Peptide mimetics may substitute for carbohydrate antigens in vaccine design applications. At present, the structural and immunological aspects of antigenic mimicry, which translate into immunogenic mimicry, as well as the functional correlates of each, are unknown. In contrast to screening peptide display libraries, we demonstrate the feasibility of a structure-assisted vaccine design approach to identify functional mimotopes. By using concanavalin A (ConA), as a recognition template, peptide mimetics reactive with ConA were identified. Designed peptides were observed to compete with synthetic carbohydrate probes for ConA binding, as demonstrated by enzyme-linked immunosorbent assay and isothermal titration calorimetry (ITC) analysis. ITC measurements indicate that a multivalent form of one particular mimetic binds to ConA with similar affinity as does trimannoside. Splenocytes from mimicope-immunized mice display a peptide-specific cellular response, confirming a T-cell-dependent nature for the mimic. As ConA binds to the Envelope protein of the human immunodeficiency virus, type 1 (HIV-1), we observed that mimicope-induced serum also binds to HIV-1-infected cells, as assessed by flow cytometry, and could neutralize T-cell line adapted HIV-1 isolates in vitro, albeit at low titers. These studies emphasize that mimicry is based more upon functional rather than structural determinants that regulate mimicope-induced T-dependent antibody responses to polysaccharide and emphasize that rational approaches can be employed to develop further vaccine candidates.

Targeting carbohydrate antigens is a major challenge in vaccine design. Carbohydrates fail to elicit memory responses, as they are T-cell-independent antigens (1–3). Conversion of a polysaccharide (PS) antigen to a thymus-dependent antigen, by covalent coupling to an immunogenic protein carrier, alters the response to PS in several important ways (4–6). However, conjugation strategies that elicit carrier-specific T- and B-cell responses do not necessarily enhance PS immunogenicity (7) nor do PS conjugates elicit responses in immunodeficient mice. Furthermore, in cases where a large number of carbohydrate antigens are required to afford protection, much like that representative of the large number of pneumococcal carbohydrate serotypes, PS conjugates will be far more complicated to produce (6).

Immunization with peptide mimetics of carbohydrate antigens can overcome the T-cell-independent nature of the immune response (8–12). Peptide antigens have an absolute requirement for T cells that can mediate memory responses upon carbohydrate boosting (11, 13). In contrast to carbohydrate conjugates, peptide mimetic conjugates can facilitate cognate interactions between B and T cells after immunization of immunodeficient mice that lack Bruton’s tyrosine kinase (10). Peptide mimetics therefore afford a vaccine approach to break tolerance to carbohydrate self-antigens (13).

Whereas peptide library screening has led to the identification of a variety of peptide mimetics of carbohydrate antigens (14, 15), concepts described for the design of small molecules may apply equally well to the design of mimetics of carbohydrate antigens (16, 17). To further facilitate concepts for a structure-assisted vaccine design, we considered, as a model system, small molecule interactions with concanavalin A (ConA). The mannose/glucose-specific lectin ConA is the most extensively studied plant lectin, known for its application as a biochemical tool and as a model protein to gain further knowledge about lectin-ligand interactions (18–20). Lectins are also particularly relevant to human immunodeficiency virus (HIV) pathogenesis. Lectin-induced inhibition of syncytium formation and infection of cells by both T-cell line adapted and primary isolates (21–27) focuses attention on oligomannosidic glycans, such as those characterized by interaction with ConA (Fig. 1). More recently the lectin DC-SIGN, expressed on dendritic cells, has been recognized to participate in facilitating HIV transmission (24, 28, 29). Consequently, defining peptide mimetics reactive with ConA may facilitate the development of immunogens to augment carbohydrate responses to HIV in future vaccine applications.

Structural studies of peptidyl-ConA complexes suggest that the carbohydrate-binding site on ConA can accommodate an extended array of carbohydrate antigens that might lend to its biological properties (30, 31). We have further defined a peptide that binds at or near the carbohydrate-binding site of ConA that displays a free energy of association comparable to those reported for core trimannoside-ConA and pentasaccharide-ConA interactions. The designed peptide elicits a robust thymus-dependent response, stimulating splenocytes from peptide-immunized mice. We observe that
the peptide, rendered as a multiple antigenic peptide (MAP), used to emulate the clustered array of Envelope protein of HIV (Env)-associated carbohydrates (32) induced an antibody response reactive with cell-bound Env protein. We observe that serum induced to the peptide mimetic paralleled neutralization results obtained by using a mannan preparation from *Saccharomyces cerevisiae* or from *Candida albicans* (33, 34), which further suggests that carbohydrate cross-reactive responses induced by peptide mimetics might be rendered even more effective immunogens.

**EXPERIMENTAL PROCEDURES**

**Epitope Mapping of ConA Ligand-binding Site**—By using the crystallographically positioned pentasaccharide structure within the ConA-binding site, we implemented the program Ligand-Design (LUDI (35), Micron Separations/Biosym Technologies), as described previously (36–39), to search a fragment library and identify amino acid residue types able to interact with ConA. This program identifies small molecular fragments in a database and then docks them into the protein-binding site in such a way that hydrogen bonds and ionic interactions can be formed between the protein and the molecular fragments. The positioning of the small fragments is based upon rules about energetically favorable non-bonded contacts and on geometry between functional groups of the protein and the ligand. The center of search was defined using the crystallographic position of the central mannose residue and searching 15 Å surrounding the centroid of the sugar for potential contact sites on ConA.

The search was performed using standard default values and a fragment library supplied with the program. Peptides were built using INSIGHTII (Micron Separations/Biosym Technologies) and accommodated in relation to the docked LUDI fragments. The peptide backbone and side chain torsion angles were rotated using a fixed docking algorithm (affinity program) within INSIGHTII, until the side chains of the peptide were approximated to the corresponding LUDI fragments. The peptide-ConA complex was subjected to energy optimization and molecular dynamic simulations, as described previously (36–39).

**Reagents and Immunizations**—Multivalent carbohydrates Lewis Y (LeY), α-d-mannose (Man-9), disialyl-biantennary (A2), asialo-biantennary (NA-2), and oligomannose 9 (Man-9), each attached to a polyacrylamide polymer of 30 kDa, were purchased from GlycoTech Corp., Rockville, MD. Methyl-α-d-mannopyranoside (Me-Man) was purchased from Sigma. Peptides were synthesized as MAP (Research Genetics, Huntsville, AL) by Fmoc (N-(9-fluorenyl)methoxycarbonyl) synthesis on polylysine groups, resulting in the presentation of eight peptide clusters. Linear peptides were synthesized by standard solid phase (Research Genetic, Huntsville, AL) and were high pressure liquid chromatography-purified. The structures were confirmed by fast atom bombardment-mass spectrometry.

**ConA** was either prepared from jack bean (*Canavalia ensiformis*) seeds (Sigma), as described previously (20), or obtained from Sigma. The concentration of ConA was determined spectrophotometrically, at 280 nm, using \( A^{1% 1 cm} = 13.7 \) (pH 7.2) and 12.4 (pH 5.2) and expressed in terms of monomer (\( M_r = 25,600 \)).

**BALB/c mice** (\( n = 4 \) per group), 4–6 weeks of age, were immunized intraperitoneally three times, at intervals of 2 weeks, with 100 μg of a respective peptide or 50 μg of LeY, each combined with 20 μg of the adjuvant QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA).
control group was immunized with QS21 alone. The LeY-expressing cell line MCF7 (ATCC, Manassas, VA) (40), without adjuvant, was also used to immunize groups of mice four times. Serum was collected at days 7 and 14 after the last immunization and stored at −80°C until use. ELISA—ELISAs were performed, as described (41). Immuno-2-plates were coated overnight at 4°C, with 100 µl of a selected peptide or carbohydrate probe to assess the binding of sera to these antigens. After blocking the plates (PBS, 0.5% FCS, and 0.2% Tween 20), serial dilutions of sera were added and resolved with anti-mouse isotype-matched HRP (Sigma). To assess the binding of ConA to carbohydrates and peptides, serial dilutions (10 to 0.6 ng/ml of ConA biotin- labeled (Sigma) to peptides was assessed in the presence of serial concentrations of MeeMan (0.8–50 m). Control wells with ConA, but not MeeMan, were also run. Plates were reacted with streptavidin-HRP (Sigma) and results calculated from triplicate measurements. Percentage of inhibition of ConA binding to peptides was calculated as 1 – (mean of test well/mean of control wells) × 100.

 Isothermal Titration Calorimetry—Isothermal titration calorimetry (ITC) was performed using an MCS isothermal titration calorimeter (MCTI, Madison, WI). In individual experiments, 400 µl of solutions of 5 µl of peptides (0.4–5.2 µm) and concentration of ConA ranging from 0.025 to 0.2 m were added from the computer-controlled microsyringe, at an interval of 4 min, into the lectin solution (cell volume = 1.3582 ml), while stirring at 350 rpm, at 27°C. Both lectin and peptide were dissolved in 100 mM HEPES buffer (pH 7.2) or 100 mM sodium acetate buffer (pH 5.2), containing 5 mM CaCl2 and MnCl2. Control experiments were performed by making identical injections of peptide into a cell-containing buffer, where protein showed insignificant heats of dilution. The experimental data were fitted to a theoretical titration curve using software supplied by Microcal Software. The quantity c = Kc M(0), where M(0) is the initial macromolecule concentration, is of importance in titration calorimetry. All experiments were performed with c values 1 < c < 200. The instrument was calibrated using the calibration kit containing RNAase A and 2-CMP, supplied by the manufacturer. Thermodynamic parameters were calculated from the equation, TΔG = RT ln Kc, where Kc and ΔG are the association constant and changes in free energy, respectively. T is the absolute temperature, and R = 1.98 kal mol−1 K−1.

 Precipitation Study—Measured volumes of known concentrations of lectins and peptides in solutions (in 100 mM HEPES buffer containing 150 mM NaCl, 5 mM CaCl2, and MnCl2) were mixed in a quartz cuvette, at room temperature, and the time-dependent development of turbidity was measured at 420 nm (42). Absorbances were monitored continuously, until they remained constant. A portion of the precipitate was treated with 400 µl MeeMan, to check whether or not the precipitation was due to the binding of the peptide to the carbohydrate-binding domain of the lectin. Absorbancy of the solution was recorded at 420 nm, before and after the addition of MeeMan.

 Cell Proliferation Assay—Spleno, s were aseptically removed and splenocytes, as the responder cells, isolated by lysis of erythrocytes. Responder cells were used for detection of cell proliferation using CellTiter 96® Aqueous One Solution (Promega, Madison, WI), based on the manufacturer’s instructions. Briefly, cells (2.5 × 104/well), were cultured in a flat-bottomed 96-well plate with 100-MAP, 20% FCS, 100 µl/mem penicillin, and 100 mM/ml streptomycin. After the 3rd day of incubation, the provided solution was added to each well, and plates were incubated for an additional 1–2 h in a humidified 5% CO2 incubator at 37°C. As an indicator of cell proliferation, absorbance was measured at 490 nm, using a 96-well plate reader (Spectra Fluor, Teco, Research Triangle Park, NC).

 Cells and Antibodies for Fluorescence-activated Cell Sorter—Sup-T1, a non-Hodgkin’s T-cell lymphoma cell line (43), and the same cells stably infected with HIV type 1, III-B (A1953), were kindly provided by Dr. A. S. Fauci. The mouse monoclonal antibody 902, specific for gp120 of HIV-1 III-B (42), was used to differentiate infected versus non-infected cells. Mouse sera were tested with dilutions ranging between 1:10 and 1:100. The secondary antibody used was anti-mouse IgG (γ-specific), fluorescein-conjugated isothiocyanate (Sigma). Cells were fixed for 30 min with 4% paraformaldehyde diluted in PBS. Acquisition of data was performed by using the FACSCAN flow cytometer and histogram analysis by using the CELLQuest software (Becton Dickin

son Immunocytometry Systems, Mansfield, MA).

Peptide Mimetics of Concanavalin A

Peptide Mimetics of Concanavalin A

Propagation of HIV-1 Isolates—CEM × 174 cells (1 × 105/ml), in RPMI 1640 media with 20% FCS, 100 µl/mem penicillin, 100 µg/ml streptomycin, 1% l-glutamine, and 1% HEPEs (R-20), were used to propagate the HIV-1 strains III-B (46–48) and M (50–51). When most cultures were virus-free, supernatants were collected and assayed for HIV-1 infectivity (CPE), virus-containing supernatants were collected after centrifugation (200 × g for 10 min) and filtration (0.45 nm filters), to be stored at −80°C, until use. Determination of the TCID₅₀—the procedure was as reported (52). Briefly, 200 µl/well of a virus isolate, diluted 1:3 in R-20, were added in sextuplicates in flat-bottomed 96-well plates. 50 µl from normal and HIV-infected human individuals (IHS, respectively). Sera were inactivated at 56°C for 1 h and sterilized by exposure to UV light. Determined dilutions of sera and viral outputs were admixed and plates were allowed to incubate for 1 h at 37°C. Then 300 µl of normal or infected wells (round-bottomed 96-well plates) containing 104 CEM×174 cells resuspended in 175 µl of R-20. Plates were incubated for 24–40 h. Control wells without virus or serum (uninfected wells) or without serum but with a selected viral isolate (infected wells) were assayed. After incubation, cells were washed, resuspended in 200 µl of R-20, and transferred to homologous flat-bottomed 96-well plates. Media were replaced at the same time in all plates, as necessary. Cultures were maintained until no further progression of CPE was observed in infected control wells, and at this time 25 µl of supernatant per well were admixed with 225 µl of 0.5% Triton X-100 (lysing solution) for p24 antigen detection by ELISA. Samples were stored at −80°C, until use. Percentage of neutralization was calculated as 1 – (mean absorbancy of test wells/mean absorbancy of control well) × 100.

ELISAs to Determine HIV-1 p24—The assay was performed by using the HIV-1 p24 Antigen Capture Assay Kit from the AIDS Vaccine Program of the NCI-Frederick Cancer Research and Development Center (Frederick, MD). Briefly, plates pre-coated with a monoclonal anti-HIV-1 p24 antigen were washed and blocked with PBS, 0.5% FCS and 0.2% Tween 20. Supernatant lysates were added in duplicates and incubated at 37°C for 2 h. A rabbit anti-HIV-1 p24 serum and a goat anti-rabbit IgG (H & L)-HRP-labeled antibody were used in successive steps. 3,3’,5,5’-Tetramethylbenzidine-peroxidase substrate (0.1 mg/ml) (Sigma), in 0.05 M phosphate-citrate buffer and 0.03% sodium perborate buffer (Sigma), was allowed to react for 20 min. Reaction was stopped with 4 N H₂SO₄, and plates were read as described (41).

Results

Peptidyl Ligands That Bind to Cona—Crystallographic analysis of ConA complexed with the trimannoside α-α-Man-(1–6)-α-α-Man-(1–3)-α-Man (55) and the pentasaccharide β-GlCNAc-(1–2)-α-Man-(1–3)-β-GlCNAc-(1–2)-α-Man-(1–4)-α-Man (56) provides a template to compare peptide-ConA complexes. Prototypic peptides that have been defined to bind ConA include 908 and 712 (Table I) (30). CD analyses of these binding analogs indicate that they share a similar CD profile (30). Secondary structure comparison of these two peptide sequences indicates similarities in tertiary class type, except in the all-β prediction, in which an extended structure spans the WYPY sequence tract of MYWYYPYASGS (Table I) (57). Although the WYPY motif can be viewed as adopting a β-turn conformation that might emulate the spatial position of the trimannoside configuration (31), an extended conformation might also be plausible for molecules to interact with ConA. An extended structure conformation is depicted in Sesbania mosaic virus coat protein (deposited in the Protein Data Bank at Rutgers University, New Brunswick, NJ) (58) for the homologue sequence WYPY. The extended structure can overlap with the pentasaccharide within the ConA-binding site (Fig. 2A).

We attempted to identify amino acid sequences that could
adopt the extended secondary profile and display an adequate interaction with the ConA site. To identify likely residue types that can interact with ConA, we used the program LUDI. We have shown previously that LUDI could be used to structurally map the binding of peptide mimetics to the combining site of anti-carbohydrate antibodies (36, 39). By using this approach, LUDI identified 153 interacting ligands for ConA, with some contacting the same sites as the pentasaccharide. In the search procedure, we identified moieties with Tyr- and Trp-like side changes and guanidinium groups that fit within the ConA site, but not always in the same fashion as the pentasaccharide (Fig. 2B). Substitution of Arg for Pro within the protopetide 908 conserves the extended structure (peptide 909 in Table I), as does a concomitant substitution of the first Tyr in the 909 peptide with a Trp residue forming the 910 sequence (Table I).

To test the ability of the peptide analogs to bind to ConA, MAP forms of the peptides 908, 909, and 910 were synthesized. The MAP forms all bound to ConA in a concentration-dependent manner, with parallel activity (data not shown). Competition analysis with solid phase 908-MAP indicated that MeoMan inhibits ConA peptide reactivity in a concentration-dependent manner, reaching a plateau of about 60% inhibition at a 1.6 mM concentration of MeoMan, a 100-fold less concentration effect than reported previously (30) (Fig. 3). As expected, lactose, as control inhibitor, did not affect ConA peptide binding (data not shown). The variant MAPs 909 and 910 displayed some differences in the MeoMan inhibition profile compared with 908-MAP. At a 1.6 mM concentration, MeoMan inhibited about 20 and 45% of ConA binding to 910-MAP and 909-MAP, respectively.

We further defined a putative peptide sequence, RYGRY, in which the Pro residue in the WYPY motif was replaced by Gly, with the first and fourth Tyr replaced by Arg, and the addition of a Tyr at the fifth position. This putative sequence was chosen because of the identification of these residues and their ConA-reactive positions by LUDI analysis. The peptide motif represented in Fig. 2C, involving the putative RYGRY tract of peptide 912 (Table I), maintains an extended secondary structure profile spanning these residues (Table I). Relative to the other class types, this peptide sequence is the same as that for peptides 908 and 712 and is perhaps more like 712, as represented in the all-β class (Table I). The putative RYGRY tract makes contact with 5 residues within ConA, as does the central mannose residue. A bifurcated hydrogen bond between the guanidinium group of Arg, at the 4th position, and Ser-21 and Tyr-12 side chains of ConA, and a bifurcated hydrogen bond between the guanidinium group of Arg, at the 1st position, and Ser-223 and Ser-168, are observed (Table II). The root mean square deviation of the ConA-peptide complex, after minimization and dynamics calculations, was found to be 1.2 Å, compared with the ConA-pentasaccharide complex, indicating that the extended structure is readily accommodated within the ConA site.

Experimentally, ConA displays higher affinity for α-d-Man-(1-6)α-d-Man-(1-3) β-d-Man constituents over β-d-GlcNAc (1-2)α-d-Man-(1-6) β-d-Man, paralleling the intermolecular interaction (interaction energy) calculation trends shown in Table II. The calculated location for the peptide mimetic is, however, not an optimal binding mode in terms of mimicking the conformational properties of the pentasaccharide and in contacting the same ConA residues, as does the pentasaccharide (Table II). Nevertheless, the interaction energy for this ConA/peptide-binding mode was found to be −75.9 kcal/mol, falling within the range of interaction energies calculated for the trimannoside constituent (Table II).

**Peptide Mimetic Competes for Carbohydrate Binding to ConA—Clustering or repeating the 912 sequence, forming the peptide 911, manifests an extended secondary structure (Table I). ELISAs carried out using various concentrations of ConA showed a concentration-dependent binding to the clustered form of the RYGRY containing peptide 911, which was inhibited by MeoMan (Fig. 4A). We observed binding of ConA to 911-MAP, at ConA concentrations lower than those required for binding to ligands tested, which included extended structure peptides, and better than that for binding to 908-MAP. This result suggests a high avidity interaction of ConA with the multivalent 911 peptide and therefore requires a higher concentration of MeoMan for inhibition of 911-MAP binding to ConA than that for the 908-MAP (Fig. 4B).

The putative monovalent peptide 912 and 911-MAP binding to ConA was studied further by ITC, to determine the binding parameters such as $K_a$ and $ΔG$. Experimental conditions were previously standardized for studies of multivalent interaction by ITC (9). The association constant ($K_a$) of 911-MAP with ConA was determined to be 1.9 nM, and $ΔG$ (5.6 kcal mol$^{-1}$) values of ConA for the monovalent 912 peptide were observed to be comparable to that observed for MeoMan (Fig. 5). Earlier, microcalorimetric studies showed that the $K_a$ and $ΔG$ values of ConA for carbohydrate ligands, such as 5Man, trimannoside, and pentasaccharide, were 0.82 × 10$^4$, 49 × 10$^3$, and 92 × 10$^3$ M$^{-1}$ and 5.3, 7.8, and 8.1 kcal mol$^{-1}$, respectively (20). In contrast, $K_a$ of ConA for 911-MAP was found to be 26 × 10$^4$ M$^{-1}$, with a value for $ΔG$ of 7.4 kcal mol$^{-1}$. These results indicate that the multivalent 911 peptide displays a comparable association constant and free energy of binding, as do oligosaccharide ligands.

To verify further the valence of the peptides for ConA, a precipitation study was carried out (Fig. 6). The number of binding site per monomer ($n_s$), as determined by ITC, suggests that peptide 912 is monovalent, whereas 911-MAP is multivalent for ConA. Multivalent lectin-ligand interactions often lead to the formation of insoluble cross-linked complexes, which can easily be monitored spectrophotometrically by measuring the absorbancy at 420 nm. The efficient precipitation by the 911-MAP form confirms that it possesses multiple binding sites for ConA. About 70% of the cross-linked complexes are re-dissolved when treated with MeoMan (400 mM). This observation clearly

| Number | Sequence | None | All-α | α/β | All-β |
|--------|----------|------|-------|-----|-------|
| 105    | GGIYPYDIYYPYDYYFD | -EE-- | -H--H-- | -E--E--H--E-- | -E--E--E--E--E-- |
| 107    | GGYRYHDIYRYDYIYRD | -EEEEEEE-- | -H--H-- | -E--E--H--E-- | -E--E--E--E--E-- |
| 911    | YRRYRGRYRSYRSGYRYSRSGS | -EEEE-- | -H-- | -E--E-- | -E--E--E-- |
| 908    | DVFYPPYASGS | -EEEE-- | -H--H-- | -E--E-- | -E--E--E-- |
| 909    | DVFYKRYASGS | -EEEE-- | -H--H-- | -E--E-- | -E--E--E-- |
| 910    | DVFWRYRASGS | -EEEE-- | -H--H-- | -E--E-- | -E--E--E-- |
| 712    | MYVYPYASGS | -EEEE-- | -H--H-- | -E--E-- | -E--E--E-- |
| 912    | RYGRYRYSRS | -EEEE-- | -H--H-- | -E--E-- | -E--E--E-- |
| 911    | GGPQPPPGQPGGGQ | -EEEE-- | -H--H-- | -E--E-- | -E--E--E-- |

### Table I

Peptides used in this study and their secondary structure properties

The secondary structure profiles were calculated from neural network calculations.
indicated that 911-MAP was bound to ConA predominantly through the carbohydrate-binding sites of the lectin, whereas the remaining ~30% of precipitation is probably due to protein-protein interactions. Peptide 912 was unable to form any detectable precipitate, which confirms its monovalent nature (Fig. 6).

**FIG. 2.** A, overlap of the extended structure of prototypic WYPY with the pentasaccharide (β-GlcNAc(1–2)-α-Man(1–3)-(β-GlcNAc(1–2)-α-Man(1–6))-Man). The Trp overlaps with the first GlcNAc(1–2) on the Man(1–3) side, with the proline residue overlapping with central α-Man(1–6) moiety. The Tyr at the 4th position in the sequence tract approximates the location of the second GlcNAc residue. Holding the Pro residue, fixed relative to the centralized mannose ring, least squares fitting of the backbone atoms, comprising the first three residues in the WYPY motif to the α1–6 linkage in the α-D-Man-(1–6)-D-Man-(1–3) α-D-Man binding mode, resulted in a root mean square deviation of 0.18 Å. B, representative placement of LUDI identified guanidinium-like moieties. C, putative 912 peptide (yellow) sitting in ConA carbohydrate-binding site, emphasizing the extended nature of the putative interacting motif.

**Binding of Serum from Immunized Mice to HIV-1 III-B-infected Cells**—The 911 peptide is predicted to have a major histocompatibility complex class II motif spanning the RYRYGRYRS sequence. Immunization with peptide 911 indicated a robust cellular response specific for peptide 911 (Fig. 7). To determine if serum antibodies react with membrane-ex-
pressed gp120/gp41, we examined serum IgG binding to con-stitutively infected cells compared with the binding to the same non-infected cells. Results in Fig. 8 show IgG antibody binding to chronically infected cells (A1953 cells). We observe that the monoclonal antibody 902 differentiates infected from non-infected cells (Fig. 8A). Immunization with control MCF7 cells induce serum reactive with the neolactoseries antigen LeY (13) and also antibodies that are potentially reactive with major histocompatibility complex class I, which shares some homology with gp120, as anti-class I antibodies bind to Env protein (Fig. 8B) (59, 60). Serum from 911-immunized mice reacted stronger with infected cells than non-infected cells (Fig. 8C). IgG from mice immunized with other formulations (LeY or QS21) did not show any significant increased binding to A1953 cells compared with their binding to Sup-T1 cells (data not shown).

**FIG. 3.** Inhibition of ConA binding to solid phase MAP by soluble MeoMan in competitive lectin-binding assay. Biotinylated ConA (0.4 μg/ml) was incubated with an increasing amount of MeoMan, and binding of free biotinylated lectin to MAPs was measured using peroxidase-labeled streptavidin. Lactose did not display any inhibition of ConA binding to the peptides.

**TABLE II**

Hydrogen bonding scheme of putative carbohydrate and peptide constituents with ConA

| Model          | Individual Residue Contact | IE  |
|----------------|---------------------------|-----|
| Mannose core   |                           |     |
| Man(1–6)       | d-Man                     | 72.8|
| Tyr-12 (SC)    | Asn-14 (SC)               | 15  (BB) |
| Leu-99 (BB)    | Arg-228 (BB)              | 100 (BB) |
| Tri-Sac        |                           |     |
| Man(1–6)       | (1–2)-GlcNAc              | 57.3|
| Tyr-12 OH (SC) | Asn-14 (SC)               |     |
| Leu-208 (BB)   | Arg-228 (BB)              |     |
| Tyr-100 (BB)   |                           |     |
| Peptide        |                           |     |
| Arg-1          | Arg-4                     | 75.9|
| Leu-99 (BB)    | Asp-16 (BB)               |     |
| Ser-168 (SC)   | Ser-21 (SC)               |     |
| Ser-223 (SC)   | Tyr-12 (SC)               |     |

**FIG. 4.** A, serial dilutions of ConA biotin-labeled were added to ELISA plates pre-coated with selected carbohydrate probes or peptide mimotopes (100 nM/well) and reacted with streptavidin-HRP. Absorbance readings at 450 nm demonstrate that ConA binds to 911 peptide more efficiently than to other peptides or to carbohydrate probes known to be reactive with ConA. B, inhibition of ConA binding to solid phase 911-MAP by soluble MeoMan in competitive lectin-binding assay. Biotinylated ConA (0.4 μg/ml) was incubated with an increasing amount of MeoMan, and binding of free biotinylated lectin to 911-MAP was measured using peroxidase-labeled streptavidin. Lactose did not display any inhibition of ConA binding to the 911-MAP.

**DISCUSSION**

Carbohydrate antigens are important targets in vaccine development. Vaccine design strategies have little utilized struc-
tural concepts to develop novel carbohydrate forms (61). The clustering and multivalent presentation of carbohydrate antigens appears relevant to induce antibody responses to natively expressed carbohydrate antigens on cell surfaces (13, 62, 63). We have shown that peptide mimeotopes can elicit carbohydrate cross-reactive immune responses to natively expressed bacterial and tumoral antigens related to those expressed on HIV-1 Env glycoprotein (9, 13, 41). To explore further the utility of targeting the Env glycoprotein and generalizing peptide design strategies (39, 64), we are optimizing peptides re-

Fig. 5. ITC profile of ConA (0.2 mM) with 912 peptide (5.2 mM), at 27 °C. Top, data obtained from 20 automatic injections, 6 μl each, of 912 peptide. Bottom, the integrated curve showing points (squares) and best fit (line). The buffer was 0.1 M HEPES with 5 mM each of CaCl₂ and MnCl₂.

Fig. 6. Profile for the kinetics of precipitation of ConA (60 μM) in the presence of 911-MAP (15 μM) and peptide 912 (squares, 911-MAP; circles, 912).

Fig. 7. 911 peptide-stimulated proliferation of splenocytes from 911 peptide-immunized mice. Mice were immunized with MAP form of 911 peptide two times at 3-week intervals. 7 days after the boost, splenocytes were collected and used for detection of cell proliferative response to MAPs 106 and 911, using CellTiter 96AQUitous One Solution (Promega, Madison, WI). MAPs were used at 5 and 1 μg/ml final concentrations. Results are given as mean ± S.D. based on three replications. Experiment was repeated three times with comparable results using pooled splenocytes from four mice. Peptide 106 (GGIYWRYDITYWRYDITYWRYD) is also in the MAP format and displays a major histocompatibility complex binding score of 2000.

Fig. 8. Binding of serum IgG from mice immunized with 911 and MCF7 to Sup-T1 cells (column 1) or to A1953 (Sup-T1 cells infected with HIV-1 III-B) (column 2). Dotted lines represent the binding of IgG from pre-immune mouse serum. A shows the binding of the monoclonal antibody 902 (mouse IgG1-k, specific for gp120 of HIV-1 III-B) to the respective cells; B shows the binding of MCF7-IgG; and C shows the binding of 911-IgG. Serum dilution used in these assays was 1:100.
performed by ITC (18, 20), and the dynamic analysis of binding of trimannoside to ConA has been assessed by p24 ELISA. Percentage of neutralization was calculated.

We identified a peptide that, rendered as a clustered and multivalent form, was reactive with ConA at lower concentrations than those required for reaction of some native oligosaccharide ligands of ConA. The 911-MAP displayed competitive inhibition with carbohydrate ligands of ConA, indicating that it binds at an overlapping carbohydrate-binding site on ConA. ITC and precipitation experiments suggest that the putative peptide 912 is monovalent for ConA, and its affinity is comparable with that of MeaMan. The MAP format of the mimeotopes, while emulating the multivalent carbohydrate structure, may still not effectively cope with micro-heterogeneity in carbohydrate structures. However, this same problem exists when immunizing with carbohydrate immunogens, since, many times, synthetic carbohydrate forms do not induce responses cross-reactive with native carbohydrate forms, requiring modifications in synthetic strategies. Likewise, cyclization of peptide mimic immunogens may further restrict carbohydrate cross-reactive responses much as it does in inducing responses to protein antigens, by limiting the polyclonal response.

In summary, these results indicate that designed peptide mimetics of carbohydrate antigens can induce functional responses that may find utility in priming strategies to further augment carbohydrate immune responses against pathogens or tumor cells (11). Although modeling does not account for multivalent interactions, as represented by MAP forms, modeling can define potential binding site constituents. Consequently, strategies that evaluate potential mimetic binding modes and thermodynamics of binding should further facilitate structure-assisted design of multivalent carbohydrate antigens. In summary, these results indicate that designed peptide mimetics of carbohydrate antigens can induce functional responses that may find utility in priming strategies to further augment carbohydrate immune responses against pathogens or tumor cells (11). Although modeling does not account for multivalent interactions, as represented by MAP forms, modeling can define potential binding site constituents. Consequently, strategies that evaluate potential mimetic binding modes and thermodynamics of binding should further facilitate structure-assisted design of surrogates for vaccine applications. Likewise, this study encourages further investigation to ascertain the mechanism(s) by which certain peptide mimics of PS antigens play their role as mimeotopes, in that they can stimulate immunity that targets PS (12).

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