Antigenic Properties of Peptide Mimotopes of HIV-1-associated Carbohydrate Antigens*

The glycan shield of the human immunodeficiency virus (HIV) envelope protein presents many potential epitopes for vaccine development. To augment immune responses to HIV, type 1 (HIV-1), envelope-associated carbohydrate antigens, we are defining peptide mimics of HIV-associated carbohydrate antigens that function as antigen mimotopes that upon immunization will induce antibodies cross-reactive with carbohydrate antigens. We have previously defined peptides with a putative sequence tract RYRY that mimic concanavalin A-binding glycans. To imitate the multivalent binding of carbohydrates, we compared the avidity of a linear (911) and cyclic peptide (D002) reactive with concanavalin A presented in a multiple antigen peptide (MAP) format. The affinity of the MAP-D002 peptide was higher than that of the peptide MAP-911, whereas the avidity of D002 peptide was lower than that of 911. Serum from mice immunized with MAP-911 had lower titer for oligomannose-9 than those elicited by MAP-D002 under the same conditions, but both immunogens elicited antibodies that can block the binding of GP120 to dendritic cells. Antibodies that bind to the studied MAPs were found in a preparation of normal human immunoglobulin for intravenous use. Those that were purified on 911 bound back to 911 and D002, whereas anti-D002 antibodies were specific only for D002. Human antibodies reactive with both mimotopes and with a mannosyl preparation were observed to bind to envelope protein. These results suggested the potential to fine-tune the antibody response to carbohydrate antigens by modifying structural features of peptide mimotope-based immunogens.

Immune targeting of carbohydrate antigens on the Env protein of HIV-1 has gained interest with the realization that the human antibody 2G12, which neutralizes a broad range of HIV-1 isolates and shows protective activity against viral challenge in animal models (1), binds a dense cluster of carbohydrate moieties on the “silent” face of the GP120 Env glycoprotein (2). Defining “protective” epitopes on HIV-associated carbohydrate antigens is a critical first step in carbohydrate-based vaccine design strategies (3–5). Crystallographic studies of 2G12 coupled with direct binding assays suggest a high binding interaction of 2G12 with mannosyl glycans at nanomolar affinity. This interaction makes use of an unusual but distinct interface between the heavy chains of 2G12 (2, 6–9). Specificity analysis of 2G12 indicates preferred interactions with Env-associated Man9GlcNAc antigen, which is also recognized by cyanovirin-N (10).

There is strong evidence that lectins bind specifically to HIV-1-associated sugar epitopes (11), permitting a better understanding of carbohydrate profiles displayed on the Env protein that may be targeted in HIV-directed drug or vaccine design applications. The lectin concanavalin A (ConA) recognizes mannosyl antigens on the Env protein (12) but might do so differently than 2G12 because ConA can tolerate a larger number of sugar residues in its carbohydrate-binding site compared with that suggested for 2G12. ConA recognizes carbohydrate structures similar to the C-type lectin DC-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN), preferring interactions with core oligosaccharide with specificity for α-1–2- and α-1–6-linked residues, whereas cyanovirin-N and 2G12 interact with terminal carbohydrates displaying specificity for α1–2–linked Man residues. ConA can also recognize α1–2–linked Man residues; therefore, ConA can recognize a larger set of mannosyl residues on HIV-1 Env than 2G12 or cyanovirin-N. These fine recognition features may affect affinity/avidity of binding of these proteins as several sugar residues of an oligosaccharide can interact with an extended binding site as observed for ConA. Alternatively, higher affinity may also be affected by multiplicity of binding sites. In this latter case, the multiplicity can compensate for weak affinity of individual sites. Therefore, as antigens display many epitopes, the interaction of ConA with mannosyl glycan expressed on the Env protein represents a model of ligand presentation.

N-Linked glycosylation patterns in HIV Env are complicated by the fact that different cell types can affect glycosylation. DC-SIGN preferentially binds to the Env protein enriched for high mannose oligosaccharides, which are typically produced by peripheral blood mononuclear cells and T cells, compared with macrophage-produced GP120, which contains more complex carbohydrates, including modification by lac-
Peptide Mimotopes of HIV-1-associated Carbohydrates

Cross-blot—A dot-blot technique was used to analyze the binding of antibody fractions to GP120 and peptides (20, 21). Polyvinylidene difluoride membrane (Millipore) was prewetted and inserted in a Miniblotter (Immunetics, Cambridge, MA). Different antibodies were added to the channels and incubated overnight at 4 °C. The membrane was washed, blocked, and inserted again in the miniblotter at 90° relative to the original orientation. Different antibody fractions were added at 1.3 μM to the channels and incubated for 4 h at room temperature. Thus each antibody fraction (∼80 μl) was in contact with all areas of immobilized antigens. This technique creates spots of equal form and size and uses very small volumes of antibodies. After additional washes the entire membrane was incubated with an alkaline phosphatase conjugate, and the reactivities were developed using 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate (Sigma). The resulting pattern was scanned and digitalized using ImageJ software as described previously (21). The average staining of each antibody was measured by measuring the height of a 50 μm square area adjacent to the immobilized spot along the axis of the channel containing the antibody was used as a background and was subtracted from the value of the specific spot.

MATERIALS AND METHODS

Reagents—Peptides were synthesized as MAPs (Research Genetics, Huntsville, AL) by Fmoc (N-(9-fluorenyl)methoxycarbonyl) synthesis on polystyrene beads, resulting in the presentation of eight peptide clusters. Antibody 2G12 was kindly provided by Dr. H. Katgering. Recombinant GFP120 was supplied by the AIDS Research and Reference Reagent Program (CM235, catalog number 2968; STHHT95, catalog number 3234; BAL, catalog number 4961; CN54, catalog number 7749). The primary isotope envelope glycoproteins were expressed in insect cells, whereas the laboratory isolate was expressed in human embryonic kidney cells (25). The bound antibodies were eluted by 0.1 M sodium acetate, pH 5.5, at 20 μl/min to the desired surface density. Low surface density corresponded to 40–100 RU and high surface density 1000–2000 RU. Spare activated sites were blocked with 1.0 M ethanolamine, pH 8.5, for 6 min. Binding of ConA was measured at 25°C. Both MAP mimetics and ConA interacted very strongly with the dextran matrix of the CM5 sensor chip. To control these nonspecific interactions, the net negative charge of the sensor chip matrix was reduced by replacing the CM5 surface by the low carboxyl Biacore CM4 sensor chip (60% net negative surface charge reduction). Furthermore, the ConA nonspecific interaction with the matrix was diminished by using high ionic strengths (0.5 M NaCl) in 10 mM phosphate-buffered, 0.005% polysorbate-20 solution, pH 7.0.

The CM4 surface abolished nonspecific attachment of ConA in 0.5 M salt, without interfering with the specific interaction of ConA with the peptide mimetics. The flow rate was kept at 50 μl/min unless otherwise noted. Binding was measured at 25°C. Binding interactions were measured without added bivalent cations nor EDTA nor EGTA. Every binding interaction included a reference blank surface but no antibody or other control surfaces. Bound analyte was removed following each binding interaction using duplicate 5-μl pulses of regeneration solution at 100 μl/min. Binding as a function of concentration was measured in triplicate samples of ConA at concentrations of 14.4–460 nM per experiment in 2-fold dilution series. The experiment was repeated using two independently immobilized ConA-free MAP immobilizations. The specific binding of ConA to the N-mannosyl epitopes was detected using 3600 RU for 911 and 2600 RU for D002. The alternative experiment, in which biotinylated-ConA was captured on SA surfaces, was carried out in order to compare the influence of orientation on the binding kinetics of ConA to the monomeric and MAP forms of D002 and 911 peptides. Biotinylated ConA was immobilized to an SA-dextran sensor chip at a final density of 1300 RU, and a blank channel was used as a control for buffer bulk shift subtraction. Binding interactions were measured by the injection of 2-fold dilutions of D002 and 911 monomeric peptides in duplicate across the immobilized ConA chip surface, followed by dissociation in running buffer. Steady state affinity analysis of association and dissociation curves was conducted at peptide concentrations ranging between 32.5 nM and 10 μM.

Binding of a fixed concentration of ConA (57.5 nM) was competed by increasing concentrations of methyl α-D-mannopyranoside. This concentration was well below the concentration that gives half of the maximum binding signal in the surface titration of D002-binding sites, measured at steady state. To establish the specificity of the competition, the experiments included an identical competition experiment with methyl α-D-mannopyranoside as a negative control. All competition experiments were run twice in triplicate. Because both methyl α-D-mannopyranoside and lactose contribute heavily to bulk refractive index changes of the binding signal, all cycles were repeated in the absence of ConA, and the bulk signal of all concentrations of carbohydrate competitor used was subtracted from the signal generated with ConA present. Triplicate bulk data were gathered in a single experiment using the same MAP surfaces as the...
Peptide Mimotopes of HIV-1-associated Carbohydrates

RESULTS

Interactions of ConA with Peptide Mimetics MAP-D002 and MAP-911—The glycemic affinity of ConA greatly increases with the number of glycans bound (22). We have argued that mimicking epitope clustering would be an important general feature in immunogen design (15). As multivalent binding depends on the spacing of binding sites and the recognition of ligands presented in particular orientations, we designed a peptide that contains repeats of the RYRY sequence, YRYRGYRSGYGRYYRGYRSGS (referred to as peptide 911). This peptide and its monomeric form YRYRGYRSG display a free energy of association comparable with those reported for core trimannoside-ConA and pentasaccharide-ConA interactions (15). To test further the role of conformation in mimotope binding, we incorporated the putative RYRY sequence tract into a cyclic peptide form. This peptide (D002) has the sequence RGGLCYRCVRYCVCVGR, which forms disulfide bridges between the 1st and 4th and between the 2nd and 3rd cysteine residues. Peptide 911 was made as a MAP on an 8-lysine core, whereas the D002 peptide was made as a MAP on a 4-lysine core.

To assess binding behavior of the immobilized mannose mimetics, SPR assays were conducted using ConA in solution. The two MAPs studied differed both in cluster structure and monomer properties. The interactions of ConA with the surface immobilized MAPs deviate from simple models as expected; however, the average stability of the complex formed under the same conditions may be compared. The binding experiments were run simultaneously for the MAP-911 and MAP-D002, at surface densities of 2000 and 3600 RU, respectively. The dissociation constant was calculated from the separate fit of the dissociation phase to a single exponential model. At the lowest concentration of ConA, the complexes at the MAP-911 surface were more stable (k_{off} = 0.00179 \pm 6.3 \times 10^{-5} \text{ s}^{-1}) than the complexes formed with MAP-D002 (k_{off} = 0.005 \pm 1.4 \times 10^{-5} \text{ s}^{-1}). At the highest ConA concentration, the lectin off-rate from MAP-911 surfaces was faster (k_{off} = 0.00594 \pm 1.0 \times 10^{-4} \text{ s}^{-1}) than at the lower ConA concentration for the same surface (k_{off} = 0.00179 \pm 6.3 \times 10^{-5} \text{ s}^{-1}) and was very similar to that from MAP-D002 surface for the same concentration of ConA (k_{off} = 0.00585 \pm 2 \times 10^{-5} \text{ s}^{-1}). The maximum association responses measured at near equilibrium after 30 min for multiple ConA concentrations indicated these interactions were complex. Scatchard analysis (Fig. 1) of the relationship v/[ConA] = K_s/(1 - v), where v is the bound fraction of the ligand, is nonlinear for MAP-911 interactions (Hill coefficient >1.3) but hardly deviates from linearity for the MAP-D002 interaction. To demonstrate the complexity of ConA/MAP-911 binding is indeed the consequence of avidity, both MAP-911 and MAP-D002 were immobilized at the same high surface density to minimize the spatial distance of D002-binding sites (23). However, only ConA-MAP-911 interactions at low and high MAP surface densities were complex and denoted the avidity of the lectin interaction. MAP-D002-ConA interactions were linear despite the possible aggregation artifacts that have been described in interaction analysis by using high surface densities of ligand (24).

To dissect the relative importance of the MAP structure, the binding of 911 and D002 monomers to ConA was studied next in the inverse orientation using monomeric 911 and D002. Biotinylated lectin was immobilized on SA-dextran surfaces, and binding of duplicate peptide monomer samples was monitored at increasing concentrations. Because of the high rate of interaction, kinetic parameters could not be determined reliably, but the equilibrium constant was derived instead by...
steady state affinity analysis at peptide concentrations ranging between 312.5 nM and 10 μM (Fig. 2). The constrained peptide (D002) showed a higher affinity (Kₐ value of 2.61 × 10⁻⁶ M for the D002 versus 1.27 × 10⁻⁵ M for the 911 monomer), although the linear peptide (911) presented multiple mimetic motifs in its sequence. The Scatchard plot for both 911 and D002 was linear (Fig. 2B) with a Hill coefficient for 911 equal to 1.000008.

**Competition of ConA Binding to MAPs by α-D-Mannopyranoside**—The competition of ConA binding to MAPs by its specific α-D-mannopyranoside ligand in solution was performed by SPR to prove the specificity of the MAP peptide interactions and to estimate the differences in their avidity. The competition analysis included the negative control (lactose) to demonstrate that only the specific ligand of ConA can compete with MAP-911 and MAP-D002 binding. Fig. 3 shows the residual ConA binding as a function of competitor (methyl α-D-mannopyranoside) and control (lactose) concentration. The competitions were run using a single concentration of ConA (57.5 nM). Because the affinity of methyl α-D-mannopyranoside for ConA in solution is a constant parameter, the ratio between sugar concentrations that yield equivalent inhibition of binding to different surfaces was used as an indirect measure of the relative affinity of ConA to the clustered mimetics. A higher concentration of specific sugar was necessary to achieve the same level of competition on a MAP-911 surface as compared with MAP-D002 surface, indicating that avidity effects and not higher affinity are responsible for the tighter binding of MAP-911 to ConA.

**Inhibition of ConA Binding to GP120 by MAP-911 and MAP-D002**—Somewhat unexpectedly, the previous experiments demonstrated lower apparent affinity of the constrained mimotope MAP containing the same motif RYRY. This observation was further confirmed by comparing the inhibition of ConA binding to recombinant GP120 by the mimotopes MAP-911 and MAP-D002. The binding of ConA to recombinant GP120 from four different isolates (including three primary isolates and one laboratory isolate) was studied by ELISA. The plate was coated with 0.2 μg/ml GP120 from different isolates and was incubated with ConA at 7.7 nM in the presence of different concentrations of the MAP mimotopes. The inhibition curves (Fig. 4) for the four GP120 strains clustered closely for each inhibitor, but the IC₅₀ for MAP-D002 was ~100 times higher than that of MAP-911, suggesting that the avidity of ConA for MAP-D002 is lower than that for MAP-911.

**MAP-D002 and MAP-911 Immunization Both Elicit Envelope-reactive Antibodies**—To confirm the antigenic mimicry of MAP-D002 with Man-9, the main structure participating both in 2G12 and ConA epitopes, groups of mice were immunized intra-peritoneally three times at 2-week intervals with either MAP-D002 or MAP-911. Sera collected after the third immunization were tested in an ELISA for binding to Man-9 immobilized on ELISA plates. As seen in Fig. 5, both MAP-D002- and MAP-911-
immunized animals produced serum antibodies that recognized Man-9. Compared with MAP-911, MAP-D002 elicited higher titers of antibodies that recognized the Man-9 structure. We also observed that the cyclic MAP-D002 peptide induced serum antibodies that inhibited GP120 binding to human dendritic cells as effectively as serum antibodies induced by peptide MAP-911 (Fig. 6) at 1:100 dilution, but the effect was not observed at higher serum dilutions (data not shown).

Normal Human Antibody Repertoire Contains Antibodies Recognizing MAP-911 and MAP-D002—Mannose-binding antibodies in the human repertoire have been demonstrated previously. Here we identified antibodies that can also cross-react with the studied MAP mimotopes. Mixtures of human IgM and IgG were isolated on affinity columns of MAP-911 and MAP-D002, and their binding to the respective antigens was compared with that of the respective flow-through fractions by SPR (Fig. 7). For this purpose, MAP-911 and MAP-D002 were immobilized on CM5 chips, and the fractions studied were allowed to interact at 0.67 μM and 5 μM/min. The antibodies purified on the affinity columns clearly showed enrichment in specific activity for the nominal antigen, as compared with the flow-through fractions at the same protein concentration. The binding to MAP-D002 was much stronger than that to MAP-911 at comparable levels of immobilized peptide. Anti-MAP-911 antibodies also bound to MAP-D002 with almost the same affinity as anti-MAP-D002 antibodies, whereas the latter were not enriched in anti-MAP-911 activity.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use. The mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.

Because the repertoire of antibodies binding to a peptide probably contains a range of specificities complementary to different conformations (thus the more flexible the peptide the richer the repertoire recruited), an attempt was made to focus on the subset of anti-MAP antibodies that are cross-reactive with mannosyl residues. To this end, an agarose-coupled mannan column was used to isolated anti-mannan antibodies from the same human immunoglobulin for intravenous use.
up with a curved Scatchard plot much like we see in Fig. 1 for the 911 peptide. This is because of the presence of multiple modes of binding to the antigen with respect to valence. In the case of MAP-D002, it effectively behaves as a monovalent form.

It is typical that a curved Scatchard plot results from valence-based heterogeneity, whereas the linear Scatchard plot in Fig. 2 emphasizes the dependence of avidity on multivalency (Fig. 1). Comparing the binding of the monomers 911 and D002 to ConA showed slightly higher affinity of the constrained peptide over the linear one, whereas in MAP format 911 showed higher avidity in direct binding and lower IC50 values in GP120 inhibition assays (Fig. 2).

Most interestingly, the IgM antibodies elicited by MAP-D002 in mice bound to Man-9 in ELISA with higher avidity than those elicited by MAP-911 (Fig. 5), which may reflect an advantage of the rigid structure of D002 either in driving the process of antibody maturation or in a more faithful representation of the mode of binding of antibodies to Man-9. Indeed, modeling of the ConA interactions with 911 shows that the peptide interacts in an extended conformation with a region on the surface of ConA, which exceeds the boundaries of the carbohydrate-binding site (15). As far as DC-SIGN interactions are considered, serum from mice immunized with either peptide could inhibit GP120 binding to human DCs incompletely and to a similar degree (Fig. 6).

Pooled human immunoglobulin (IgM and IgG) for intravenous use (a representative repertoire of clinically healthy individuals) contained antibodies that bind to both MAP-911 and MAP-D002 (Fig. 7). In this case the spontaneous reactivities to MAP-D002 and MAP-911 bound both to MAP-D002, but only anti-MAP-911 bound to MAP-911. The higher apparent affinity of both fractions to MAP-D002 may be due to its constrained conformation. Thus, in humans a spontaneous (due to natural antibody or, more probably, to pre-exposure to xenogenic carbohydrate epitopes) antibody reactivity exists that may be represented by a range of specificities covering both mimotopes. A prime and boost strategy may capitalize on the existence of this set of clones, first recruiting a maximal repertoire range reactive with MAP-911 (and less so by MAP-D002) and further focusing on an antigen of interest (e.g. high density Man-9 epitopes such as those found on the silent face of HIV Env), because antibodies that recognize both mannans and the respective mimotopes are found naturally (Fig. 8). Alterna-
tively constrained conformation immunogens like D002 may be used in the boosting phase to select for higher affinity antibodies. These concepts are presently being tested.

Acknowledgment—We thank Charlotte Read Kensil of Antigenics Inc. for the QS-21.

REFERENCES
1. Ferrantelli, F., Hofmann-Lehmann, R., Rasmussen, R. A., Wang, T., Xu, W., Li, P. L., Montefiori, D. C., Cavacini, L. A., Kattinger, H., Stiegler, G., Anderson, D. C., McClure, H. M., and Ruprecht, R. M. (2003) AIDS 17, 301–309
2. Scanlan, C. N., Pantophlet, R., Wormald, M. R., Saphire, E. O., Calarrese, D., Stanfeld, R., Wilson, I. A., Kattinger, H., Dwek, R. A., Burton, D. R., and Rudd, P. M. (2003) Adv. Exp. Med. Biol. 535, 205–218
3. Lee, R. T., and Lee, Y. C. (2000) Glycoconj. J. 17, 543–551
4. Li, H., and Wang, L. X. (2004) Org. Biomol. Chem. 2, 483–488
5. Dudkin, V. Y., Orlova, M., Geng, X., Mandal, M., Olson, W. C., and Danishefsky, S. J. (2004) J. Am. Chem. Soc. 126, 9560–9562
6. Wang, L. X., Ni, J., Singh, S., and Li, H. (2004) Chem. Biol. 11, 127–134
7. Calarrese, D. A., Scanlan, C. N., Zwick, M. B., Deechongkit, S., Mimura, Y., Kunert, R., Zhu, P., Wormald, M. R., Stanfeld, R. L., Roux, K. H., Kelly, J. W., Rudd, P. M., Dwek, R. A., Kattinger, H., Burton, D. R., and Wilson, I. A. (2003) Science 300, 2065–2071
8. Lee, H. K., Scanlan, C. N., Huang, C. Y., Chang, A. Y., Calarrese, D. A., Dwek, R. A., Rudd, P. M., Burton, D. R., Wilson, I. A., and Wong, C. H. (2004) Angew. Chem. Int. Ed. Engl. 43, 1000–1003
9. Sanders, R. W., Venturi, M., Schiffner, L., Kalyanaraman, R., Kattinger, H., Lloyd, K. O., Kwong, P. D., and Moore, J. P. (2002) J. Virol. 76, 7293–7305
10. Pantophlet, R., Wilson, I. A., and Burton, D. R. (2003) J. Virol. 77, 5889–5901
11. Gattegno, L., Ramdani, A., Jouault, T., Saffar, L., and Gluckman, J. C. (1992) AIDS Res. Hum. Retroviruses 8, 27–37
12. I. M., and Mitchell, W. M. (1987) AIDS Res. Hum. Retroviruses 3, 285–282
13. Lin, G., Simmons, G., Pohlmann, S., Baribaud, F., Ni, H., Leslie, G. J., Haggarty, B. S., Bates, P., Weissman, D., Hoxie, J. A., and Doms, R. W. (2003) J. Virol. 77, 1337–1346
14. Agadjanyan, M., Dam, T. K., Luo, P., van Vliet, S. J., Vandenbreuck-Grauls, C. M. J. E., Geijtenbeek, T. B. H., and van Koyck, Y. (2003) J. Immunol. 170, 1635–1639
15. Cunto-Amesty, G., Dam, T. K., Luo, P., Monzavi-Karbassi, B., Brewer, C. F., Van Cott, T. C., and Kieber-Emmons, T. (2001) J. Biol. Chem. 276, 30490–30498
16. Agadjanyan, M., Luo, P., Westerink, M. A., Carey, L. A., Hutchins, W., Steplewski, Z., Weiner, D. B., and Kieber-Emmons, T. (1997) Nat. Biotechnol. 15, 547–551
17. Monzavi-Karbassi, B., Cunto-Amesty, G., Luo, P., Lees, A., and Kieber-Emmons, T. (2001) Biologics 29, 249–257
18. Monzavi-Karbassi, B., Shamloo, S., Kieber-Emmons, M., Jousheghany, F., Luo, P., Lin, K. Y., Cunto-Amesty, G., Weiner, D. B., and Kieber-Emmons, T. (2003) Vaccine 21, 753–760
19. Monzavi-Karbassi, B., Luo, P., Cunto-Amesty, G., Jousheghany, F., Pushov, A., Weissman, D., and Kieber-Emmons, T. (2004) Arch. Virol. 149, 75–91
20. Ray, S., Ghirlando, R., Yamaguchi, M., and Muramoto, K. (2004) J. Mol. Recognit. 17, 127–134
21. Robinson, W. E., Jr., Montefiori, D. C., and Mitchell, W. M. (1987) AIDS Res. Hum. Retroviruses 3, 285–282
22. N., and Chermann, J. C. (1995) J. Exp. Med. 182, 149–158
23. Poluektov, A., Oldfoss, S., Sjogren-Janssen, E., Jeansson, S., Sjolund, I., Akerblom, L., Hansen, J. E., and Hu, S. L. (1992) J. Gen. Virol. 73, 3999–3105
24. Turville, S. G., Arthos, J., Donald, K. M., Lynch, G., Naif, H., Clark, G., Hart, D., and Cunningham, A. L. (2001) Blood 98, 2482–2488
25. van Koyck, Y., and Geijtenbeek, T. B. (2003) Nat. Rev. Immunol. 3, 697–709
26. Pantophlet, R., Wilson, I. A., and Burton, D. R. (2003) J. Virol. 77, 5889–5901
27. Gattegno, L., Ramdani, A., Jouault, T., Saffar, L., and Gluckman, J. C. (1992) AIDS Res. Hum. Retroviruses 8, 27–37
28. Ezekowitz, R. A., Kuhlman, M., Gropman, J. E., and Byrn, R. A. (1989) J. Exp. Med. 169, 185–196
29. Kieff, E., and Chermann, J. C. (1995) AIDS Res. Hum. Retroviruses 11, 87–95
30. Hansen, J. E., Nielsen, C. M., Nielsen, C., Hveegaard, P., Mathiesen, L. R., and Nielsen, J. O. (1992) J. Gen. Virol. 73, 9560–9562
31. Bolmstedt, A., Olofsson, S., Sjoblom, I., Kehayov, I., Hristova, S., Lacroix-Mens, T., Giltiay, N., Kazatchkine, M. D., and Kaveri, S. V. (2002) Int. Immunol. 14, 453–461
32. Dam, T. K., Roy, R., Das, S. R., Oscarsen, S., and Brewer, C. F. (2000) J. Biol. Chem. 275, 14223–14230
33. Mulder, K. M., Arndt, K. M., and Pluckthun, A. (1998) Anal. Biochem. 261, 149–158
34. Mygind, N., Skov, J., and Hripcsak, G. (2001) Anal. Biochem. 297, 248–254
35. Myszka, D. G. (1999) J. Mol. Recognit. 12, 279–284
36. Carlen, S., and Roderick, R. (2002) Org. Biomol. Chem. 17, 2543–2549
37. Anderson, D. C., McClure, H. M., and Ruprecht, R. M. (2003) AIDS 17, 301–309
38. Lee, R. T., and Lee, Y. C. (2000) Glycoconj. J. 17, 543–551
39. Lees, E. M., and Chermann, J. C. (1995) J. Exp. Med. 182, 149–158