Peptides may substitute for carbohydrate antigens in carbohydrate-specific immunological reactions. Using the recognition properties of an anti-Lewis Y (LeY) antibody, BR55-2, as a model system, we establish a molecular perspective for peptide mimicry by comparing the three-dimensional basis of BR55-2 binding to LeY with the binding of the same antibody to peptides. The peptides compete with LeY, as demonstrated by enzyme-linked immunosorbert assay and Biacore analysis. The computer program LUDI was used to epitope map the antibody-combining site, correlating peptide reactivity patterns. This approach identified amino acids interacting with the same BR55-2 functional residue groups that recognize the Fuc(1–3) moiety of LeY. Molecular modeling indicates that the peptides adopt an extended turn conformation within the BR55-2 combining site, serving to overlap the peptides with the LeY spatial position. Peptide binding is associated with only minor changes in BR55-2, relative to the BR55-2-LeY complex. Anti-peptide serum distinguishes the Fuc(1–3) from the Fuc(1–4) linkage, therefore differentiating disaccharide-related neolactoseries antigens. These results further confirm that peptides and carbohydrates can bind to the same antibody-binding site and that peptides can structurally and functionally mimic salient features of carbohydrate epitopes.
Peptide Mimicry of Lewis Y

A structure-based computer-screening approach was used to assemble an epitope map of the potential amino acids that could interact with the BR55-2 combining site (9, 27). This mapping confirmed the participation of previously identified YRY and WRY motifs reacting with BR55-2 (28) and suggested a new putative sequence, FSLLW, that adopted an extended turn-like structure within the BR55-2 combining site. The accuracy of this approach was corroborated by the isolation of this putative sequence within a peptide selected from a random peptide display library (29). Furthermore, vaccination with the phage-isolated peptide containing the FSLLW sequence induced a highly specific anti-LeY response that distinguished the Fuc(1–3)Gal from the Fuc(1–4)Gal linkage that is observed in neolactoseries antigen homologs (Table I). These results provide further evidence, at the molecular level, that surrogate antigens can bind by similar mechanisms as the carbohydrate that they mimic, inducing specific immune responses.

**EXPERIMENTAL PROCEDURES**

**Preparation of Antibodies against Carbohydrate-mimicking Peptides**—Peptides were synthesized as multiple antigen peptides (MAPs) (Research Genetics, Huntsville, AL), by Fmoc (N-(9-fluorenylmethoxycarbonyl) synthesis on polystyrene groups, resulting in the presentation of eight peptide clusters. For generation of polyclonal serum, Balb/c mice (n = 4 per group), 4–6 weeks of age, were immunized intraperitoneally with 50 μg of the respective MAPs and 20 μg of QS-21 adjuvant (Aquila Biopharmaceuticals Inc., Farmington, MA) three times at intervals of 2 weeks. Serum was collected at 3 and 14 days after the last immunization and stored at −20 °C.

**Biacore Analysis**—Biotinylated, multivalent LeY-PAA (Glycotope Inc.) was captured on a Biacore 2000 gold surface via streptavidin (1000 RU amine-coupled, according to the standard amine coupling procedure recommended by the manufacturer) and immobilized on a non-cross-linked carboxymethylated matrix (CMS sensor chip, Biacore, Inc.). The antigen employed consisted of a polymer of n four (difucosylated) hexose units in an acrylamide matrix containing m biotin/n sugars. The LeY surface density was kept below 1000 RU (480 and 700 RU, in two independent experiments), to reduce steric hindrance and minimize diffusion-controlled kinetics of binding. Ligand-biotin and mAb-biotin were captured on a control surface, as described for LeY.

Solution inhibition assays were designed to rank and evaluate the apparent equilibrium constants for dissociation of the BR55-2 (IgG2a isotype) complexes with LeY, peptides, and the negative control competitor Leb. The solution competitions consisted of preincubation of a fixed concentration of BR55-2 with increasing amounts of antigen, control antigen, or peptidomimetics. Identical molar ratios of BR55-2 and competitors were chosen to standardize the inhibition assays and allow for ranking and calculation of free BR55-2 in solution with respect to the same duplicate titration curve for BR55-2 to LeY. The following assumptions are inherent to the calculation of BR55-2 inhibition by LeY antigen and peptides: 1) From the macroscopic point of view, the immobilized LeY surface is homogeneous. The analyte (BR55-2) is considered available at uniform concentration at all times, the interaction obeys the principles of mass action kinetics, and the conservation law applies at all times, namely (LeY) t = (LeY-BR55-2) t + (LeY) f; 2) From the kinetic standpoint, the interaction is complex. BR55-2 binding was first fit to the simple 1:1 interaction model and then to the bivalent interaction model using BIA evaluation 3.0. A simple interaction could not fit data generated in direct BR55-2 binding to LeY, whereas the bivalent model fit was preferred by a decrease of 89-fold in Ch2 distri-bution of experimental residuals around the modeled binding and the dissociation portions of the curves at all doses (global fit) using BIA evaluation 3.0 software.

In inhibition experiments, the steady state signal, indicative of the total mass of BR55-2 bound to the sensor surface LeY at equilibrium, was taken as a measure of free BR55-2. Titration curves were used to calculate the concentration of free BR55-2 binding to the LeY surface using nM-μM BR55-2. Equilibrium and inhibition analysis was carried out by considering solution free BR55-2 as the only signal-generating species based on the assumption that species with one competitor bound site and one free site, which are both in equilibrium with surface bound LeY and competitor or LeY in solution, are not favored in the presence of a polymeric competitor. Competition experiments were tested and carried out either in phosphate-buffered saline or in 2–7% Me2SO as indicated.

**ELISA Assays**—Solid phase ELISA was performed to assess the binding of anti-carbohydrate monoconal antibodies and polyclonal anti-peptide serum to MAPs or to a variety of carbohydrate-synthesized probes incorporated into a PAA matrix (GlycoTech, Rockville, MD). For peptide ELISA, respective MAPs were used to coat Immulon 2 plates (2 μg/well) and reacted with 0.2 μg of the anti-LeY monoclonal antibody BR55-2. For peptide inhibition, plates were coated overnight with LeY-PAA, at 0.1 μg/well. The mAbs (0.1 μg/well) or serum were admixed with varying concentrations of MAPs and incubated for 15 min on ice reaction with LeY-coated plates. For evaluation of serum anti-carbohydrate activity, Immulon 2 plates were coated with a variety of carbohydrate probes that included Fuc(1–4)GlcNAc, LeY, Galβ(1–3)Gal, Galβ(1–3)Galα, sialyl-Lea, Lea, sialyl-LeX, LeX, LeX-pentasaccharide, and Leb-hexasaccharide. Plates were coated overnight with 2 μg/well of the respective probes at 4 °C and blocked (5). Serial dilutions of the respective antisera were added and resolved with 100 μl/well of 1:10000 mouse-isotype matched horseradish peroxidase (Sigma) or horse-radish peroxidase-labeled IgG and IgM reactive Anti-Mouse antibody (Sigma), diluted in blocking buffer, and incubated at 37 °C for 1 h. Absorbency at 450 nm was read using a Dynatech MR5000 ELISA reader, after 15 min of color development. All results were calculated from triplicate measurements.

**Epitope Mapping of BR55-2 Combining Site**—Using the positioned LeY structure within the BR55-2 combining site, we implemented the program Ligand-Design (LUDI (31) MSI/Biosym Technologies), as described previously (9, 27, 32), to search a fragment library and identify amino acid residue types able to interact with BR55-2, in a manner similar to that of LeY. The search was performed using standard default values and a fragment library supplied with the program. This program identifies small molecular fragments in a data base 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 nonbonded contacts and on the geometry between functional groups of the protein and the ligand. The center of search was defined using the crystallographic LeY position. In this approach, the OH-3’ position on the LeY structure was used as the sampling point. Peptides were built using INSIGHTII (MSI/Biosym Technologies) and accommodated in relation to the docked LUDI fragments. The peptide backbone and side chain torsional angles were modeled 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-BR55-2 complex was subjected to energy optimization and molecular dynamics simulations, as described previously (9).

**RESULTS**

**Ligand Search Highlights Heavy Chain Interaction with Mimics**—Structural studies on Lewis antigens have generally

### Table I

| Antigen Structure | Neolactoseries core antigen structures |
|-------------------|--------------------------------------|
| LeY               | (Fuc(1–3)Galβ1) → 4(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer |
| Leb               | (Fuc(1–3)Galβ1) → 4(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer |
| LeX               | Galβ1 → 4(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer |
| eLeX              | NeuNAca2–3Galβ1 → 4(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer |
| Lea               | Galβ1 → 3(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer |
| Sialyl-Lea        | NeuNAca2–3Galβ1 → 3(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer |

**Antigen Structure**

- **LeY**: (Fuc(1–3)Galβ1) → 4(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer
- **Leb**: (Fuc(1–3)Galβ1) → 4(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer
- **LeX**: Galβ1 → 4(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer
- **eLeX**: NeuNAca2–3Galβ1 → 4(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer
- **Lea**: Galβ1 → 3(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer
- **Sialyl-Lea**: NeuNAca2–3Galβ1 → 3(Fuc(1–3)GlcNAcβ1) → 3Galβ1 → 4Glcβ1 → Cer
substantiated that their structures maintain well defined conformations with relatively long lifetimes (19, 33–35). Hard sphere or rigid geometry calculations, albeit without solvent, provide a good picture of the steric repulsion that modulates the conformational properties of the Lewis antigens. Consequently, structure-based drug-design approaches (31, 36, 37) offer the ability to establish potential interaction profiles (epitope maps) to elucidate the molecular basis for peptide mimicry of the LeY antigen in binding to BR55-2.

As a further illustration of this approach, definition of residues involved in the anti-LeY monoclonal antibody B3 (9). The computer screening search with LUDI identified 231 interacting ligands for BR55-2 that reduce to the functional group types shown in Table II. The majority of LeY contacts occur with main chain atoms, with some involvement of side chains, as do interactions with the LUDI identified ligands. Not unexpectedly, all BR55-2 residues that interact with LeY are identified in the LUDI search, emphasizing a large number of contacts with the heavy chain of BR55-2, as compared with the light chain.

As a further illustration of this approach, definition of residue types, shown in Table II, can be combined with bulky hydrophobic amino acids occupying the LeY spatial volume. In Fig. 1A, representative, nonoverlapping organic ligands are shown positioned within the BR55-2 combining site, relative to the BR55-2-LeY tetrasaccharide identified crystallographically. The majority of contacts occur with main chain atoms, with some involvement of side chains, as do interactions with the LUDI identified ligands. Not unexpectedly, all BR55-2 residues that interact with LeY are identified in the LUDI search, emphasizing a large number of contacts with the heavy chain of BR55-2, as compared with the light chain.

| MC, main chain; SC, side chain. |

| Amino acid types identified computationally | Guanidinium | Tyr | Trp | Ser/Thr | His | NH/CO | Fuc(1–2) | Fuc(1–3) |
| Light chain |  |  |  |  |  |  |  |  |
| Heavy chain |  |  |  |  |  |  |  |  |
| A100(MC), M96(MC), Y35(SC) | A52A(SC), D31(MC) | Y35(SC), D31(SC), D31(MC) | Y35(SC), S92(MC) | A100(MC), M96(MC), Y33(SC) | D31(MC), N52A | S92(SC) | S27E(SC) | S27E |

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| Peptide Mimicry of Lewis Y |
|--------------------------|
| **TABLE II** |
| **Ligand/epitope hydrogen bond contact residues on BR55–2** |

| BR55-2 | Amino acid types identified computationally | Guanidinium | Tyr | Trp | Ser/Thr | His | NH/CO | Fuc(1–2) | Fuc(1–3) |
| Light chain |  |  |  |  |  |  |  |  |
| Heavy chain |  |  |  |  |  |  |  |  |
| A100(MC), M96(MC), Y35(SC) | A52A(SC), D31(MC) | Y35(SC), D31(SC), D31(MC) | Y35(SC), S92(MC) | A100(MC), M96(MC), Y33(SC) | D31(MC), N52A | S92(SC) | S27E(SC) | S27E |

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FIG. 1. Identification and placement of the putative FSLLW sequence interacting with BR55-2. In A, space filling of BR55-2 (gold color) is shown with nonoverlapping residue types identified by LUDI in comparison with LeY within the BR55-2 combining site. B illustrates optimized FSLLW peptide in the BR55-2 combining site relative to the LUDI identified fragments.
Neural net secondary structure predictions across several structural classes indicate that peptides K61107, K61106, and K61104 display discernable structures that span multiple residues in any of the classes shown, whereas K61105 does not display extended structures that span more than four consecutive residues. Peptides K61109 and K61111 do not display extended structures that span more than four consecutive residues in any of the structural classes shown. Peptide K61106, K61107, and K61104 display similar extended structures spanning over more than four consecutive residues.

### Table III

| Peptide Sequence | Method of Identification | Secondary Structure Predictions (nnPredict) | Motif |
|------------------|--------------------------|-------------------------------------------|-------|
| K61105            | Synthetic design         | 222                                       | E, E, E, E |
| K61106            | Synthetic design         | 222                                       | E, E, E, E, E |
| K61107            | Synthetic design         | 222                                       | E, E, E, E, E |
| K61108            | Synthetic design         | 222                                       | E, E, E, E, E |
| K61109            | Synthetic design         | 222                                       | E, E, E, E, E |
| K61110            | Synthetic design         | 222                                       | E, E, E, E, E |
| K61111            | Synthetic design         | 222                                       | E, E, E, E, E |

**Peptide Mimicry of Lewis Y**

At increasing concentrations of peptide competitor K61104, the kinetics of binding of free BR55-2 to LeY changed showing 2-fold faster off rates. It seems plausible that upon binding of competitor to BR55-2 in solution, the LeY polymer and multivalent K61107 peptide have a hydration radius that disable the binding of a second K61107 in solution or sensor surface LeY molecule. K61104 has the smallest hydration radius. One way to explain the observed change in kinetics as a function of competitor concentration is that the species with one K61104 polymer may bind to surface LeY, with the true kinetics of binding for the second site. By extension, mostly free BR55-2 would bind to the LeY surface accounting for a homogeneous kinetics. Because the stability of the complex at the sensor surface can be explained by cooperative interactions of the full-length BR55-2 molecule, we may also conclude that K61104 binds BR55-2 in such a way that the polymer does not promote a higher valency for IgG binding to the LeY surface, as would happen in the case of LeY or K61107 competition. This also results in the faster off rate measured (always the slowest rates are measured).

**Induction of Anti-carbohydrate-specific Immune Response with FSLW-containing Peptide**—Because the FSLW sequence tract was identified both computationally and by phage display, we decided to test the immunological mimicry of the K61104 peptide. Balb/c mice were immunized with MAP K61104 administered with the adjuvant QS-21. The anti-K61104 serum displayed LeY reactivity predominately IgM isotype, as observed in our previous studies using MAP peptide forms (28). The amount of carbohydrate reactive IgM induced by the peptides was found to be similar (~5 μg/ml) to that induced by the respective multivalent synthetic LeY (6). The IgG response induced by the peptide emulates the IgG response induced by the synthetic carbohydrate forms (~2 μg/ml). The IgM fraction of the anti-K61104 serum displayed a 3-fold increase in reactivity for LeY over Leb (Fig. 4A). This level of dilution was also observed using multivalent IgG antibody horseradish peroxidase-labeled antimouse antibody (data not shown), indicating that the IgG fraction does not contribute significantly to LeY binding. At 1:50 serum dilution (Fig. 4B), higher levels of reactivity are observed for LeY, with about the same level of reduced reactivity for Leb-hexasaccharide, LeX-pentasaccharide, sLeX, Lea, and sialyl-Lea.

To further define the minimal determinant that distinguishes selectively for LeY over its homologs, anti-K61104 serum was screened against a variety of LeY substituents (Fig. 4B). The best reactivity was observed with the Fuca1–3GlcNAc
moiety (Fig. 4B), reflecting the spatial association of the peptide with BR55-2, in comparison with other moieties on LeY. Most importantly, the anti-K61104 distinguishes the Fuc(1–3) from the Fuc(1–4) GlcNAc linkage, as demonstrated by a significantly reduced reactivity with Fuc(1–4)GlcNAc. This selective interaction sets apart reactivities between Leb and LeY, because reactivity is observed for the H1 type constituent of Leb (Fig. 4B). The cross-reactivity of the anti-peptide serum for LeY, in a specific manner, suggests a structural mimicry between the K61104 peptide and LeY. However, it also appears that serum antibody recognizes both fucosyl groups much like BR55-2, because anti-K61104 serum selectively binds to LeY relative to sLeX (Fig. 4B). It has been found that anti-K61104 serum displays reduced mediation of complement-dependent cytotoxicity, in the presence of rabbit complement, for targeting the sLeX expressing Meth A fibrosarcoma cell line (6).

**DISCUSSION**

Peptide mimotopes have been used to better define the fine specificity of anti-carbohydrate antibodies (15, 41) and to elicit protective antibodies targeting carbohydrate epitopes (3, 5, 6). An analysis of the details of the mimicry between peptide and carbohydrate ligands is relevant for defining structure/function correlates necessary in vaccine design applications. To increase the level of mimicry between the peptide and carbohydrate antigen, it is important to understand the structural basis of carbohydrate antigenicity. Specificity in protein-carbohydrate recognition is achieved by a combination of multivalency and geometry. General structural features associated with molecular recognition and interaction are the stacking of aromatic side chains against sugar rings, the presence of hydrogen bond networks in which sugar OH groups act as both acceptors and donors, and the coordination of multiple hydrogen bonds with water molecules. The structure of the complex of LeY with BR55-2 provides a molecular basis to understand peptide binding to the antigen-combining site and to explain peptide competition with LeY for BR55-2 binding.

BR55-2 can bind to some peptides that contain the sequence tract (W/Y)RY, and such binding is mediated by the Arg residue that mimics the spatial position of Fucose(1–3) by contacting the same atoms within BR55-2 as complexed LeY (Table II). The mimicry for Fucose(1–3) by the guanidinium group of Arg might be a basis for partial mimicry of LeY by (W/Y)RY-containing peptides. We have shown that the Arg containing peptides K61106 and K61107 also bind to the anti-sLeX antibody FH-6 (28). sLeX shares the Fucose(1–3) moiety with the LeY homolog (Table I). The single substitution of Pro for Arg, in the (W/Y)RY tract, reduces BR55-2 binding (Fig. 2), further demonstrating that specificity of binding can be determined by the identity of the peptide side chain constituents of the motif. These data provide strong evidence that peptides and carbohydrates can bind to the same antibody-binding site, whereas changes in peptide presentation lend to fine specificity of anti-carbohydrate antibodies.

Conformational studies indicate that the (W/Y)(R/L)Y and (W/Y)PY motifs can adopt extended turn-like structures (3, 9, 38), suggesting that a particular peptide structure is required for polysaccharide mimicry. The extended turn characteristics of mimicking peptides are implicated as a binding mode conformation, as evidenced in a crystallographic structure of a peptide that mimics a cryptococcus carbohydrate epitope, in complex with an anti-cryptococcal antibody (7). Using a structure-based design approach, we identified another sequence, FSLLLW, which displayed a binding mode structure of the extended turn type in which the FSL residues spatially mimic the Fucose(1–3)GlcNAc moiety of LeY for binding to BR55-2. This sequence tract was identified in a peptide isolated by phage display screening with BR55-2 (29). These results suggest that we can develop putative peptide binding motifs theoretically, which resemble peptides isolated by phage isolation. The peptide K61104 did not display FH-6 binding (data not shown), implying that the FSLLLW containing peptide interaction with BR55-2 is specific. K61104 competed with LeY for binding to BR55-2 (Fig. 2), again indicating that the peptide and LeY can bind to the same binding site on BR55-2.

It is possible that LeY, expressed on tumor cell surfaces, is displayed as “oligosaccharide patches” or as “clustered” epitopes. Immunization with a multivalent tetrasaccharide
form of LeY induces serum that cross-reacts with natively expressed LeY (6), whereas the same tetrasaccharide in monovalent form attached to carrier protein does not induce antibodies cross-reactive with tumor expressed LeY (22). These observations present a viewpoint that multivalent synthetic form of LeY might be representative of the LeY structure naturally found on tumor cells. Consequently, we examined the interaction of multivalent forms of LeY and peptides with full-length anti-LeY monoclonal antibody BR55-2.

Because valence plays a key role in protein-carbohydrate

**FIG. 4. Reactivity of the K61104 serum with neolactoseries constituents.** A, titration of the IgM portion of the derived anti-peptide serum against LeY and Leb. Detection was with horseradish peroxidase-labeled anti-mouse IgM isotype as detecting antibody. B, profile of the anti-peptide serum cross-reactivity with neolactoseries associated carbohydrate probes.
interactions, methods that allow monitoring the kinetic parameters that mediate these interactions is essential (42). It is also possible that multivalent probes contain "neo" epitopes that complicate analysis. To validate the use of multivalent forms, multivalent LeY was first examined for binding to BR55-2, where it was found that multivalent LeY can bind to BR55-2 in both a one- and two-site model (data not shown). The one binding site model gives a dissociation constant (K_M) of 8 μM, which is similar to that observed for a Fab fragment of the anti-LeY antibody BR96 (9.9 μM) (43) and similar to that obtained previously for BR55-2 binding to tumor cells, as estimated by ELISA (44). Consequently, this result indicates that the multivalent form of LeY can emulate a single site interaction model.

Using surface plasmon resonance methods we assessed the kinetics of binding of K61104 and K61107 peptides to BR55-2 (Fig. 3). These data, along with our ELISA results, show that the peptides are binding to the same binding site as LeY with approximately the same affinity. Based upon the relative ranking of K61104 and K61107 (Fig. 3) for BR55-2 binding, it appears that the K61107 peptide competes with LeY better than K61104 does. This result is consistent with those assessed from ELISA (Fig. 2). There is a trend toward a change in the kinetics of binding of BR55-2, dependent on the concentration of LeY, K61107, or K61104 competitor, particularly toward faster off rates. K61104 showed a clear cut change in the binding to species of BR55-2 (suspected to be with a single free site), where steady state is attained faster because of an increase in the off rate and binding curves that fit to a 1:1 interaction model. The bivalency of BR55-2 of binding in the present analysis may arise from the ability of murine antibodies to bind cooperatively through a Fc-dependent mechanism (45). Although such interactions are typically attributed to the IgG3 isotype, some IgG2a antibodies may still be affected by such interactions. This aggregation effect would be a measure of the avidity of BR55-2 to the multivalent probes rather than the resolution of the affinity of each binding event on a bivalent antibody with two nonindependent binding sites.

The way in which the FSLIIF sequence interacts with the BR55-2 heavy and light chain, emulating LeY binding, suggests that this putative sequence can function as a LeY mimic, inducing anti-LeY reactive antibodies. Immunization with the K61104 peptide leads to a LeY specific immune response (Fig. 4) that is mediated by selective interaction with the Fuc(1–3) GlcNAc moiety (Fig. 4B). The immune response induced by the K61104 peptide is more specific than those induced by synthetic LeY formulations.

These studies are suggestive that peptides can bind to isolating antibodies by the same mechanisms as the original carbohydrate antigen. This finding does not negate previous results suggestive that peptides are specific for their isolating monoclonal antibodies (41). This conclusion was reached by comparing panels of antibodies that recognize essentially the same carbohydrate epitope and consensus peptides isolated by the respective anti-carbohydrate antibodies. Here, we are examining one antibody, and it may be speculated that the K61104 peptide will not cross-react with other anti-LeY antibodies, nor should it. Panels of anti-LeY antibodies with different reactivities clearly suggest that LeY-reactive antibodies recognize different epitopes on the LeY antigen (19). Vaccination with the K61104 peptide will nevertheless induce subsets of antibodies with the specificity that BR55-2 has for LeY. Furthermore, our findings imply that peptides with low affinity/avidity for isolating antibodies can still induce highly specific anti-carbohydrate immune responses. Previous studies on anti-idiotypic antibodies have shown that immune responses to mimics have little to do with affinity, a property associated mostly with antigenic mimicry (46, 47). Furthermore, a peptide mimic of the polysaccharide cryptococcal epitope, displaying high affinity for the isolating antibody, did not induce a high titer anti-carbohydrate immune response, suggesting that the peptide was only a partial mimic (7).

Although current procedures for predicting ligand-antibody interactions are limited, mainly because of the conformational flexibility of ligands and antibodies and because of the role of solvents in mediating ligand recognition and binding, the utilization of a crystallographically determined starting position can, nevertheless, lend to discrimination of differences in binding orientations of analogs. Although modeling does not account for multivalent interactions as represented in using MAP forms, multivalency by itself does not lead to BR55-2 binding as we showed in Fig. 2, in which MAP peptide K61111 does not bind to BR55-2. Modeling was used to define how the antibody-binding site might be recognized by peptide or by LeY. It is well known that peptides adopt different conformations under different conditions. There is no a priori way to know that a MAP form will affect the conformational presentation of a peptide (Table III). But if a peptide within the MAP form binds to an antibody, it is argued that it does so because the presented peptide adopts a binding mode conformation conducive to antibody binding. Consequently, strategies that evaluate binding modes of mimics within antibody combining sites provides relevant information as to the construction of an effective mimicope. Most likely this requires an improvement of the fitting between the bound peptide and the antibody, by maximizing particular interactions within the antibody heavy and light chains (7, 8). Three-dimensional structures of antibodies in complex with peptides will facilitate structure-based design of peptide surrogates in vaccine applications. Strategies for improving the complementary association between peptide mimotopes and antibody-combining sites have been suggested (7).

The results presented here support the suggestion by Young et al. (7) that molecular imprinting can be sufficient to generate a molecular mimetism from an immunological point of view. These studies further demonstrate a rational approach for the identification of peptidomimetics that can be used to direct the diversity of combinatorial libraries or to optimize strategies oriented to elicit a particular subset of antibodies in the context of an immune response.

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