Multiple Antigenic Mimotopes of HIV Carbohydrate Antigens

Relating Structure and Antigenicity

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Carbohydrate mimetic peptides are designable, and they can carry T-cell epitopes and circumvent tolerance. A mimic-based human immunodeficiency virus (HIV) vaccine can be a viable alternative to carbohydrate-based antigens if the diversity of epitopes found on gp120 can be recapitulated. To improve existing mimics, an attempt was made to study the structural correlates of the observed polyspecificity of carbohydrate mimetic peptides based on the Y(P/R)Y motif in more detail. A carbohydrate mimetic peptide, D002 (RGGLCYCRYRYCVGKR), bound a number of lectins with different specificities. Although this peptide reacted strongly with both lotus and concanavalin A (ConA) lectins, it bound to lotus stronger than ConA. By varying the central motif RYRY, five versions were produced in multiple antigen peptide format, and their avidity for lotus and ConA lectins was tested by surface plasmon resonance. Although the kinetic parameters were similar, the version based on the central motif RYRY had an optimal affinity for both lectins as well as improved avidity for wheat germ agglutinin and phytohemagglutinin. Thus, as far as lectin specificity is concerned, YPYRY had improved multiple antigenic properties. Both RYRY and YPYRY precipitated antibodies from human IgG for intravenous use that bound to gp120 in vitro and immunoprecipitated gp120 from transfected CHO-PI cells. Thus, Y(P/R)Y motifs mimic multiple carbohydrate epitopes, many of which are found on HIV, and preimmunize human IgM antibodies that bind to HIV carbohydrates cross-react to a comparable extent with both RYRY and YPYRY carbohydrate mimetic peptides.

Glycosylation accounts for roughly 50% of the molecular mass of the major envelope (Env) protein of human immunodeficiency virus type 1 (HIV-1) (1). In its native quaternary conformation Env exposes mostly its glycosylated surface. The latter represents a diverse array of glycans, dominated by N-linked structures (high mannose, complex, or hybrid structures) and sialosyl residues (2–4). Early observations that lectins and carbohydrate-reactive antibodies inhibit virus entry into cells (5–9) highlight the potential importance of targeting Env-associated glycans in vaccine strategies. This notion is further supported by the existence of a human neutralizing monoclonal antibody 2G12 with protective activity against viral challenge in animal models (10), which binds a virus-specific conformational epitope in the cluster of oligomannose glycans of gp120 Env (11).

Such an antibody response could be a viable strategy for a vaccine if it could induce broadly reactive anti-HIV responses (12). However, targeting a set of carbohydrate structures as diverse and variable as the ones found on Env of HIV may be daunting. It will require a multivalent vaccine approach to induce antibodies with polyreactivity for the diverse glycoforms of Env (13–15). An alternative approach to induce polyreactive antibodies is based upon carbohydrate mimic peptides (16). Using peptide mimics of carbohydrates as immunogens offers the possibility of designing T-cell-dependent immunogens for eliciting stronger, long term carbohydrate-reactive antibody responses (16–21). Peptide mimics are also easier to synthesize and modify than carbohydrate antigens.

We have observed that similar structural motifs can mimic Env-associated mannose and fucosylated lactosamines (22–24). The YPY motif was proposed as a mannose mimic (19, 25). A similar motif, YRY, was initially implicated in the mimicry of the major C polysaccharide, a 2-9 sialic acid (major C polysaccharide) of Neisseria meningitidis (26). We have further optimized mimotopes on the basis of YRY (e.g. in peptide 911YRYRGRYSGGRYRGRYS) in terms of binding to concanavalin A (ConA) and eliciting anti-mannose antibodies (22, 23). Recently, we demonstrated that the YRY containing cyclic peptide D002 (RGGLCYCRYRYCVGKR) is a mimic of oligomannose antigens that elicit antibodies reactive to oligomannose-9, and serum of D002 immunized mice was able to suppress the binding of HIV gp120 to human dendritic cells (27). At the same time, the peptides 105 (GGIYYPYDIYYPYDVYPYDIYYPYDIYYPYDV) and 107 (GGIYRYDIYRYDIYRYD) have been found to mimic a large variety of fucosylated lactosamines (16, 23, 24). Carbohydrate mimetic peptides based on the YYRY and the YYPY motifs conjugated to proteasomes elicit antibodies that bind Env of HIV-1 and inhibit HIV infection of target cells.
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(16). Thus, mimicry of diverse carbohydrate antigens is built on a small set of amino acids.

Most probably these residues interact with carbohydrate-binding sites on antibodies and lectins through a degenerate alphabet of simple bonding patterns, mostly of hydrogen bonds and \( \pi \)-stacking, that are common with carbohydrate antigens. Therefore, refining the specificity of the mimotopes with respect to the target structures (but not for the templates) may be impossible (29), and it may be advisable rather to use a controlled level of degeneracy of recognition to provide structures that elicit a repertoire of antibodies of predetermined polysizepecificity (multiple antigen mimotopes (MAM)) (30). Previously we have shown that different peptide motifs rank in their lectin polyreactivity in the following order: RYRY > YYRYD > YPYRD > YWRYD (28). It is possible that such carbohydrate mimics elicit a broader range of anti-carbohydrate antibodies than predicted from the initial characterization of these motifs. Here we further explore the possibility of using Y(R/P)Y-based carbohydrate mimetic peptides as MAM for HIV vaccine development by studying the polyreactivity of these motifs.

MATERIALS AND METHODS

Reagents—Biotinylated *Griffonia simplicifolia* lectin I (catalog number B-1105), biotinylated peanut agglutinin (catalog number B-1075), biotinylated *Vicia villosa* lectin (VVL) (catalog number B-1235), biotinylated jacalin (catalog number B-1155), biotinylated *Lotus tetragonolobus* lectin (catalog number B-1325), biotinylated PHA-E (catalog number B-1125), biotinylated *Erythrina cristagalli* lectin (catalog number B-1145), biotinylated *Sambucus nigra* lectin (catalog number B-1305), and biotinylated ConA were obtained from Vector Laboratories (Burlingame, CA). Gp120MN was from Advance Biotechnologies Inc. (Columbia, MD; catalog number 14-129-050).

The peptides RYRY (RGGLCYCRYRVCVCG; D002), PYRY (RGGLCYCPYRVCVCG), PYPY (RGGLCYCPPY-CVCVGR), YPYR (RGGLCYCYPYRVCVCG), YRYPY (RGGLCYCYPYPCVCVGR), and 911 (YRYRYRYSRYSRYSRYSRYS) were synthesized as 8-mer multiple antigen peptides (MAPs) by Biosynthesis, Inc. (Lewisville, TX).

Cell Lines—Human immunodeficiency virus Env protein (gp120) expressing Chinese hamster ovary (CHO-PI) (catalog number 2284) cells and CHO cells transfected only with an empty vector (CHO-EE) (catalog number 2238) were from AIDS Research and Reference Reagent Program, National Institutes of Health (31). CHO-PI cells are cotransfected with HIV-1 env and rev expression vectors. CHO-PI expresses the HXB2 envelope protein with a glycoprophosphatidylinositol anchor. The HXB2 env gene lacks complete rev and tat genes and was introduced using the vector pEE14 (Celltech), which expresses glutamine synthetase. The cells were cultured according to the protocol available in the data base.

ELISA—For the lectin panel binding, 4HBX microtiter plates (Immulon) were coated with 5 \( \mu g/ml \) (0.3 \( \mu M \)) peptide RYRY or YPYRY in methanol overnight at room temperature by evaporation. After blocking with 0.5% fetal bovine serum in PBS, 0.05% Tween 20, biotinylated lectins were incubated in the microtiter plates at dilutions between 50 pm and 10 \( \mu M \) in duplicate wells. Streptavidin-peroxidase conjugate was used as a secondary reagent with \( p \)-nitrophenyl conjugate as a substrate, and the absorbance was read at 405 nm. Saturation binding curves (of the type: concentration \( \times \) maximal level/(\( K + \) concentration) + background) were fitted to the experimental data with the help of the nonlinear regression unit of the Statistica for Windows (StatSoft, Tulsa, OK).

Anti-human IgG conjugates of alkaline phosphatase were used as secondary reagents, and 0.1% \( p \)-nitrophenyl phosphate in 0.1 M diethanolamine, pH 9.5, was used as a chromogenic substrate and read at 405 nm. All binding experiments were carried out at room temperature.

Biosensor Experiments—Because of the highly hydrophobic nature of the multiple antigenic peptides used, the assay was designed following the method used by MacKenzie et al. (32). The peptides were coupled to a hydrophobic biosensor chip HPA, covered with a lipid monolayer. The liposome suspension was prepared by drying in a rotary evaporator 4 ml of 7.5 mM solution of dimyristoylphosphatidylcholine (Sigma) in a 1:1 mixture of chloroform and methanol followed by addition of 60 ml of distilled water to the flask and sonication for 15 min at 37 °C. The liposome suspension was cleared by centrifugation for 10 min at 4000 \( \times g \), aliquoted, and frozen. The surface plasmon resonance binding assay was carried out using Biacore 3000 (Biacore, Uppsala, Sweden). The surface of an HPA chip (Biacore, Uppsala, Sweden) was washed by a 5-min injection of 40 mM \( n \)-octyl \( \beta \)-D-glucopyranoside followed immediately by the injection of the liposome suspension at 2 \( \mu l/min \). The liposome layer was considered continuous when the sensogram reached a plateau. To stabilize the lipid-coated surface, the liposome injection was followed by a 3-min injection of 10 mM NaOH. This procedure yielded typically a signal of 1500–2000 resonance units. Solutions of lophylized MAP dissolved in distilled water were injected over the surface of the chip to at ligand density 800 resonance units.

Lectins were then injected at 20 \( \mu l/min \) flow rate in HBS-N buffer (Biacore) for 4 min at a flow rate of 20 \( \mu l/min \) (ConA, 200, 300, 400, and 500 nM or lotus lectin-6, 18.7, 31.2, and 43.7 nM) followed by 4 min of dissociation phase before regeneration. The surface was regenerated using 10 mM NaOH delivered in three pulses of 4 min each. The sensorsgrams of the binding were analyzed after subtracting the binding to a liposome-covered channel with no peptide. Data preparation for analysis and fitting of the direct binding data were performed using BIAEvaluation 3.1. The lectins displayed complex kinetics of binding to the studied peptides, which sometimes required fitting using the bivalent analyte model and mass transfer. In the cases when bivalent binding model was used, only the set of the constants for the first phase were used. The \( \chi^2 \) criterion of the goodness of fit varied between 0.1 and 1.4 for the different experiments.

Precipitation of Mimotope-reactive Human IgG Antibodies—Human IgG binding to RYRY and YPYRY was isolated from the intravenous immunoglobulin preparation (IVlg) Sandoglobulin (Novartis) by precipitation. After extensive dialysis against PBS to eliminate the sugar excitant, 100 mg of IVlg were mixed with 0.4 mg of the MAP of interest slowly dissolved in distilled
water with constant mixing. The mixture was incubated for 30 min at 37 °C before adding polyethylene glycol 4400 to 3.5% and incubating further for least 4 h at 4 °C. The precipitate was collected by centrifugation, rinsed, and dissolved in 30 ml of 0.05 M glycine/HCl buffer, pH 2.7. This solution was concentrated in Amicon ultrafiltration concentrators (Millipore, Billerica, MA) with molecular mass cutoff of 100 kDa, resuspended in the same buffer, and concentrated again three times to separate the lower molecular weight MAP from the IgG. The resulting solution was dialyzed against PBS and concentrated again to 0.5 mg/ml IgG. No MAP was detectable in the dialyzed samples as analyzed by SDS-PAGE.

**Cross-blots**—A dot-blot technique was used to analyze the binding of antibody fractions to gp120 and peptides (27, 33). Polyvinylidene difluoride membrane (Millipore) was pre-wetted and inserted in a miniblotter (Immunetics, Cambridge, MA). Recombinant gp120 (SF162, 7363, AIDS Reagents, National Institutes of Health) was added to the channels and incubated overnight at 4 °C. The membrane was washed, blocked for 1 h at room temperature with PBS, Tween containing 2% bovine serum albumin, 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. 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). In the inhibition experiments the 0.33 μM of the antibodies were mixed with 2500 μM of different sugars. The resulting pattern was scanned and digitized using Image software as described previously (33). The average staining of the area adjacent to the analyzed 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.

**Immunoprecipitation**—CHO-PI or CHO-EE cells were incubated with 0.2 mg/ml RYRY or YPYRY precipitated fractions of IVlg or nonfractionated IVlg, washed, lysed, and cleared by centrifugation at 10,000 × g. The clear extract was incubated with 20 μl of protein G-Sepharose beads (Sigma), which were washed and boiled with sample buffer, 1% Nonidet P-40, pH 7, with a protease inhibitor mixture (Pierce). The proteins were resolved by SDS-PAGE on a 4–12% BisTris gradient gel (Invitrogen), blotted onto PVDF membrane by semi-dry transfer, and probed with deglycosylated gp120 Env2-3-specific serum (AIDS reagents). Peroxidase-conjugated secondary antibody was used to detect the binding of the anti-gp120 antibody, and the reaction was developed by enhanced chemiluminescence.

**Statistics**—The staining intensities in the cross-blot experiment were compared using Mann-Whitney U test (p < 0.05). The half-maximal binding of lectins in MAP ELISA was compared using the standard error of the estimate provided by Statistica for Windows (StatSoft). The logit linearized data for the binding of 2G12 to peptides were compared by General Linear Models (GLM) followed by post hoc pair wise comparisons Statistica (StatSoft) with significance level of p < 0.05.

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**RESULTS**

**RYRY Is a Polyreactive Mimotope**—The reactivity of the mannose mimotope peptide RYRY (also known as D002) with ConA has been studied previously (27). To determine the degree of specificity of the mimicry, we further tested the reactivity of RYRY with a panel of lectins (Fig. 1 and Table 1) with specificity relevant to the repertoire of carbohydrate epitopes.
found on HIV-1 Env. Microtiter plates were coated with RYRY and incubated with serial dilutions of different biotinylated lectins. The concentrations of lectins that yielded half-maximal binding were used to compare the binding of the diverse biotinylated ligands. All lectins tested bound to the peptide but the apparent affinities (which could be assessed only comparatively) grouped in three groups with approximately 1 order of difference. The maximal levels of binding differed (lotus > PHA > ConA > MAA > GS-1 > VVL > WGA) with lower maximal binding seen with the lectins with higher half-maximal binding concentration (data not shown).

Effects of YPY/YRY Motif Permutations on Lectin Cross-reactivity—Based on the similarity of proline- and arginine-based motifs, the effect of concatenating and permutating the YRY/YPY motifs on cross-reactivity for LeX and mannose was further explored. Five variants were synthesized that had the same flanking regions (RGGLCYCXXXCCVCVGR) responsible for the cyclization but with the following core motifs: PYPY, YRYPY, YPYRY, YPYPY, and PYRY. To quantitate the binding of each peptide to ConA and lotus lectin, the kinetic parameters of the interaction were determined next by surface plasmon resonance (Fig. 2). The peptides in MAP format were adsorbed on a hydrophobic biosensor chip HPA covered with a liposome monolayer. The lectins were injected over the surface thus prepared in different concentrations. The parameters are based on the calculations from two matrices.

The kinetic parameters showed dependence on the concentration for the two lower concentrations (data not shown); therefore, the comparison was performed based on the mean of the values for the two higher concentrations of each parameter. At these two concentrations, the values of each of the parameters for a given peptide converged.

All peptides had high apparent affinity for both lectins (\(K_D\) in the range 0.2–8.3 nM). Lotus lectin bound to the peptides with higher association rates and higher affinity than ConA, but the dissociation rates were comparable on average (Fig. 2). The high association rates of YPYPY and YPYRY and the very low dissociation rate of YRY contributed to the higher affinity these three peptides had for lotus as compared with PYPY, PYRY, and YRYPY. The pattern of avidities of these two lectins with the panel of RYRY versions differed with RYRY ranking first in binding to lotus and 4th in binding to ConA. Ranking the peptides according to affinity showed that YPYRY had, on the average, higher ranks for both lectins than the rest of the peptides. Although the parameters varied specifically for the two lectins the avidities of all peptides were close.

Lectin Binding Profile for YPYRY Compared with RYRY—The changes in the binding to a panel of lectins that accompanied the modification of the main motif of the peptide from RYRY (D002) to YPYRY were analyzed next. The concentration yielding half-maximal binding to YPYRY of each panel of lectins was determined by ELISA as done previously for RYRY (Figs. 3 and 4 and Table 2). Maximal binding capacity could indicate the abundance of the mimicking conformation in the ensemble, and indeed lower affinity lectins also showed lower maximal values. In this experiment, however, the conclusion is formally imprecise because of possible differences in the specific biotin activity in the conjugates of the different lectins. Therefore, we considered only the concentrations yielding half-maximal binding as an invariant parameter of binding. Thus, the range of apparent affinities was the same for both peptides, but the ranking of the lectins was altered. For both peptides there were two groups of lectins with markedly different apparent affinity. Three of five lectins in the high binding group for YPYRY had improved binding compared with RYRY. Lotus lectin binding was reduced but remained in the range of high binding lectins because its avidity for RYRY exceeded considerably that of any other tested lectin. Thus, the YPYRY motif had an increased avidity for three of five lectins (WGA, ConA, and PHA) as com-
pared with the prototype RYRY-containing peptide, and at the same time retained high binding to two others (jacaline and lotus), which is considered an improvement of its MAM polyspecificity because these lectins represent diverse specificities.

The Normal Human IgG Repertoire Contains Antibodies Cross-reactive with Mannose/Fucose MAM and gp120—Previously we showed that IVIg as pools of human IgG and IgM, representative of the repertoire in the healthy human population, contain antibodies that cross-react with the RYRY-D002 peptide and HIV Env (27). We used the same approach to compare the antigenic properties of the RYRY and YPYRY motifs. The human IgG antibodies were precipitated from dialyzed Sandoglobulin by adding water solutions of the MAP peptides and using polyethylene glycol to precipitate the immune complexes. The precipitate was centrifuged, washed, dissolved in glycine/HCl, pH 2.7, and dialyzed by repeated concentration by

### TABLE 2

| Lectins | YPYRY Mean | YPYRY S.D. | RYRY Mean | RYRY S.D. |
|---------|------------|------------|------------|------------|
| Lotus   | 64.3       | 12.2       | 13.8       | 4.7        |
| PHA     | 19.2       | 3.4        | 49.4       | 14.2       |
| Jacaline| 52.7       | 15.2       | 55.3       | 95.6       |
| MAA     | 701.5      | 89.3       | 75.7       | 32.1       |
| ConA    | 32.3       | 5.7        | 79.9       | 41.6       |
| WGA     | 50.6       | 12.3       | 352.0      | 146.1      |
| VVL     | 908.3      | 25.4       | 535.2      | 175.4      |
| GS-1    | 683.2      | 78.7       | 592.3      | 124.4      |
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microfiltration (cutoff 100 kDa). Finally the antibodies were dialyzed against PBS. These antibodies were compared with antibodies isolated from IVIg using a mannan-agarose column. All fractions, including nonfractionated IVIg, were tested at 0.1 mg/ml against RYRY, YPYRY, and gp120 in cross-blot. The reactivities were measured densitometrically (Fig. 5). All fractions were enriched in reactivities to the isolating antigen and to gp120. Although both peptide-reactive fractions cross-reacted with the alternative peptide, anti-YPYRY antibodies appeared to have higher avidity that is consistent with the higher association rate found in the SPR experiment. All three fractions had similar reactivity to gp120 with anti-mannan antibodies nonsignificantly exceeding the other two fractions.

Because the isolated antibodies could also be binding to protein epitopes on gp120, the participation of carbohydrate structures in the epitopes on gp120 was confirmed by inhibition assay using fucose, GalNAc, and α-methylmannopyranoside (molar ratio 1:15,000) in cross-blot (Fig. 6). These three sugars represent the diverse nature of the N-associated glycan moieties. The binding to gp120 of both antibody fractions was inhibited by all three sugars. The high inhibitor to ligand ratios were necessary to overcome the high avidity of the binding. These data further suggest that IVIg contains mimotope-recognizing antibodies that cross-react with gp120, and their binding to the latter is dependent on a variety of carbohydrate structures.

Because the presentation of gp120 adsorbed on polystyrene microtiter plates or on PVDF membrane may vary considerably compared with the membrane presentation of the oligomer, the binding of the isolated fractions of IgG to gp120 was confirmed next by immunoprecipitation of membrane antigens bound by the antibodies. The studied antibodies were incubated with CHO-PI or CHO-EE cells, and the immune complexes were immunoprecipitated after washing the antibody-treated cells and extracting by Nonidet P-40-based lysis buffer. Protein G-Sepharose beads were added to the lysates, incubated, washed, and eluted by boiling in reducing sample buffer. The proteins thus precipitated were resolved by SDS-PAGE in a 4–12% gradient gel and subjected to Western blot. Mouse Env2-3-specific serum was used to detect the presence of gp120 in the immunoprecipitated material (Fig. 7). A band of 110 kDa, recognized by the gp120-specific antibody, was present only in the precipitate of the CHO-PI but not in the CHO-EE cell lysates and in none of the lysates of cells treated with nonfractionated IVIg at the same IgG concentration.

Lectin Data Do Not Predict Binding by the Human Antibody 2G12—Because the broadly neutralizing human antibody to gp120-2G12, binds to a mannose epitope on the lectin shield, we next tested the predictions of the lectin binding experiments with respect to the capacity of the studied peptides to mimic the 2G12 epitope. Surprisingly, 2G12 showed significant binding only to the peptide PYPY (Fig. 8). The binding curves were compared by GLM followed by post hoc comparisons.

DISCUSSION

The binding of a number of different lectins to D002 peptide supported the hypothesis that the YRY motif does not specifically mimic mannose, but it can also be a basis
for carbohydrate mimicry of broader specificities. The potential of YPY and YRY motifs to mimic multiple carbohydrate epitopes was demonstrated by the strong binding of both ConA and lotus lectin to five cyclic peptides, variants of D002 RGGLC YCRYRCVCGVR, in which the core motif of YRY was replaced by PYPY, PYR, YRY, PYRY, and YPY. The measured kinetic parameters varied within a narrow range. This indicates that the mimicry is related either to the flanking regions or to the composition rather than to the exact sequence of the core motifs.

Carbohydrate-binding proteins often use multiple binding sites, which compensate for the low intrinsic affinity. This produces graded responses with a single type of interaction and increases the specificity of the recognition (34, 35). Although avidity measurements are sensitive to many factors like ligand density, analyte concentration, mobility in the lipid membranes, etc., SPR with artificial membrane-type chips provide an environment closer to the conditions for the protein/carbohydrate interactions on the cellular membrane. The peptides used by us were in a MAP format, and their multimeric nature and the solid phase provided conditions for avidity binding, similar to the lectin/carbohydrate interactions. The presence of avidity binding was supported by the complex binding curves and the high apparent affinities ($K_D$) that were in the same range as those measured for the binding of H2G10 antibody to asialog-GM1 immobilized in liposomes on HPA chip by Harrison et al. (36).

The range of the apparent association rate constants was also comparable with that of some bivalent anti-carbohydrate antibodies (37). The affinities of the binding of ConA and lotus lectin to the RYRY and YPYRY peptides are in the same range or up to an order higher than those for carbohydrate polymers (38, 39). At the same time the affinity of ConA for a YRY-based linear MAP 911 is very close to that of the pentasaccharide ligand, 2.6 $\times$ 10$^4$ M$^{-1}$ (22). Studying WGA binding, Shinohara et al. (40) also observed that immobilizing the sugar ligand favors avidity binding. Thus, our data are consistent with avidity interaction with intrinsic constants and manner of multiple binding similar to those of the lectin/carbohydrate interactions.

ConA has been found to form a complex mesh when binding to multimeric polysaccharide molecules (38). It is possible that the lectins bound the MAPs in the lipid layer forming similar mesh, which may be facilitated by the lateral mobility of the MAP molecules in the lipid layer thus presenting a system very similar to the membrane-bound glycoproteins.

ConA and lotus had different patterns of kinetic parameters when binding to the array of RYRY variants. Lotus lectin had better binding to all peptides due to higher association rates. The motifs YPPY and YPYRY had the highest association rates for lotus, followed by PYRY, whereas PYRY had the lowest indicating the preference for three tyrosine residues and a possible entropic advantage of the more rigid structure of the PPY trimer.

Those peptides that had lower association rates for lotus lectin (YRPY, PYRY, and YPPY) bound ConA with higher association rates indicating some lectin specificity. The motif YRPY had optimal apparent affinity to both lectins and was considered a better candidate for a multiple antigenic mimotope. Because the mechanisms of its binding to lotus and ConA seemed different (the association rates for lotus lectin were 29 times higher than for ConA), it was hypothesized that YPYRY may represent an example of polyspecific structure that captures multiple salient features of the carbohydrate epitopes and presents different subsets of these features in different molecular interactions. This hypothesis was supported also by the pattern of reactivity of YPYRY with the panel of lectins used to test RYRY polyspecificity. The difference in the two patterns indicated specific propensities for each of the two peptides to mimic different carbohydrate epitopes. RYRY reactivity seemed skewed to lotus lectin, whereas YPYRY bound better to four of five lectins binding RYRY strongly and a more uniform avidity for that group, whereas the difference from the low binding group was increased. Thus, the YPYRY-based peptide is potentially a better MAM than the one based on the RYRY motif.

By definition, mimicry is but an approximation of the target structure, and those parts of the mimic that differ from the target could engage in additional bonds. In this way peptides that mimic carbohydrates can exhibit specificity for the template, used to select or construct them, by bonding that is not
related to the target (29). In other cases the imperfect similarity to the target is accompanied by a structural and functional mimicry of several different targets (antigens) (41). It seems that some degree of polyspecificity is intrinsic to both carbohydrate recognition (42–44) and carbohydrate mimicry (41). It is possible that improving the MAM character of a peptide mimic also leads to a better fidelity of the carbohydrate mimicry by changing the ratio of carbohydrate emulating versus template-specific bonding and or conformations. The existence of conformational epitopes, common for structurally distinct carbohydrates, has been discussed previously (16). Proteins interact with carbohydrates mostly through hydrogen bonds and pyranose π-stacking. To mimic this interaction it is necessary to provide the same bonding pattern by a peptide. This may not be trivial for most of the amino acids because of the lower number of hydrogen bonds per amino acid compared with the monosaccharides. Only arginine seems to have advantage in this respect. On the other hand proline provides no hydrogen bond but has a spatial similarity with a pyranose ring that also makes it preferable (21). It also reduces the flexibility of the peptides favoring existing mimotope conformations. Thus, the capacity of the YPYRY motif to mimic multiple carbohydrate epitopes is understandable. At the same time the dissimilar kinetic data for YPYRY and YRYPY indicates a level of structural specificity and/or a role for the flanking sequences in the recognition.

Previously we showed that IgG and IgM preparations for intravenous use, as representative probes of the human antibody repertoire, contain antibodies that cross-react with mannan, RYRY, and gp120 (27). Although the gp120 binding may occur through superantigen type of interaction with the most abundant family V,3 (45, 46), the small yield (low proportion of highly reactive antibodies) and the correlation of this reactivity with anti-carbohydrate reactivity argues in favor of a more specific binding. Thus, the naive human repertoire contains B-cell clones that may produce an effective gp120 response by an appropriate immunization. Expanding this paradigm, the capacity of RYRY and YPYRY peptides to precipitate from a human IgG preparation (Sandoglobulin) antibodies that are enriched both in peptide and gp120 reactivity was demonstrated and compared with that of mannan. The possibility of activating the polyreactive antibodies by low pH treatment is discussed elsewhere (47), but in a similar setting it has been shown that affinity purification indeed selects specific antibodies as the low pH treatment of the effluent does not induce reactivity, comparable with the isolated antibodies (33). Because of the low probability for IgG to originate from B1 cells, finding anti-mimic preimmune IgG antibodies raises the chances that B2 clones exist that can be boosted by a vaccine. Furthermore, the binding of the isolated IgG to a CHO cell line expressing gp120 demonstrated that the specificity of the antibodies might be immunologically relevant, although the antibodies bound also to nontransfected cells (data not shown). RY-specific mouse serum, which inhibited the binding of gp120 expressing CHO cells to dendritic cells, was also found to bind better but not specifically to CHO-PI cells as compared with CHO-EE cells (30). Therefore, the recognition of gp120 on CHO cells was confirmed by immunoprecipitation using human preimmune anti-MAM IgG and Western blotting with gp120-specific antibody. The lack of differences between preimmune human IgG antibodies recognizing RYRY and YPYRY peptides in these tests may be due to the polyreactivity of both the MAM and the recognizing antibodies. On the other hand 2G12, a mannos-binding and gp120-specific human IgG antibody, bound only to the PYPY version and not to RYRY or YPYRY. So far this is the first carbohydrate mimetic peptide that 2G12 binds to in our hands, although we have not proven the competition of PYPY with oligomannose 9 yet. It is possible that this structure has properties that facilitate antibody binding, because an anti-LeX antibody-E5, bound best to it too.3

A strong anti-carbohydrate response could exert a pressure on the virus to reduce its glycan shield and expose more inhibitory epitopes. The presence of preimmune antibodies that bind to the glycan shield in the normal human repertoire itself may be a factor shaping the evolution of the virus before the development of specific responses. Although natural anti-HIV IgM antibodies derived from phage display library (48) and by immunoaffinity purification (49) were found to have no neutralizing capacity in vitro, polyclonal anti-carbohydrate antibodies do neutralize (50–52), and natural antibodies have been found to exert profound effect on survival in influenza infection in mice (53). In vivo, natural, or cross-reactive anti-carbohydrate antibodies may interfere rather with cell adhesion to dendritic cells (30). In the course of the infection, the number of N-glycosylation sites on HIV Env increases (54). We hypothesize that soon after infection the virus adapts to the first line of defense, including anti-carbohydrate antibodies, by reducing the number of N-glycosylation sites, but later the changes in the repertoire of antibodies (55) probably dictates expansion of the glycan shield. The changes in the density of the glycan cluster may indicate anti-carbohydrate selection pressure occurring very early in the infection. At the same time it is obvious that alone innate immunity mechanisms are insufficient to maintain long and potent pressure on the glycan shield composition. The early isolates have 25–28 sequons as compared with 30–33 in late isolates from the same patient (54), whereas virus adapted to high concentrations of mannose-binding lectins has only 16 sequons (56). Therefore, the strategy outlined above may help amplify adaptive anti-carbohydrate responses to the point that they impose a substantial reduction of the glycan shield (similar to the one caused by lectins or even stronger), which could be accompanied by induction of antibody responses to neutralizing protein epitopes to be uncovered in the process. A similar treatment, based on lectins, has been proposed already (57), but when possible, antibody response with the same properties will be preferable to introduction of exogenous protein due to the shorter half-life and possible undesirable interactions of the latter.

Acknowledgments—We thank the Arkansas Biosciences Institute for funding the University of Arkansas for Medical Sciences SPR facility.

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