Serum glycan-binding IgG antibodies in HIV-1 infection and during the development of broadly neutralizing responses

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Background: The HIV-1 envelope is covered with glycans that provide structural integrity and protect conserved regions from host antibody responses. However, these glycans are often the target of broadly neutralizing antibodies (bNAbs) that emerge in some HIV-infected individuals. We aimed to determine whether antiglycan IgG antibodies are a general response to HIV-1 infection or specific to individuals who develop bNAbs.

Methods: IgG binding to glycans was assessed using arrays that contained 245 unique components including N-linked carbohydrates, glycolipids, and Tn-peptides. Sera from 20 HIV-negative and 27 HIV-positive women (including 12 individuals who developed bNAbs) were profiled longitudinally. HIV-1 gp120 proteins were used to compete for binding to the array.

Results: Antiglycan IgG antibodies fluctuated over a 3-year period, irrespective of HIV infection. However, HIV-positive individuals had elevated binding to 40 components on the array that included Man_8, Man_9, Tn-peptides, heat shock protein, and glycolipids. Competition experiments confirmed that a proportion of these glycan-binding IgG antibodies were HIV-1-specific, some of which were higher in individuals who developed bNAbs.

Conclusions: HIV-1 infection is associated with elevated levels of IgG antibodies to specific glycans. Furthermore, some antiglycan IgG antibodies were more abundant in individuals with bNAbs, suggesting a unique phenotype that may be informative for HIV vaccine design.   Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

Introduction

A comprehensive understanding of the immune response to HIV-1 infection is critical for the development of a vaccine. Antibodies targeting the viral envelope can be detected as early as 12 days postinfection, and even though these bind gp41, they are unable to neutralize the virus [1]. These are followed by strain–specific

neutralizing antibodies from 4 weeks postinfection, which typically target gp120 and persist throughout infection [2]. In a subset of HIV-infected individuals, broadly neutralizing antibodies (bNAbs), capable of neutralizing a large number of HIV strains, develop after 2−3 years of infection [2]. Understanding the factors that regulate bNAb development is a major research endeavour.

The trimeric HIV envelope glycoprotein is heavily glycosylated with between 25 and 30 N-linked glycosylation sites that account for half of its molecular mass, known as the glycan shield [3,4]. Although glycans are attached to the viral envelope through the host cell glycosylation pathways, they undergo limited processing, resulting in inefficiently trimmed glycans [5]. The glycan shield on trimeric gp120 is mostly comprised of densely packed high mannose, particularly $Man\alpha_{1-2}Man$ terminating glycans such as $Man_{6-9}GlcNAc_2$, and complex N-linked glycans, although some O-linked glycans do occur [3,4,6−8]. In addition to protecting immunogenic sites on the virus, these glycans are also essential for glycoprotein folding and are thus often conserved [3−5].

A significant number of bNAbs target glycan-rich epitopes on the HIV envelope [9]. The N332 supersite on the V3 and V4 loops includes Man_{7-9} and are targeted by 2G12 and some of the PGT antibodies (PGT125−131 and PGT135), whereas complex type glycans (A2, N2, and NA2) are targeted by PGT121−123 [10,11]. The N160, N156, and/or N173 sites of V2 region have high mannose N-linked glycans and sialylated N-linked glycans, which are bound by PG9/PG16 and the CAP256-VRC26 lineage [12−14]. High mannose N-linked glycans have also been observed at the gp120-gp41 interface (targeted by 8ANC195 and 35O22) and CD4-binding sites (targeted by HJ16 and PGV04) [3,9,15−17]. Despite the identification and characterization of a number of glycan-binding monoclonal bNAbs, the polyclonal serum antiglycan antibody responses to HIV infection in humans have not been well studied.

Glycan arrays have been developed to study the glycan-binding profiles of antibodies and have proven to be particularly useful in determining the types of glycan structures that are bound by HIV-1 bNAbs [10,11,18−25]. In addition, the antiglycan antibody responses in sera of nonhuman primates after Ad5hr-simian immunodeficiency virus (SIV) vaccination and SIV infection [26] and HIV vaccine responses in rabbits have been assayed on arrays [20,27,28]. Here, we studied serum antiglycan IgG antibody profiles of HIV-negative and HIV-positive individuals, including those who develop bNAbs, over 3 years of infection. Our aim was to determine whether elevated levels of serum antiglycan IgG antibodies are observed during HIV infection or unique to individuals who make bNAbs.

Methods

Serum samples and ethics statement

Sera from 47 South African Black women (27 HIV-positive and 20 HIV-negative) taken at four time-points (yearly for 3 years and, when available, prior to infection) were obtained from the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 002 Acute Infection Study [29] and the 004 Tenofovir Gel Trial [30] cohorts. Among the 27 HIV-positive individuals, 12 developed bNAbs and 12 did not develop bNAbs, despite being infected for at least 2 years and having similar antigenic loads (Supplementary Table 1, http://links.lww.com/QAD/B157) [31]. Sera were tested against a panel of 18 viruses comprising 6 subtype A, 6 subtype B, and 6 subtype C viruses. Individuals with bNAbs were capable of neutralizing 33−94% (median 53%) of the panel, whereas those without bNAbs neutralized only 0−6%. Three individuals whose sera neutralized 17−22% of the panel viruses were termed intermediate neutralizers. The HIV-positive samples were selected based on neutralization breadth and matched for viral load. Samples from 20 HIV-negative individuals were used as controls to assess normal fluctuations in glycan-binding over a 3-year period. Control reference serum, consisting of pooled human sera, used to assess reproducibility of the arrays, was purchased from Valley Biomedical Products and Services (Winchester, Virginia, USA). All human serum samples were heat-inactivated for 30 min in a 56°C water bath prior to binding on the glycan arrays. Similar experiments using samples from macaques showed no inhibitory effect on antibody function or antiglycan antibody profiles [26,32]. All time-points from the same individual were run on the same day to minimize intraindividual variability, and samples from individuals with and without bNAbs were intermixed to minimize experimental biases. In addition, all samples were run in duplicate and averaged. This study received ethics clearance from the University of the Witwatersrand (M080470 and M111104). The CAPRISA Acute Infection study in adult women received ethical approval from the Universities of KwaZulu-Natal (E013/04), Cape Town (025/2004), and the Witwatersrand (MM040202), and informed consent was obtained from all study participants.

Monoclonal antibodies

Seven well characterized bNAbs were used to assess the ability of the glycan arrays to detect HIV-specific glycan-binding. PG16 and 2G12 were obtained from the NIH AIDS Reagent Program. PGT121, PGT125, PGT126, PGT128, and PGT130 were provided by IAVI's Neutralizing Antibody Center (Scripps Research Institute, La Jolla, California, USA).

Glycan arrays

Glycan arrays were composed of 337 components (of which 245 were unique structures, many of which were

printed at multiple densities on the array surface), including *N*-linked carbohydrates, glycolipids, and glycopeptides (Supplementary Table 2, http://links.lww.com/QAD/B157). Included in the array were ten controls (BSA, HSA, linkers, and fluorochromes) that were used to assess technical reproducibility and were excluded from downstream analysis. The arrays were generated as previously described [26], with the exception of the addition of a washable fluorescent dye, DyLight649 (0.7 μg/ml; ThermoScientific, Waltham, Massachusetts, USA) to the print buffer as an indicator of successful liquid deposition and spot morphology. Triton X-100 concentration was reduced to 0.0001% instead of 0.006% to produce smaller spots allowing additional glycans to be spotted onto the plate. The array format and binding assay were analyzed for reproducibility and validated with numerous antibodies and lectins as previously described [33–37]. All array experiments were done using sera diluted 1:50 in the absence of calcium. Other studies using these arrays have ruled out competition by lectins, such as mannose-binding lectin (MBL), under these conditions (unpublished data). Slides were scanned at 10 μm resolution with a Genepix 4000A microarray scanner (Molecular Devices Corporation, Sunnyvale, California, USA) and analyzed with Genepix Pro 6.0 software as previously reported [33].

gp120 competition assay

To determine if glycan-binding profiles were HIV-specific, sera with high Man_8 binding levels were incubated with purified monomeric ConC gp120 (100 μg/ml) or PBS alone at room temperature for 15 min. Monomeric ConC gp120 was expressed as described in Gray *et al.* [38] and purified using a *Galanthus Nivalis* lectin (Stratagene, La Jolla, California, USA). Sera incubated with or without gp120 were run on the same glycan array slide to minimize variability. A total of three wells (six replicate spots) were analyzed.

Statistical and data analysis

All fold-change differences were calculated based on relative fluorescence unit (RFU) binding. Pearson correlations were used to calculate the coefficient of determination (r^2) of glycan-binding between and within individuals. Two methods were used to assess levels of glycan-binding in HIV-positive samples relative to HIV-negative samples. The first method identified array components with binding levels at least two-fold higher than those observed in the HIV-negative samples and present in at least two HIV-infected individuals. In the second method, we identified components with at least two-fold increased binding over two consecutive time-points in postinfection samples in at least two individuals compared to matched preinfection samples. Two-sampled *t* tests were used to compare the binding profiles of gp120 or PBS-treated sera in the gp120 competition assay and between the individuals who made bNAbs and those who did not. The resulting *P* values were corrected for multiple testing using a Bonferroni

correction and *P* values 0.05 or less were considered significant.

Results

Detection of monoclonal antibody binding on glycan arrays

Seven well characterized monoclonal antibodies (mAbs) with broadly neutralizing activity were used to assess the array's ability to detect HIV-specific glycan-binding. These mAbs targeted high mannose *N*-linked glycans and complex glycans on the V2 and V3 loops of gp120 [10,11,24,39,40]. A cut-off of at least 3000 ($log_2 11$) relative fluorescence values (RFUs) was used in order to compare our data to a previously published study [11].

Six of the seven mAbs bound to at least two glycans (Fig. 1) confirming previous reports [10,11,24,39,40]. The only mAb with no binding was PG16, which is known to target a trimer-specific epitope and has previously failed to bind on arrays [17]. The mAb 2G12 and the PGT antibodies, except PGT121, bound high mannose *N*-linked glycans Man_8 and Man_9 to varying levels. PGT121 bound NA2 (other glycans A2, A2F, and N2 previously associated with PGT121 [10,11] were not part of this array). In addition, we found binding to thyroglobulin by PGT125, PGT128, and PGT130, whereas fatty acid-binding protein (FABP), ManT (branched Man_3 glycan), and galactose-α (Gal-α) were recognized by single mAbs. On the basis of these results, we concluded that the array used in this study was capable of capturing HIV-specific glycan-binding antibodies.

Natural fluctuations in antiglycan IgG antibodies over 3 years

To identify changes associated with HIV infection, it was necessary to first define natural fluctuations in serum antiglycan IgG antibodies in HIV-negative individuals. Previous longitudinal studies of human sera on glycan arrays have been limited to 1−3 months [36], and because bNAbs take years to develop, we aimed to understand natural fluctuations in glycan-binding IgG antibodies over a longer timeframe. We therefore profiled 80 serum samples from 20 HIV-negative individuals (from the CAPRISA 002 and 004 cohorts) collected yearly for 3 years (time-points labeled as 0, 1, 2, and 3 years).

As might be expected, we observed greater fluctuations over 3 years than seen in previous studies (data not shown) [36,41]. For all HIV-negative samples, 10% of the glycans varied by 2.6-fold (typically used as a cut-off for healthy individuals [36]), 3% by 4-fold, and only 0.5% showed more than 10-fold changes. Fluctuations varied considerably between individuals with some (e.g. CAP11) showing fewer glycan changes between time-points, and
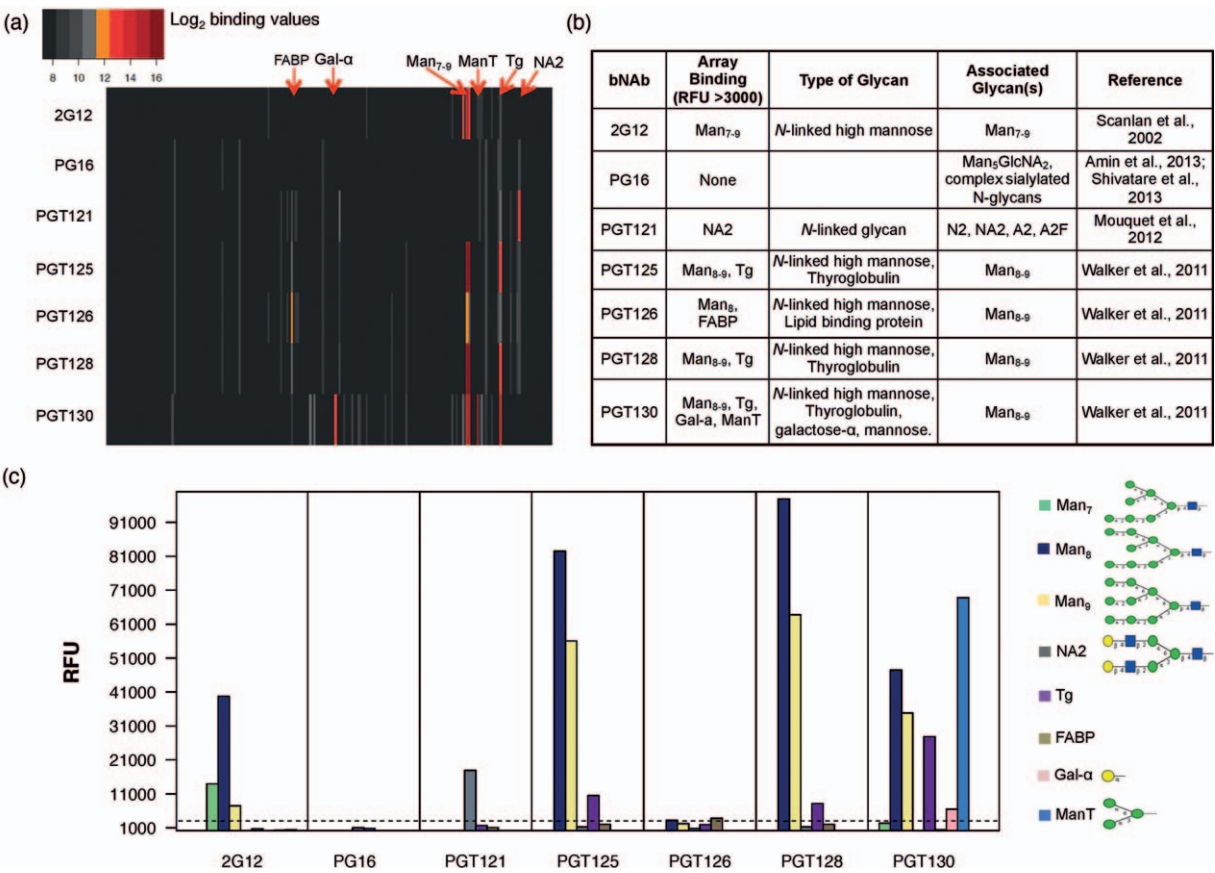
Fig. 1. Monoclonal antibody (mAb) glycan-binding profiles. (a) Heatmap of binding by each mAb on the glycan array. Shown are the $\log_2$-binding values of the seven mAbs for all glycans on the array. (b) Table comparing glycan-binding profiles from this array to previous studies. Listed are the glycans that had RFU-binding values at least 3000 ($\log_2 11$, the cut-off used in previous studies). (c) Glycan-binding profiles of all seven mAbs for all glycans with RFU-binding at least 3000. Shown are the RFU-binding values for each of the mAbs tested for all eight glycans observed with binding at least 3000 on this array. RFU, relative fluorescence unit.

others (CAP39 and CAP54) with more drastic changes (Fig. 2a). By comparison, HIV-positive individuals showed similar fluctuations over time including some with more drastic changes (Fig. 2b). Despite these fluctuations, the overall IgG antibody glycan-binding profiles from the same individual at different time points were highly correlated based on Pearson correlations, with coefficient of determination (r^2) values typically at least 0.9, irrespective of HIV infection. This is in contrast to correlations seen between different individuals, which showed more variation, where r^2 values were typically 0.8 or less (Fig. 2c).

Although substantial changes could be observed in some individuals at certain time-points irrespective of HIV infection, antiglycan antibody signals generally oscillated rather than continually increased or decreased throughout the 3 years of study. This result suggests a fluctuation about a steady-state level for essentially all the antibody populations measured in these individuals. Thus, we concluded that comparing a single time-point could result in a high proportion of false-positives, and that

longitudinal sampling was necessary to accurately determine glycan–binding changes specifically related to HIV infection.

Defining HIV-associated glycan-binding responses

We devised two methods that used data from HIV-negative samples to identify responses associated with HIV-1 infection. In the first method, we compared 88 longitudinal samples from 27 HIV-positive individuals to 80 longitudinal samples from the 20 HIV-negative individuals. Binding needed to be at least two-fold higher than the highest level seen within the HIV-negative individuals and observed in at least two HIV-positive individuals to be considered elevated. In the second method, we focused on changes relative to preinfection, which were only available for 11 of the 27 HIV-infected individuals, selecting array components with at least two-fold increased binding in two consecutive time-points (to account for temporal oscillations) in postinfection samples.
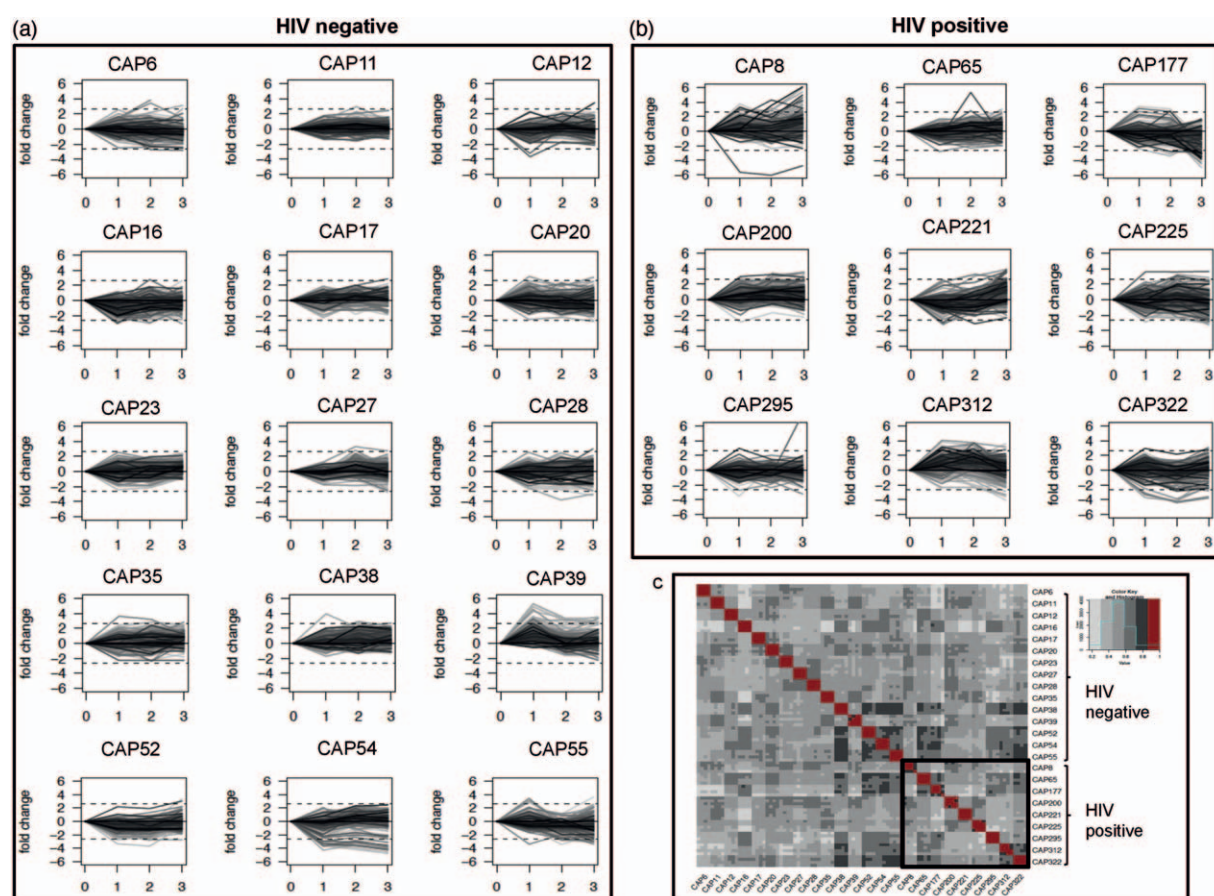
Fig. 2. Fluctuations in longitudinal antiglycan serum IgG. (a) Longitudinal fluctuations in HIV-negative individuals. Shown are fold-change differences in longitudinal serum IgG antibodies compared to year 0 for 15 of 20 HIV-negative individuals for whom all four time-points were available. Each line represents a different glycan spotted on the array. (b) Longitudinal fluctuations in HIV-positive individuals. Shown are the fold-change differences in longitudinal serum IgG antibodies compared to matched preinfection samples for nine of 27 HIV-infected individuals for which all four time-points were available, where each line represents a different glycan. (c) Correlation of longitudinal samples within and across individuals. Shown are the r^2 values of Pearson correlations comparing the RFU values of all glycans on the arrays for each of the four time-points for each of the participants shown in (a) and (b). The heat map is ordered so that all four time-points per individual are next to each other (time-points not labeled), with the HIV-positive individuals in the bottom right corner. The correlations shown in the diagonal (mostly in red) represent each time-point within an individual, those outside the diagonal in gray represent the correlations compared across individuals.

A combination of these two methods identified 40 (out of 245) array components that were elevated in HIV-positive samples, including high mannose *N*-linked glycans, Tn–peptides, glycolipids, glycoproteins, Tf–peptides, and Lewis antigens (Supplementary Table 3, http://links.lww.com/QAD/B157). Most (36/40, 90%) were elevated in postinfection samples compared to matched preinfection samples. Nine out of the 40 components were identified using both methods, and included Man_8, 4 Tn–peptides [Ac-A-Tn(Thr)-S-G, Ac-S-Tn(Thr)-Tn(Thr)-G, Ac-S-Tn(Thr)-G-G, and Ac-P-Tn(Thr)-T-G], 2 glycolipids (GT2 and GT3), heat shock protein (HSP), and Fuc-b (Fig. 3). The highest levels of binding against Man_8 were observed at 3 years postinfection, with two individuals having 15-fold and

11.5–fold higher binding than the HIV–negative samples, respectively (Fig. 3a). In addition, these values were 6−8–fold higher than the median of 220 previously studied healthy individuals from the United States [42]. Other *N*-linked high mannose glycans (Man_5 and Man_9), identified using only the first method, also showed very high levels of binding at 3 years postinfection, 53-fold and 7-fold, respectively. Increased binding to the Tn–peptides were observed throughout HIV infection, with the exception of Ac-P-Tn(Thr)-T-G, in which increased binding was mostly observed at 1 year postinfection (Fig. 3e). Likewise, binding against the two glycolipids and Fuc-b were observed throughout infection, whereas elevated binding to HSP was mostly observed at 1 and 3 years postinfection (Fig. 3f−i).
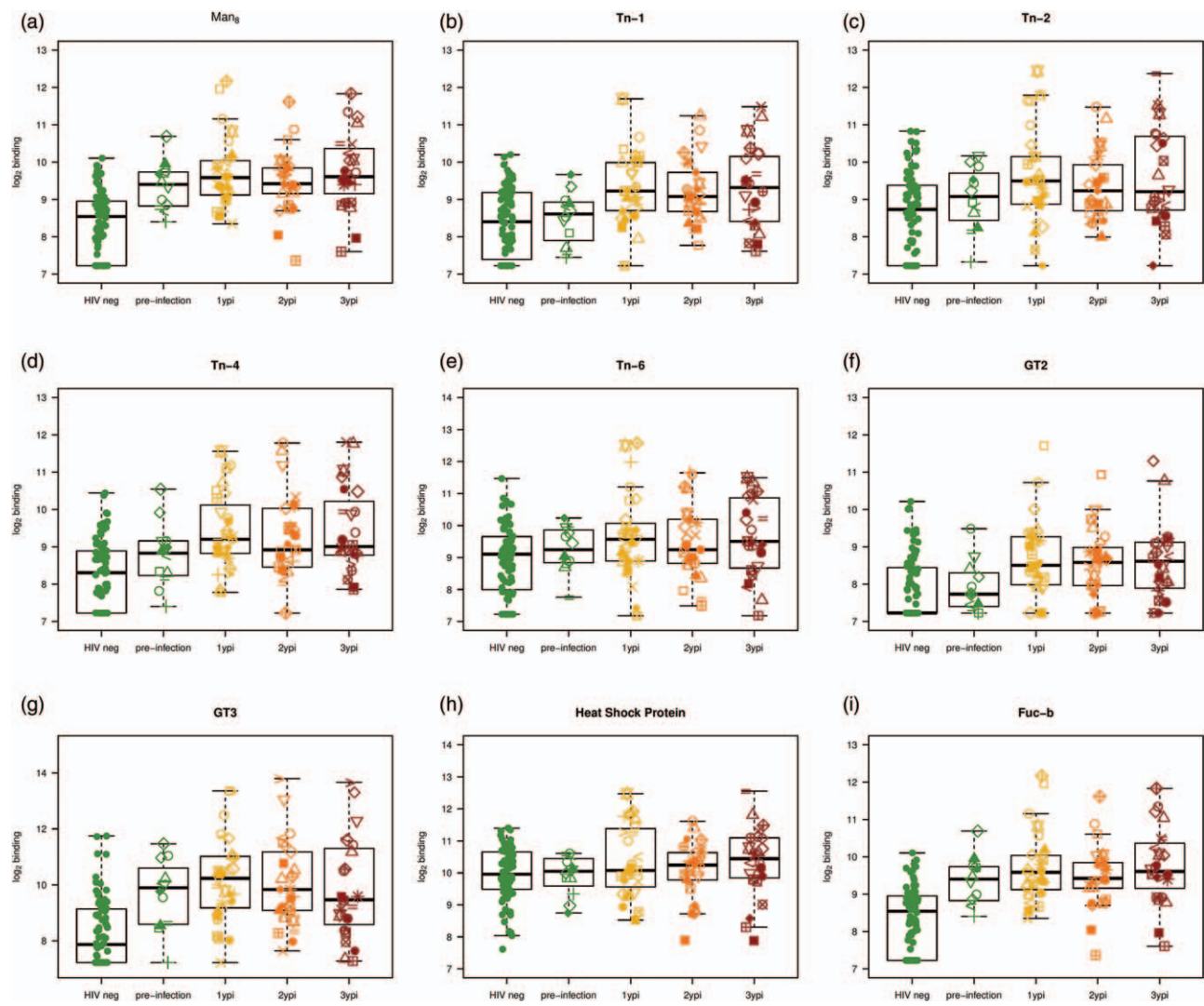
Fig. 3. Longitudinal glycan-binding profiles during HIV-1 infection. Shown are the log_2-binding values for all serum samples. HIV-negative and preinfection (green), 1 year postinfection (ypi, in yellow), 2 years postinfection (orange), and 3 years postinfection (red). Each HIV-positive individual is shown using a different symbol. Tn-1 refers to Ac-A-Tn(Thr)-S-G, Tn-2 Ac-S-Tn(Thr)-Tn(Thr)-G, Tn-4 Ac-S-Tn(Thr)-G-G, and Tn-6 Ac-P-Tn(Thr)-T-G.

Competition with gp120 suggests some glycan-binding IgG antibodies to be HIV-specific

Since increased glycan–binding may be due to hyper–gammaglobulinemia associated with HIV infection, we used a competition assay to determine what proportion of these antibodies was HIV-specific. Three–year postinfection sera from eight HIV–positive individuals (highlighted in Supplementary Table 1, http://links.lww.com/QAD/B157), with high levels of binding to Man_8, found on the HIV-1 trimer [3], were incubated with the HIV glycoprotein gp120 prior to glycan array binding (data from NA3, NA4, NGA2B, Sialyl LeA, or the Tf-peptides were not available in this experiment). As a control, gp120-incubated sera showed a 21.4-fold reduction in median binding to gp120 compared to the PBS–incubated sera (Fig. 4). We saw a 10.9-fold decrease in binding to Man_8 following gp120 incubation,
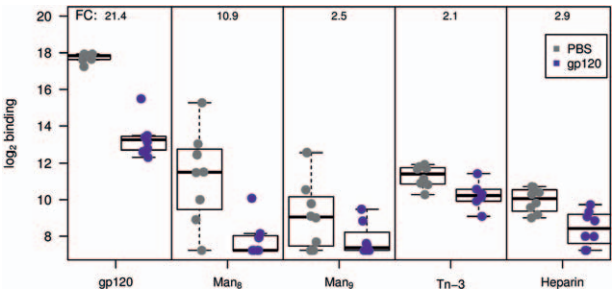

Fig. 4. Reduced glycan-binding following gp120 incubation. Shown are log_2-binding values for 3 year postinfection sera from eight HIV-1-infected individuals incubated with PBS (gray) or gp120 (purple) prior to binding on the glycan array. The mean RFU fold-change differences (FCs) associated with each glycan are shown above the plot. Tn-3 refers to Ac-S-Tn(Thr)-A-G. RFU, relative fluorescence unit.

and more than two-fold decreases in binding to Man_9, Tn–3 [Ac-S-Tn(Thr)-A-G], and heparin. These data suggested that at least a proportion of these glycan-binding IgG antibodies are HIV-specific.

Unique HIV-specific antiglycan IgG profiles in individuals who develop broadly neutralizing antibodies

To determine if the elevated levels of binding observed during HIV infection were unique to individuals who developed bNAbs, the binding levels for the 40 array components that were elevated during infection were compared between the individuals that made bNAbs and individuals who did not develop bNAbs (Supplementary Table 1, http://links.lww.com/QAD/B157). For this analysis, we used samples from 10 bNAb and 11 nonbNAb individuals at 3 years postinfection (Fig. 5). Although there were no statistically significant differences between the two groups, individuals who developed bNAbs tended to have higher levels of glycan-binding antibodies, particularly to some high mannose (Man_3 and NA4), Tn-peptides (Tn–1, Tn–2, Tn–3, and Tn–6), and HSP90. These data suggest that individuals who develop bNAbs may have a unique serological profile that could be informative for vaccine design.

Discussion

In this study, we used glycan microarray technology to profile IgG responses over 3 years of HIV-1 infection. We observed an increase in glycan-binding antibodies upon infection to 40 components on the array, including high mannose *N*-linked glycans, Tn-peptides, and glycolipids, some of which were confirmed to be HIV-specific following a reduction in binding after gp120 incubation. Furthermore, individuals who made bNAbs tended to have elevated levels of glycan-binding compared to those who did not, despite chronic HIV infection. This study is the most comprehensive analysis of antiglycan IgG antibody responses induced by HIV infection and highlights a potentially unique glycan-binding profile in individuals who develop bNAbs.

Since glycans are targeted by the human immune system, we first profiled serum IgG antibodies in HIV-negative individuals with similar demographics over a 3-year time frame to determine baseline antiglycan IgG antibody levels. These profiles were highly correlated within an individual at different time-points, with antibody subpopulations fluctuating about a steady state, suggesting a highly co-ordinated and controlled response to antigenic stimuli. As expected, these fluctuations varied considerably from one individual to another and were greater than those observed in earlier studies over a short time period [36,41]. This analysis highlights the importance of having matched controls when studying antiglycan responses to infection.

Two different approaches were devised to account for natural glycan-binding variation, which identified a number of IgG responses that were the result of HIV infection. Antibodies to high mannose glycans, such as Man_8, were one of the most noteworthy responses, with certain individuals producing exceptionally high signals compared to HIV-negative control individuals. In addition, these antibodies were partially depleted by preincubation with gp120, providing support that they are HIV-specific. Responses to high mannose *N*-linked glycans tended to occur during chronic infection, with the highest levels of binding seen at 3 years postinfection. This time frame is in line with the development of bNAbs, although responses to Man_8 and Man_9 did not show a significant association with breadth possibly because these are also targeted by strain-specific antibodies.

In addition to high mannose antibodies, we observed HIV-associated responses to Tn-peptides, glycolipids, glycoproteins, Lewis antigens, Tf-peptides, and other array components, some of which were also higher among individuals with bNAbs. Increased binding to Tn-peptides has also been observed following SIV vaccination and SIV infection using this same array [26]. Tn-peptides are precursors of *O*-linked glycans, and in normal mammalian cells the Tn-portion of the *O*-linked glycans are masked during the glycosylation pathway. However, in HIV infection, the normal glycosylation pathways are altered revealing the Tn-portion of these *O*-linked glycans on the glycan shield potentially rendering them more immunogenic [26]. Interestingly, anti–Tn-peptide antibodies have been associated with the neutralization of HIV-1 by preventing fusion of the virus into infected cells [43,44]. Furthermore, a recent study has revealed how *O*-linked glycans on viral surfaces are used to initiate immune responses at mucosal surfaces [45].

Similarly, other glycans that we identified have previously been associated with HIV. Ganglioside glycosphingolipids are found on the HIV-1 envelope and have been associated with the uptake of HIV-1 into mature dendritic cells [46−49]. These glycolipids attach to the cellular receptor Siglec-1 enabling the incorporation of HIV-1 into the dendritic cell and the subsequent transfer to $CD4^+$ T cells [49]. Thyroglobulin (which was also bound by three of the PGT mAbs) is a glycoprotein produced by the thyroid gland that is dysregulated in HIV-infected individuals [50−54]. Thyroglobulin is highly glycosylated and binds very strongly to ConA, suggesting that the binding noted in this array may be through high mannose. Finally, heparin has previously been observed to inhibit viral infectivity including HIV-1, and thus its elevation among those with bNAbs is of interest [55−58].

HIV-1 infection has been associated with a wide range of B-cell dysfunctions, including hypergammaglobulinemia (increased levels of circulating IgG), malignancies,
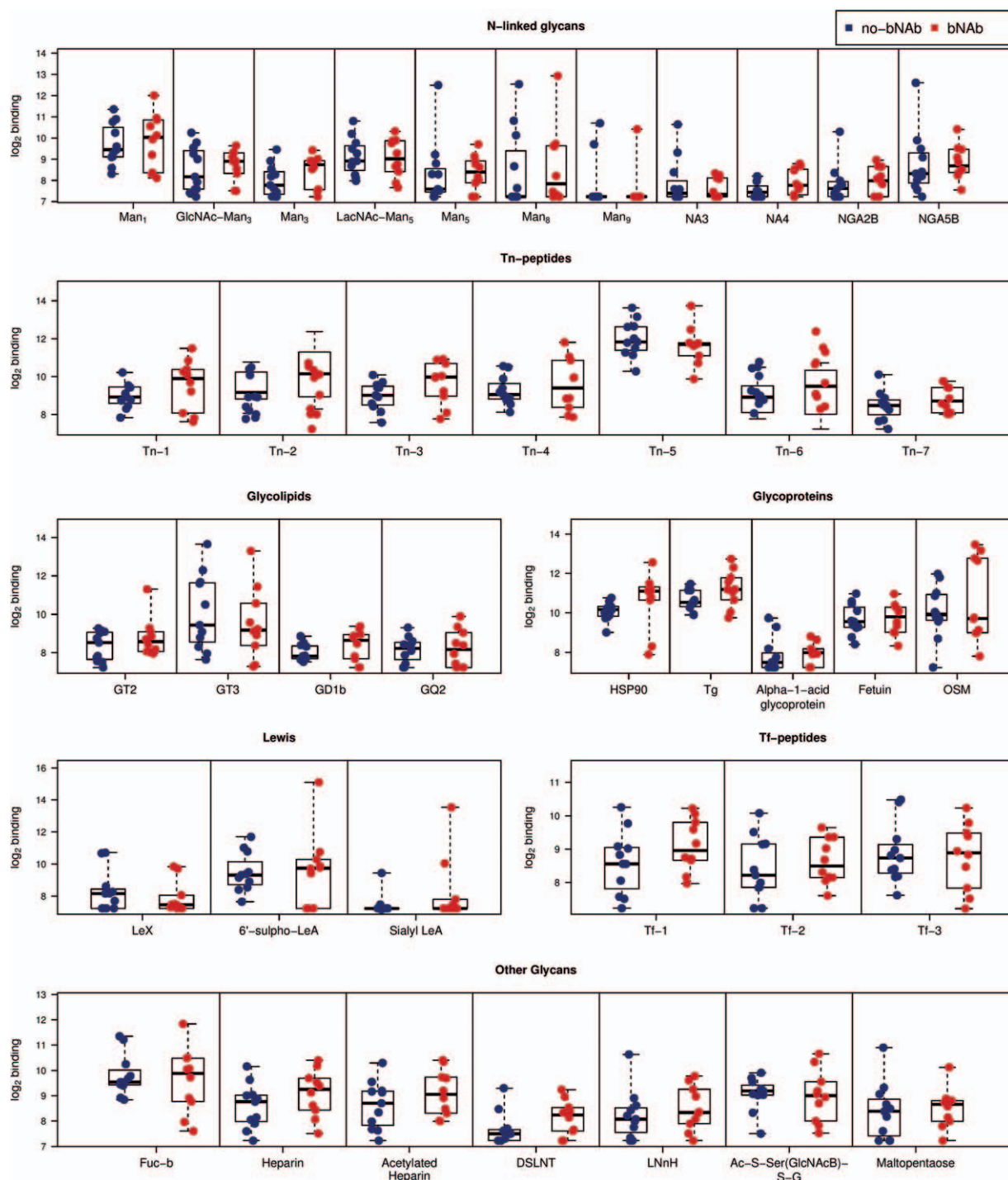
Fig. 5. Differences in glycan-binding between individuals with and without bNAbs. Shown are the $\log_2$-binding values at 3 years postinfection for the individuals who developed bNAbs ($n = 10$) compared to those who did not develop bNAbs ($n = 11$) despite chronic HIV infection, for each of the 40 array components that were elevated during HIV infection. Tn-1 refers to Ac-A-Tn(Thr)-S-G, Tn-2 Ac-S-Tn(Thr)-Tn(Thr)-G, Tn-3 Ac-S-Tn(Thr)-A-G, Tn-4 Ac-S-Tn(Thr)-G-G, Tn-5 Ac-S-Tn(Thr)-S-G, Tn-6 Ac-P-Tn(Thr)-T-G, Tn-7 Ac-Tn(Ser)-Tn(Ser)-Tn(Ser)-G, Tf-1 DTVPLPTAHGTSASSTG, Tf-2 DTVPLP-TF(Thr)-AHGTSASSTG, and Tf-3 DTVPLPTAHGT-TF(Ser)-ASSTG. bNAbs, broadly neutralizing antibodies.

exhaustion, an increase in subsets of B cells not found in healthy individuals, and polyclonal activation [1,59,60]. Although we observed a number of changes in HIV-infected individuals, the overall IgG antibody profiles and the average signals on the array were largely unchanged within a given individual before and after acquiring HIV infection. Therefore, the changes we identified using our two strategies are not simply a result of global changes in

antibody levels or general dysregulation of antibody production, but rather a specific response to HIV infection. It is possible that greater changes might be observed if all antibodies were assessed and not just those that bind glycans.

The study had some limitations. First, we were only able to assess the HIV specificity of antibodies using gp120 and thus would not have detected glycan-binding antibodies to epitopes in gp41 or those present only on the trimeric envelope structure. In addition, only samples with high levels to Man$_8$ were used in the gp120 competition assay and not those with high levels to other array components. It is likely therefore that we have underestimated the proportion of glycan-binding IgG antibodies that are HIV-specific. Second, we used stringent criteria to identify HIV-associated IgG responses. Although our goal was to minimize false-positives, this approach could have missed HIV-associated responses that occurred infrequently or were below our cut-off. Third, the array contained only a small fraction of the glycans found in the human glycome and thus some important responses may not have been detected. Lastly, the comparison of individuals who develop bNAbs to those who do not may be limited by our sample size; increased numbers may show more statistically significant results.

Nonetheless, this study is the first to highlight natural fluctuations in a diverse spectrum of glycan-binding IgG antibodies over a 3-year time frame in healthy and HIV-infected individuals. Through this, we have shown that HIV induced glycan-binding antibodies against *N*-linked glycans, which are known to be targets of bNAbs, but also against *O*-linked glycans and glycolipids, whose role in the HIV envelope trimer is less clear. Since the responses to *N*-linked high mannose glycans were seen later in infection, whereas responses to Tn-peptides and glycolipids were seen constantly throughout infection, further investigation into these glycans may be warranted.

Acknowledgements

Conflicts of interest

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