glomerulosclerosis: Remission of Nephrotic Syndrome With Angiotensin Converting Enzyme Inhibitor. *Nephrology Dialysis Transplantation.* **17:**144-147.

HIKI, Y., KOBAYASHI, Y., OOKUBU, M. AND KASHIWAGI, N. 1990. The Role of HLA-DR4 In The Long-Term Prognosis of IgA Nephropathy. *Nephron.* **54:**264-265.

HIKI, Y., KOBAYASHI, Y., OOKUBU, M., OBATA, F. AND KASHIWAGI, N. 1991. Association of HLA-DQw4 With IgA Nephropathy In The Japanese Population. *Nephron.* **58:**109-111.

HIKI, Y., KOBAYASHI, Y., TATENO, S., SADA, M. AND KASHIWAGI, N. 1982. Strong Association of HLA-DR4 With Benign IgA Nephropathy. *Nephron.* **32:**222-226.

HIKI, Y., TANAKA, A., KOKUBO, T., IWASE, H., NISHIKIDO, J., HOTTA, K. AND KOBAYASHI, Y. 1998. Analysis of IgA1 Hinge Glycopeptides In IgA Nephropathy By Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. *Journal of American Society of Nephrology.* **9:**577-582.

HILME, E., HANSSON, L., SANDBERG, L., SODERSTROM, T. AND HERLITZ, H. 1993. Abnormal Immune Function In Essential Hypertension. *Journal of Hypertension.* **11:**989-994. [Abstract]

HOECKER, G. 1986. Recollection of a Chilean Member of The Very Exclusive H-2 Club. *Immunogenetics.* **24:**345-349.

HORIE, A., HIKI, Y., ODANI, H., YASUDA, Y., TAKAHASHI, M., KATO, M., IWASE, H., KOBAYASHI, Y., NAKASHIMA, I. AND MAEDA, K. 2003. IgA1 Molecules Produced by Tonsillar Lymphocytes are Under-*O*-Glycosylated In IgA Nephropathy. **42:**486-496.

HUANG, C.C., HU, S.A., LIN, J.L AND WU, J.H. 1989. HLA and Chinese IgA Nephropathy In Taiwan. *Tissue Antigens.* **33:**45-47. [Abstract]

HUGHES, A.L. AND YEAGER, M. 1998. Natural Selection At Major Histocompatibility Loci of Vertebrates. *Annual Review of Genetics.* **32:**415-435.

HUNG, K.Y., CHEN, W.Y., YEN, T.S., YANG, C.S., FERNG, S.H. AND KAO, C.L. 1996. Adult Primary IgA Nephropathy and Common Virus Infections. *Journal of Infection.* **32:**227-230.

HUTTER, H. AND DOHR, G. 1998. HLA Expression on Immature and Mature Human Germ Cells. *Journal of Reproductive Immunology.* **38:**101-122.

HVIID, T.V.F., SØRENSEN, S. AND MORLING, N. 1999. Polymorphisms In The Regulatory Region Located More Than 1.1 Kilobases 5' to The Start Site of Transcription, The Promoter Region and Exon 1 of The HLA-G Gene. *Human Immunology.* **60:**1237-1244.

JANER, M. AND GERAGHTY, D.E. 1998. The Human Major Histocompatibility Complex: 42,221 bp of Genomic Sequence, High-Density Sequence-Tagged Site Map, Evolution and Polymorphism For HLA Class I. *Genomics.* **51:**35-44.

JANEWAY, C.A., TRAVERS, P., WALPORT, M. AND SHLOMCHIK, M.J. 2005. Antigen Presentation to T Lymphocytes. *In*: E. Lawrence (ed.), Immunobiology: The Immune System in Health and Disease, 6th Edition. Garland Science Publishing. NY, USA.

JAWETZ, E., MELNICK, J.L. AND ADELBERG, E.A. 1982. Antibody-Mediated and Cell Mediated (Hypersensitivity and Immunity) Reactions, p.175-188. *In*: Review of Medical Microbiology. Lange Medical Publications. California.

JEFFERY, K.J.M AND BANGHAM, C.R.M. 2000. Review: Do Infectious Diseases Drive MHC Diversity? Microbes and Infection. **2:**1335-1341.

JIA, Q. AND PESTKA, J.J. 2005. Role of Cyclooxygenase-2 In Deoxynivalenol-Induced Immunoglobulin A Nephropathy. *Food and Chemical Toxicology.* **43:**721-728.

JORGE, R., SCOTT, I.U., DARÉ, A., FRANCO, R.F. AND FLYNN JR., H.W. 2002. Hemiretinal Vein Occlusion Associated With Membranous Glomerulonephritis. *American Journal of Ophthalmology.* **133:**415-416.

JUST, J.J. 1995. Genetic Predisposition To HIV-1 Infection and Acquired Immune Deficiency Virus Syndrome: A Review of The Literature Examining Associations With HLA. *Human Immunology.* **44:**156-169.

KANAUCHI, M., KAWANO, T. AND DOHI, K. 2000. Serum IgA Levels In Patients With Diabetic Nephropathy and IgA Nephropathy Superimposed Diabetes Mellitus. *Diabetes Research and Clinical Practice.* **48:**113-118.

KASHIWABARA, H., SHISHIDO, H., TOMURA, S., TUCHIDA, H. AND MIYAJIMA, T. 1982. Strong Association Between IgA Nephropathy and HLA-DR4. *Kidney International.* **22:**377-382.

KHOSHNOODI, J. AND TRYGGVASON, K. 2001. Congential Nephrotic Syndromes. *Current Opinion in Genetics and Development.* **11:**322-327.

KIEPIELA, P., LESLIE, A.J., HONEYBORNE, I., RAMDUTH, D., THOBAKGALE, C., CHETTY, S., RATHNAVALU, P., MOORE, C., PFAFFEROTT, K.J., HILTON, L., ZIMBWA, P., MOORE, S., ALLEN, T., BRANDER, C., ADDO, M.M., ALTFELD, M., JAMES, I., MALLAL, S., BUNCE, M., BARBER, L.D., SZINGER, J., DAY, C., KLENERMAN, P., MULLINS, J., KORBER, B., COOVADIA, H.M., WALKER, B.D. & GOULDER, P.J.R. 2004. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. *Nature.* **432:** 769-774.

KIMBALL, J.W. 1986. Transplantation Immunity, p. 502-519. *In*: Introduction to Immunology 2nd Edition. Macmillan Publishing Co., USA.

KISHI, Y., TAKAI, K., AKAO, J., ISOYAMA, N., MATSUMURA, M., UCHIYAMA, K., TSUCHIDA, M., SUGA, A. AND NAITO, K. 2004. Two Cases of Recurrent IgA Nephropathy Following Kidney Transplantation. *Transplantation Proceedings.* **36:**2160-2161.

KLOUDA, P.T., MANOS, J., ACHESON, E.J., DYER, P.A., GOLDBY, F.S., HARRIS, R., LAWLER, W., MALLICK, N.P. AND WILLIAMS, G. 1979. Strong Association Between Idiopathic Membranous Nephropathy and HLA-DRw3. *The Lancet.* 770-771.

KOBAYASHI, Y., CHEN, X-M., HIKI, Y., FUJII, K. AND KASHIWAGI, N. 1985. Association of HLA-DRw8 and DQw3 With Minimal Change Nephrotic Syndrome In Japanese Adults. *Kidney International.* **28:**193-197.

KOHARA, M., NAITO, S., ARAKAWA, K., MIYATA, J., CHIHARA, J., TAGUCHI, T. AND TAKEBAYASHI, S. 1985. The Strong Association of HLA-DR4 With Spherical Mesangial Dense Deposits In IgA Nephropathy. *Journal of Clinical Laboratory Immunology.* **18:**157-160. [Abstract]

KOMATSU, H., FUJIMOTO, S., HARA, S., SATO, Y., YAMADA, K. AND ETO, T. 2005. Multivariate Analysis of Prognostic Factors and Effect of Treatment In Patients With IgA Nephropathy. *Renal Failure.* **1:**45-52.

KORBET, S.M., GENCHI, R., BOROK, R.Z. AND SCHWARTZ, M.M. 1996. The Racial Prevalence of Glomerular Lesions In Nephrotic Adults. *American Journal of Kidney Diseases.* **27:**647-651.

KOSELJ-KAJTNA, M., ROTT, T., KOSELJ, M., FERLUGA, D., HVALA, A., VIZJAK, A. AND KANDUS, A. 2002. Recurrent Immunoglobulin A Nephropathy Complicated With De Novo Membranous Glomerulonephritis In Renal Allografts. *Transplantation Proceedings.* **34:**3117-3118.

KOSELJ, M., ROTT, T., KANDUS, A., VIZJAK, A. AND MALOVRH, M. 1997. Donor Transmitted IgA Nephropathy: Long-Term Follow-Up of Kidney Donors and Recipients. *Transplantation Proceedings.* **29:**3406-3407.

KOSTYU, D.D., HANNICK, L.I., TRAWEEK, J.L., GHANAYEM, M., HEILPERN, D. AND DAWSON, D.V. 1997. HLA Class I Polymorphism: Structure and Function and Still Questions. *Human Immunology.* **57:**1-18.

KOTHARI, M.L. AND MEHTA, L.A. 1984. White/Might Has Rights: Apartheid and Health. *Journal of Postgraduate Medicine.* **30:**65-68.

KRASOWSKA-KWIECIEN. A., SANCEWICZ-PACH, K., AND POGAN A. 2001. Class II Human Leukocyte Antigens In Minimal Change Nephrotic Syndrome and Focal Segmental Glomerulosclerosis-Preliminary Study. *Polski merkuriusz lekarski.* **10:**256-258. [Abstract]

KRISTENSEN, B.O. 1981. Immunogenetic Markers In Essential Hypertension. *Clinical Experimental Hypertension.* **3:**763-774. [Abstract]

KRIZ, W. 2003. The Pathogenesis of 'Classic' Focal Segmental Glomerulosclerosis – Lessons From Rat Models. *Nephrology Dialysis Transplantation.* **18[Suppl. 6]:**vi39-vi44.

KRIZ, W. 2005. TRPC6 - A New Podocyte Gene Involved In Focal Segmental Glomerulosclerosis. *Trends In Molecular Medicine.* **11:**527-530.

KRIZ, W. AND KAISSLING, B. 1985. Structural Organization of The Mammalian Kidney, p. 265-306. *In*: D.W. Seldin and G. Giebisch (eds.), The Kidney: Physiology and Pathophysiology Volume I. Raven Press. NY.

KUPER, L. 1950. Some Demographic Aspects of White Supremacy In South Africa. *The British Journal of Sociology.* **1:**144-153.

KWOH, C., SHANNON, M.B., MINER, J.H. AND SHAW, A. 2006. Pathogenesis of Nonimmune Glomerulopathies. *The Annual Review of Pathology: Mechanisms of Disease.* **1:**349-374.

LAGUERUELA, C.C., BUETTNER, T.L., COLE, B.R., KISSANE, J.M. AND ROBSON, A.M. 1990. HLA Extended Haplotypes In Steroid-Sensitive Nephrotic Syndrome of Childhood. *Kidney International.* **38:**145-150.

LAI, A.S.H. AND LAI, K.N. 2005. Molecular Basis of IgA Nephropathy. *Current Molecular Medicine.* **5:**475-487.

LAI, K.N. 2002. Future Directions In the Treatment of IgA Nephropathy. *Nephron.* **92:**263-270.

LAI, K.N., SHUTE, J.K., LINDLEY, I.J., LAI, F.M., YU, A.W.Y., LI, P.K.T. AND LAI, C.K.W. 1996. Neutrophil Attractant Protein-1/Interleukin 8 and Its Autoantibodies In IgA Nephropathy. *Clinical Immunology and Immunopathology.* **80:**47-54.

LAST, R.J. 1990. Abdomen, p.295-418. *In*: R.M.H. McMinn (ed.), Last's Anatomy Regional and Applied. Longman Group. UK.

LAURENT, M.R. AND WELSH, K.I. 1984. The Human Major Histocompatibility Complex (MHC), p.1-25. *In*: G.S. Panayi and C.S David (eds.), Immunogenetics. Butterworth & Co (Publishers) Ltd. UK.

LEBEDEVA, T.V., HUANG, A., JANZEN, M., LAU, M. AND YU, N. 2003. Identification of Novel HLA Class I Alleles Using Single Allele Sequencing. *Tissue Antigens.* **62:**433-435.

LEE, N., LLANO, M., CARRETERO, M., ISHITANI, A., NAVARRO, F., LOPEZ-BOTET, M. AND GERAGHTY, G.E. 1998. HLA-E Is a Major Ligand For The Natural Killer Inhibitory Receptor CD94/NKG2A. *Immunology.* **95:**5199-5204.

LEDERMAN, S. AND SUCIU-FOCA, N. 1999. CD4+ and CD8+ Regulatory T Lymphocytes To Arbitrate The Outcomes of Immune Responses. *Human Immunology.* **60:**553-561.

LEFFELL, M.S. 2002. MHC Polymorphism: Coping With The Allele Explosion. *Clinical and Applied Immunology Reviews.* **3:**35-46.

LESAVRE, P. AND GRÜNFELD, J.P. 1996. Idiopathic Focal Segmental Glomerulosclerosis – New Lessons From Kidney Transplantation. *The New England Journal of Medicine.* **334:**914-915.

LETAVERNIER, E., BRUNEVAL, P., MANDET, C., VAN HUYEN, J-P.D., PÉRALDI, M-N., HELAL, I., NÖEL, L-H. AND LEGENDRE, C. 2007. High Sirolimus Levels May Induce Focal Segmental Glomerulosclerosis *De Novo*. *Clinical Journal of The American Society of Nephrology*. **2:**326-333.

LI, P.K-T., BURNS, A.P., SO, A.K.L., PUSEY, C.D., FEEHALLY, J. AND REES, A.J. 1991. The DQw7 Allele at The HLA-DQB Locus Is Associated With Susceptibility To IgA Nephropathy In Caucasians. *Kidney International*. **39:**961-965.

LI, C.K. AND ISENBERG, D.A. 2006. Systemic Lupus Erythematosus. *Medicine*. **34:**445-452.

LI, L. AND BOUVIER, M. 2005. Biochemical and Structural Impact of Natural Polymorphism In The HLA-A3 Superfamily. *Molecular Immunology*. **42:**1331-1344.

LIM, C.S., ZHENG, S., KIM, Y.S., AHN, C., HAN, J.S., KIM, S., LEE, J.S., CHAE, D-W., KOO, J.R., CHUN, R.W., NOH, J.W. 2001. Th1 and Th2 Predominanace and Proinflammatory Cytokines Determine The Clinicopathological Severity of IgA Nephropathy. *Nephrology Dialysis Transplantation*. **16:**269- 275.

LIPSITCH, M., BERGSTROM, C.T. AND ANTIA, R. 2003. Effect of Human Leukocyte Antigen Heterozygosity on Infectious Disease Outcome: The Need for Allele-Specific Measures. *Biomed Central Medical Genetics*. **4:**2-10.

MACKAY, I. AND POWELL, W. 2006. Methods For Linkage Disequilibrium Mapping In Crops. *Trends In Plant Science*. **12:**57-63.

MAENAKA, K AND JONES, E.Y. 1999. MHC Superfamily Structure and The Immune System. Current Opinion in Structural Biology. **9:**745-753.

MAFFEI, A., PAPADOPOULOS, K. AND HARRIS, P.E. 1997. MHC Class I Antigen Processing Pathways. *Human Immunology*. **54:**91-103.

MAFFEI, A. AND HARRIS, P.E. 1998. Peptides Bound To Major Histocompatibility Molecules. *Peptides*. **19:**179-198.

MAKHATADZE, N.J. 1998. Tumor Necrosis Factor Locus: Genetic Organisation and Biological Implications. *Human Immunology*. **59:**571-579.

MALE, D., COOKE, A., OWEN, M., TROWSDALE, J., AND CHAMPION, B. 1996. Class I and Class II Molecules of The MHC. *In*: L. Cook (ed.), Advanced Immunology, 3rd Edition. Mosby. Italy.

MALHOTRA, S.L. 1970. Dietary Factors Causing Hypertension In Indian. *The American Journal of Clinical Nutrition*. **23:**1353-1363.

MARGULIES, D.H. 1999. The Major Histocompatibility Complex. *In*: W.E. Paul (ed.), Fundamental Immunology, Fourth Edition. Lippincott-Raven, Philadelphia.

MATTHEWS, V.B., WITT, C.S., FRENCH, M.A.H., MACHULLA, H.K.G., De La CONCHA, E.G., CHEONG, K.Y., VIGIL, P., HOLLINGSWORTH, P.M., WARR, K.J., CHRISTIANSEN, F.T. AND PRICE, P. 2002. Central MHC Genes Affect IgA Levels In The Human: Reciprocal Effects In IgA Deficiency and IgA Nephropathy. *Human Immunology*. **63:**424-433.

MATHIS, B.J., KIM, S.H., CALABRESE, K., HAAS, M., SEIDMAN, J.G., SEIDMAN, C.E. AND POLLAK, M.R. 1998. A Locus For Inherited Focal Segmental Glomerulosclerosis Maps To 19q13. *Kidney International*. **53:**282-286.

McCLUSKEY, J. AND AU PEH, C. 1999. The Human Leukocyte Antigen and Clinical Medicine: An Overview. *Reviews in Immunogenetics*. **1:**3-20.

McKAY, D.B., MILFORD, E.L AND TOLKOFF-RUBIN, N.E. 2000. Clinical Aspects of Renal Transplantation, p.2542-2605. *In:* B.M Brenner (ed.), Brenner and Rectors: The Kidney, 6th Edition, Volume 2. W.B. Saunders Company. Philadelphia.

MIDDLETON, D., CURRAN, M. AND MAXWELL, L. 2002. Natural Killer Cells and Their Receptors. *Transplant Immunology*. **10:**147-164.

MIGUELES, S.A., SABBAGHIAN, M.S., SHUPERT, W.L., BETTINOTTI, M.P., MARINCOLA, F.M., MARTINO, L., HALLAHAN, C.W., SELIG, S.M., SCHWARTZ, D., SULLIVAN, J. & CONNORS, M. 2000. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proceedings of The National Academy of Sciences*. **97:** 2709-2714.

MITCHISON, A., BRUNNER, M.C. AND SCHNEIDER, S.C. 1996. Tolerance-of-Self and Protection against Autoimmunity, pp. 1-17. *In*: A.S Mustafa, R.J. Al-Attiyah, I. Nath and T.D Chugh (eds.), T-Cell Subsets and Cytokines Interplay in Infectious Diseases. Basel, Karger. Berlin, Deutschland.

MOBBS, R.J., TUCK, R.R. AND HURLEY, B. 2000. Chronic Inflammatory Demyelinating Polyneuropathy Associated With Membranous Glomerulonephritis: Case Report. *Journal of Clinical Neuroscience.* **7:**454-455.

MODIFICATION OF DIET IN RENAL DISEASE STUDY GROUP, HEBERT, L.A., KUSEK, J.W., GREENE, T., AGODOA, L.Y., JONES, C.A., LEVEY, A.S., BREYER, J.A., FAUBERT, P., ROLIN, H.A. AND SHIN-RU WANG WRITING GROUP. 1997. Effects of Blood Pressure Control on Progressive Renal Disease In Blacks and Whites. *Hypertension.* **30:**428-435.

MUIRHEAD, N. 1999. Management of Idiopathic Membranous Nephropathy: Evidence-Based Recommendations. *Kidney International.* **55[suppl. 70]:**S-47-S-55.

MULLIS, K.B. AND FALOONA, F.A. 1987. Specific Synthesis of DNA *in Vitro* Via a Polymerase-Catalyzed Chain Reaction. *Methods in Enzymology.* **155:**335-350.

NAKAMURA, T., USHIYAMA, C., SUZUKI, S., HARA, M., SHIMADA, M., EBIHARA, I. AND KOIDE, H. 2000. The Urinary Podocyte As a Marker For The Differential Diagnosis of Idiopathic Focal Segmental Glomerulosclerosis and Minimal-Change Nephrotic Syndrome. *American Journal of Nephrology.* **20:**175-179.

NICHOLLS, K.M., FAIRLEY, K.F., DOWLING, J.P. AND KICAID-SMITH, P. 1984. The Clinical Course of Mesengial IgA Associated Nephropathy In Adults. *Quarterly Journal of Medicine.* **210:**227-250.

NIKOLICH-ŽUGICH, J., FREMONT, D.H., MILEY, M.J. AND MESSAOUDI, I. 2004. The Role of MHC Polymorphism In Anti-Microbial Resistance. *Microbes and Infection.* **6:**501-512.

NOBLE, J.A., VALDEZ, A.M., LANE, J.A., GREEN, A.E. AND ERLICH, H.A. 2006. Linkage Disequilibrium With Predisposing DR3 Haplotypes Accounts For Apparent Effects of Tumor Necrosis Factor and Lymphotoxinα Polymorphisms on Type 1 Diabetes Susceptibility. *Human Immunology.* **67:**999-1004.

NOMOTO, Y., ENDOH, M., MIURA, M., SUGA, T., TOMINO, Y., SAKAI, H., NOSE., Y. AND TSUJI, K. 1984. IgA Nephropathy Associated With HLA-DR4 Antigen. *American Journal of Nephrology.* **4:**184-187.

NOWACK, R. BIRCK, R. AND VAN DER WOUDE, F.J. 1997. Mycophenolate mofetil For Systemic Vasculitis and IgA Nephropathy. *The Lancet*. **349:**774.

NUÑEZ-ROLDAN, A., VILLECHENOUS, E., FERNANDEZ-ANDRADE, C. AND MARTIN-GOVANTES, J. 1982. Increased HLA-DR7 and Decreased DR2 In Steroid-Responsive Nephrotic Syndrome. *New England Journal of Medicine*. **306:**366-367.

OLSON, J.L. 2001. Renal Disease, p. 481-494. *In*: T.G. Parslow, D.P. Sites, A.I. Terr and J.B. Imboden (eds.), Medical Immunology. The McGraw-Hill Companies, Inc. USA.

PARHAM, P., LOMEN, C.E., LAWLOR, D.A., WAYS, J.P., HOLMES, N., COPPIN, H.L., SALTER, R.D., WAN, A.M. AND ENNIS, P.D. 1988. Nature of Polymorphism In HLA-A, -B, and –C Molecules. *Proceedings of The National Academy of Sciences*. **85:**4005-4009.

PARHAM, P. AND OHTA, T. 1996. Population Biology of Antigen Presentation by MHC Class I Molecules. *Science*. **272:**67-74.

PARK, C.Y., KIM, D.M., CHO, Y.S., YOON, S.H., CHUNG, J.H., CHUNG, C.H. AND KIM, H.L. 2004. A Case of Focal Segmental Glomerulosclerosis Associated With Aplastic Anemia. *Journal of Korean Medical Sciences*. **19:**898-900.

PARK, M., LEE, J.W. AND KIM, C. 2007. Correspondence Analysis Approach For Finding Allele Associations In Population Genetic Study. *Computational Statistics and Data Analysis*. **51:**3145-3155.

PATTISON, J.M., SIBLEY, R.K. AND KRENSKY, A.M. 1997. Mechanisms of Allograft Rejection, p. 331-354. *In*: E.G Neilson and W.G Couser (eds.), Immunologic Renal Diseases. Lippincott-Raven. Philadelphia, NY.

PAYNE, R. 1977. The HLA Complex: Genetics and Implications In The Immune Response, p. 20-31. *In*: J. Dausset and A. Svejgaard (eds.), HLA and Disease. Williams and Wilkins Co., Baltimore, USA.

PETROVSKY, N. AND BRUSIC, V. 2004. Virtual Models of The HLA Class I Antigen Processing Pathway. *Methods*. **34:**429-435.

PLAZA, J.J., HERRERO, G., BARAT, A., LOUTAIF, J.J., HERNANDO, L., VALLADO, P. AND OLIVA, H. 1982. Membranous Glomerulonephritis As a Complication of Oral Gold Therapy. *Annals of Internal Medicine*. **97:**563-564.

POLLAK, V.E., ROSEN, S., PIRANI, C.L., MUEHRCKE, R.C. AND KARK, R.M. 1968. Natural History of Lipid Nephrosis and Membranous Glomerulonephritis. *Annals of Internal Medicine.* **6:**1171-1196.

PORTO, I., LEONE, A.M., CREA, F. AND ANDREOTTI, F. 2005. Inflammation, Genetics and Ischemic Heart Disease: A Focus on the Major Histocompatibility Genes. *Cytokine.* **29:**187-196.

POZZI, C., BOLASCO, P., FOGAZZI, G., ANDRULLI, S., ALTIERI, P., PONTICELLI, C. AND LOCATELLI, F. 1999. Corticosteroids In IgA Nephropathy: A Randomized Controlled Trial. *Lancet.* **353:**883-887.

POZZI, S., LONGO, A. AND FERRARA, G.B. 1998. HLA-B Locus Sequence-Based Typing. *Tissue Antigens.* **53:**275-281.

PUOANE, T., STEYN, K., BRADSHAW, D., LAUBSCHER, R., FOURIE, J., LAMBERT, V. AND MBANANGA, N. 2002. Obesity In South Africa: The South African Health and Demographic Survey. *Obesity Research.* **10:**1038-1048.

RAJAGOPALAN, S. AND LONG, E.O. 1999. A Human Histocompatibility Leukocyte Antigen (HLA)-G-Specific Receptor Expressed on All Natural Killer Cells. *The Journal of Experimental Medicine.* **189:** 1093-1099.

REES, A.J. 1997. Immunogenetics, p. 99-125. *In*: E.G. Neilson and W.G. Couser (eds.), Immunologic Renal Diseases. Lippincott-Raven. USA.

REMUZZI, G., BENIGNI, A. AND REMUZZI, A. 2006. Mechanisms of Progression and Regression of Renal Lesions of Chronic Nephropathies and Diabetes. *Journal of Clinical Investigation.* **116:**288-296.

RITZ, E. 2006. Heart and Kidney: Fatal Twins? *The American Journal of Medicine.* **119:**31s-39s.

RODRÍGUEZ-PINTO, D. 2005. B Cells As Antigen Presenting Cells. *Cellular Immunology.* **238:**67-75.

ROSSINI, M. AND FOGO, A.B. 2004. Interpreting Segmental Glomerular Sclerosis. *Current Diagnostic Pathology.* **10:**1-10.

ROSSMAN, M.D., STUBBS, J., LEE, C.W., ARGYRIS, E., MAGIRA, E. & MONOS, D. 2002. Human Leukocyte Antigen Class II Amino Acid Epitopes: Susceptibility and Progression Markers for Beryllium Hypersensitivity. *American Journal of Respiratory Critical Care Medicine.* **165:**788-794.

RUDER, H., SCHÄRER, K., OPELZ, G., LENHARD, V., WALDHERR, R., MÜLLER-WIEFEL, D.E., WINGEN, A-M. AND DIPPELL, J. 1990. Human Leukocyte Antigens in Idiopathic Nephrotic Syndrome In Children. *Pediatric Nephrology.* **4:**478-481.

ŞAHINER, S., AYLI, M.D., YÜKSEL, C. AND ÖNEÇ, K. AND ABAYLI, E. 2004. Membranous Nephropathy Associated With Acute Myeloid Leukemia. *Transplantation Proceedings.* **36:**2618-2619.

SAIKI, R.K., WALSH, P.S., LEVENSON, C.H. AND ERLICH, H.A. 1989. Genetic Analysis of Amplified DNA With Immobilized Sequence-Specific Oligonucleotide Probes. *Proceedings of the National Academy of Sciences.* **86:**6230-6234.

SAPER, M.A., BJORKMAN, P.J. AND WILEY, D.C. 1991. Refined Structure of The Human Histocompatibility Antigen HLA-A2 at 2·6 Å Resolution. *Journal of Molecular Biology.* **219:**277-319.

SAVIN, V.J., SHARMA, R., SHARMA, M., McCARTHY, E.T., SWAN, S.K., ELLIS, E., LOVELL, H., WARADY, B., GUNWAR, S., CHONKO, A.M., ARTERO, M. AND VINCENTI, F. 1996. Circulating Factor Associated With Increased Glomerular Permeability To Albumin In Recurrent Focal Segmental Glomerulosclerosis. *New England Journal of Medicine.* **334:**878-883.

SAYEGH, M.A., PERKINS, D.L. AND CARPENTER, C.B. 2000. Transplantation Immunobiology, p. 2518-2541. *In*: B.M Brenner (ed.), Brenner and Rectors: The Kidney, 6th Edition. W.B. Saunders Company. USA.

SCHAFER, P.H., PIERCE, S.K. AND JARDETZKY, T.S. 1995. The Structure of MHC Class II: A Role for Dimer of Dimers. Seminars in Immunology. **7:** 389-398.

SCHNAPER, H.W. 1999. Focal Segmental Glomerulosclerosis, p. 1003-1026. *In*: E.G. Neilson and W.G. Couser (eds.), Immunologic Renal Disease. Lippincott-Raven. Philadelphia.

SCHWARTZ, B.D. 1987. The Human Major Histocompatibility Complex, p. 50-64. *In*: D.P. Sites, J.D. Stobo, H.H. Fudenberg and J.V. Wells (eds.), Basic and Clinical Immunology, 6th Edition. Appleton and Lange. USA.

SCHWARTZ, M.M. AND LEWIS, E.J. 2002. Rewriting The Histological Classification of Lupus Nephritis. *Journal of Nephrology.* **15[suppl. 6]:**S11-S19.

SEEDAT, Y.K., NAICKER, S., RAWAT, R. AND PARSSO, I. 1984. Racial Differences In The Causes of End-Stage Renal Failure In Natal. *South African Medical Journal.* **65:**956-958.

SEGARRA, A., VILA, J., POU, L., MAJÓ, J., ARBÓS, A., QUILES, T. AND PIERA, L.L. 2002. Combined Therapy of Tacrolimus and Corticosteroids In Cyclosporin-Resistant or -Dependant Idiopathic Focal Glomerulosclerosis: A Preliminary Uncontrolled Study with Prospective Follow-up. *Nephrology Dialysis Transplantation.* **17:**655-662.

SHARMA, M., SHARMA, R., MCCARTHY, E.T. AND SAVIN, V.J. 1999. "The FSGS Factor:" Enrichment and *in Vivo* Effect of Activity From Focal Segmental Glomerulosclerosis Plasma. *Journal of The American Society of Nephrology.* **10:**552-561.

SIMPSON, E., SCOTT, D., JAMES, E., LOMBARDI, G., CWYNARSKI, K., DAZZI, F., MILLRAIN, M. AND DYSON, P.J. 2002. Minor H Antigens: Genes and Peptides. *Transplant Immunology.* **10:**115-123.

SINGH, D., WEST, K., KAMAL, K., GUPTA, R., BELITSKY, P., KIBERD, B AND LAWEN, J. 2006. Early Recurrence of Primary Focal Segmental Glomerulosclerosis In an Older Cadaveric Renal Allograft Recipient Resistant To Plasmapheresis. *Urology.* **67:**200.e1-200.e3.

SLOAN, R.P., SCHWARTZ, M.M., KORBET, S.M., BOROK, R.Z. AND THE LUPUS NEPHRITIS COLLABORATIVE STUDY GROUP. 1996. Long-Term Outcome In Systemic Lupus Erythematosus Membranous Glomerulonephritits. *Journal of The American Society of Nephrology.* **7:**299-305.

SNELL, R.S. 2004. The Abdomen Part II, p. 213-325. *In*: Clinical Anatomy, 7th Edition. Lippincott Williams and Wilkins. USA.

STERN, L.J., BROWN, J.H., JARDETZKY, T.S., GORGA, J.C., URBAN, R.G., STROMINGER, J.L. AND WILEY, D.C. 1994. Crystal Structure of The Human Class II MHC Protein HLA-DR1 Complexed With An Influenza Virus Peptide. *Nature.* **368:**215-221.

STEVANOVIĆ, S. 2002. Structural Basis of Immunogenecity. *Transplant Immunology.* **10:**133-136.

SULLIVAN, K.A. AND AMOS, B.D. 1986. The HLA System and Its Detection, p. 835-846. *In*: N. R. Rose, H. Friedman and J.L. Fahey (eds.), Manual of Clinical Laboratory Immunology, 3rd Edition. American Society For Microbiology. Washington, DC.

SULLIVAN, L.C., HOARE, H.L., McCLUSKEY, J., ROSSJOHN, J. AND BROOKS, A.G. 2006. A Structural Perspective on MHC Class 1b Molecules In Adaptive Immunity. *Trends In Immunology.* **27:**413-420.

SUZUKI, K., HIRANO, K., ONODERA, N., TAKAHASHI, T. AND TANAKA, H. 2005. Acute IgA Nephropathy Associated With *Mycoplasma pneumoniae* Infection. *Pediatrics International.* **47:**583-585.

SVEJGAARD, A. 1986. HLA and Disease, p. 912-920. *In*: N. R. Rose, H. Friedman and J.L. Fahey (eds.), Manual of Clinical Laboratory Immunology, 3rd Edition. American Society For Microbiology. Washington, DC.

SVEJGAARD, A. AND RYDER, L.P 1977. Associations Between HLA and Disease, p. 46-71. *In*: J. Dausset and A. Svejgaard (eds.), HLA and Disease. Williams and Wilkins Co., Baltimore, USA.

SWAMINATHAN, S., LEUNG, N., LAGER, D.J., MELTON, L.J., BERGSTRALH, E.J., ROHLINGER, H. AND FERVENZA, F.C. 2006. Changing Incidence of Glomerular Disease In Olmsted County, Minnesota: A 30-Year Renal Biopsy Study. *American Society of Nephrology.* **1:**483-487.

TAKAHASHI, K., TAKASAKI, S., MORITA, C., HAYASHIDA, K., MATSUI, M., TAMECHIKA, Y. AND HISHIBASHI, H. 2001. Autoimmune Hepatitis With Membranous Glomerulonephritis. *Journal of Gastroenterology and Hepatology.* **16:**356-359.

TAKEUCHI, Y., YOSHIKAWA, M., TSUKAMOTO, N., SHIROI, A., HOSHIDA, Y., ENOMOTO, Y., KIMURA, T., YAMAMOTO, K., SHIIKI, H., KIKUCHI, E. FUKUI, H. 2003. Cronkhite-Canada Syndrome With Colon Cancer, Portal Thrombosis, High Titre of Antinuclear Antibodies, and Membranous Glomerulonephritis. *Journal of Gastroenterology.* **38:**791-795.

THOMAS, M., IBELS, L.S. AND ABBOT, N. 1985. IgA Nephropathy Associated With Mastitis and Haematuria. *British Medical Journal.* **291:**867-868.

THORSBY, E. 1997. Invited Anniversary Review: HLA Associated Diseases. *Human Immunology.* **53:**1-11.

TIERCY, J.-M. 2002. Molecular Basis of HLA Polymorphism: Implications In Clinical Transplantation. *Transplant Immunology.* **9:**173-180.

TRINH, C.H., SMITH, D.P., KALVERDA, D.P., PHILLIPS, S.E.V. AND RADFORD, S.E. 2002. Crystal Structure of Monomeric Human β-2 Microglobulin Reveals Clues To Its Amyloidogenic Properties. *Proceedings of The National Academy of Sciences.* **99:**2754-2759.

TROWSDALE, J. 2005. HLA Genomics In The Third Millennium. *Current Opinion in Immunology.* **17:**498-504.

TRYGGVASON, K., PATRAKKA, J. AND WARTIOVAARA, J. 2006. Hereditary Proteinuria Syndromes and Mechanisms of Proteinuria. *New England Journal of Medicine.* **354:**1387-1401.

TÚRI, S., NÉMETH, I., TORKOS, A., SÁGHY, L., VARGA, I., MATKOVICS, B. AND NAGY, J. 1997. Oxidative Stress and Antioxidant Defense Mechanism In Glomerular Diseases. *Free Radical Biology and Medicine.* **22:**161-168.

TURKA, L.A. 1997. Normal Immune Responses, p.65-94. *In*: E.G Neilson and W.G Couser (eds.), Immunologic Renal Diseases. Lippincott-Raven. Philadelphia, NY.

TUSSEY, L.G., ROWLAND-JONES, S., ZHENG, T.S., ANDROLEWICZ, M.J., CRESSWELL, P., FRELINGER, J.A. AND McMICHAEL, A.J. 1995. Different MHC Class I Alleles Compete For Presentation of Overlapping Viral Epitopes. *Immunity.* **3:**65-77.

VALDIVIA, P., GONZALEZ RONCERO, F., GENTIL, M.A., JIMÉNEZ, F., ALGARRA, G., PEREIRA, P., RIVERA, M., SUÑER, M., CABELLO, V., TORO, J. AND MATEOS, J. 2005. Plasmapheresis For The Prophylaxis and Treatment of Recurrent Focal Segmental Glomerulosclerosis Following Renal Transplant. *Transplantation Proceedings.* **37:**1473-1474.

VAN ES, L.A. 1997. Immunoglobulin A Nephropathy. *In*: E.G. Neilson and W.G. Couser (eds.), Immunologic Renal Diseases. Lippincott-Raven. Philadelphia, NY.

VAN ROOD, J.J., DE VRIES, R.R.P. AND BRADLEY, B.A. 1981. Genetics and The Biology of The HLA System, p. 59-113. *In*: M.E Dorf (ed.), The Role of the MHC in Immunobiology. Garland Publishing Inc. NY, USA.

VARGAS-ARENAS, R.E., PELLINO, M.L., PÉREZ, C., HERNÁNDEZ, D.E., PINTO, J.A. AND TAPIA, F.J. 2002. Glomerular Lesions In Venezuelan Mestizos With Human Immunodeficiency Virus Infection. *International Congress Series*. **1237:**207-217.

VARNEY, M.D., GAVRILIDIS, A. AND TAIT, B.D. 1999. Polymorphisms In The Regulatory Regions of The HLA-DPB1 Gene. *Human Immunology*. **60:**955-961.

VAUVERT, T., HVIID, F. AND CHRISTIANSEN, O.B. 2005. Linkage Disequilibrium Between Human Leukocyte Antigen (HLA) Class II and HLA-G-Possible Implications For Human Reproduction and Autoimmune Diseases. *Human Immunology*. **66:**688-699.

WALLACE, R.A., SANDERS, G.P. AND FERL, R.J. 1996. Thermoregulation, Osmoregulation and Excretion, p. 740-763. *In*: L. Covello and K. Trost (eds.), Biology, The Science of Life, Fourth Edition. HarperCollins Publishers Inc. NY.

WARDLE, E.N. 2004. Is IgA Nephropathy Induced By Hyperproduction of Interferon-α? *Medical Hypotheses*. **62:**625-628.

WATTS, C. 2001. Antigen Processing In The Endocytic Compartment. *Current Opinion In Immunology*. **13:**26-31.

WEEKS, D.E. AND LATHROP, G.M. 1995. Polygenic Disease: Methods For Mapping Complex Disease Traits. *Trends In Genetics*. **11:**513-519.

WENDA, S., DAUBER, E.M., DORNER, G., REISACHER, R.B.K., GLOCK, B. AND MAYR, W.R. 2006. Linkage Disequilibria Between 6 STR Loci Situated In The HLA Region On Chromosome 6. *International Congress Series*. **1288:**804-806.

WILLIAMS, T. M. 2001. Human Leukocyte Antigen Gene Polymorphism and Histocompatibility Laboratory. *Journal of Molecular Diagnostics*. **3:**98-104.

WILSON, C.B., YAMAMOTO, T. AND WARD, D.M. 1987. Renal Diseases, p. 495-515. *In*: D.P. Sites, J.D. Stobo, H.H. Fudenberg and J.V. Wells (eds.), Basic and Clinical Immunology, 6th Edition. Appleton and Lange. USA.

WINN, M.P., CONLON, P.J., LYNN, K.L., HOWELL, D.N., SLOTTERBECK, B.D., SMITH, A.H., GRAHAM, F.L., BEMBE, M., QUARLES, L.D., PERICAK-VANCE, M.A. AND VANCE, J.M.

1999. Linkage of a Gene Causing Familial Focal Segmental Glomerulosclerosis To Chromosome 11 and Further Evidence of Genetic Heterogeneity. *Genomics*. **58:**113-120.

WOLFE, S.L. 1995. The Cell Surface and Intercellular Communication p. 150-177. *In*: E. Silge (ed.), Introduction to Cell and Molecular Biology. Wadsworth Publishing Company. USA.

WOOLEY, P.H. AND DAVID, C.S. 1984. Major Histocompatibility Complex Genes and Their Role in Autoimmune and Infectious Diseases, p.1-25. *In*: G.S. Panayi and C.S David (eds.), Immunogenetics. Butterworth & Co (Publishers) Ltd. UK.

WRIGHT, P., NIMGAONKAR, V.L., DONALDSON, P.T. AND MURRAY, R.M. 2001. Schizophrenia and HLA: A Review. *Schizophrenia Research*. **47:**1-12.

YAQOOB, M. 2005. Renal Disease, p. 605-687. *In*: P. Kumar and M. Clark (eds.), Clinical Medicine, Sixth Edition. Elsevier Saunders. Philadelphia.

YOSHIKAWA, N. AND ITO, H. 1999. Combined Therapy With Prednisolone, Azathioprine, Heparin-Warfarin, and Dipyridamole For Paediatric Patients With Severe IgA Nephropathy-Is It Relevant For Adult Patients? *Nephrology Dialysis Transplantation*. **14:**1097-1099.

ZACHARIAS, M. AND SPRINGER, S. 2004. Conformational Flexibility of The MHC Class I α1 - α2 Domain In Peptide Bound and Free States: A Molecular Dynamics Simulation Study. *Biophysical Journal*. **87:**2203-2214.

ZAVAZAVA, N. AND EGGERT, F. 1997. MHC and Behavior. *Immunology Today*. **13:**8-10.

ZHANG, C., ANDERSON, A. AND DeLISI, C. 1998. Structural Principles That Govern The Peptide-Binding Motifs of Class I MHC Molecules. *Journal of Molecular Biology*. **281:**929-947.

APPENDIX

Dilutions for DNA to a concentration of 15ng/µl were done as follows using a DNA sample with a concentration of 95.3ng/µl.

$$\frac{15}{95.3} = 7.9\ \mu l\ DNA + 42.1 \mu l\ H_20 = 50 \mu l$$

$$\frac{10}{7.9} \times 42.1$$

$$= 53.3\ \mu l\ H_20$$

$\therefore$ 7.9 µl DNA with a concentration of 95.3ng/µl + 53.3 µl H_20 nuclease-free H_20 gives a concentration of 15ng/µl DNA.