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We performed a scanning mutagenesis study of heavy chain complementarity-determining region (CDR) residues to identify how mutations affected binding of the anti-carcinoma mAb BR96 to Ag, Lewis Y, and to an anti-Id Ab (anti-Id). By ELISA, we demonstrated that the anti-Id bound close to the Ag binding site of BR96, but the anti-Id and Ag sites were not identical. Immunoblot analysis and screening of light and heavy chain CDR libraries with multiple mutations in each CDR suggested that the heavy chain had greater involvement in anti-Id binding. We then analyzed contributions of individual residues in the heavy chain CDRs to binding of Ag and anti-Id. In a filamentous phage vector containing BR96 V region sequences, mutations were introduced by codon-based mutagenesis at single positions within the three heavy chain CDRs. The resulting libraries of Fab fragments had all amino acids represented at a CDR position. We evaluated the expressed Fabs for binding to Ag and anti-Id by plaque lift assay. We identified the positions with mutations that had the greatest negative effect on binding to the anti-Id and to Ag and analyzed them on the basis of the BR96 x-ray structure. The residues most important for binding to the anti-Id were located in heavy chain CDR1 and CDR2 and were peripheral to the residues within the Lewis Y binding pocket. The Journal of Immunology, 1998, 160: 2353–2359.

Anti-Id Abs recognize antigenic determinants in the V region of other Igs. Anti-Id reagents are used frequently in research and in support of clinical studies with therapeutic Abs. As therapeutic agents in themselves, anti-Ids have been investigated since Jerne’s proposal of the Ab/anti-idiotypic network (1). This led to the concept that, if an anti-Id resembled or mimicked the Ag recognized by the Ab, the anti-Id might substitute as Ag to stimulate an immune response, essentially acting as a vaccine (2–7). An anti-Id would be a reasonable alternative to an Ag-based vaccine, particularly if the Ag were difficult to produce and purify in large quantities. The potential utility of anti-Ids for this purpose has prompted studies of the structural relationship between the Ag binding site of an Ab and the sites to which anti-Ids bind (8–10).

The three-dimensional structure of Abs alone or complexed with Ag has been defined for numerous mAbs by x-ray crystallography. Few studies have examined mAbs complexed with an anti-Id (9, 11), or have compared the detailed interaction of an Ab to Ag and anti-Id (8, 10, 12). The ultimate demonstration that an anti-Id represents a good mimic of Ag is empirical, i.e., to immunize an animal with the anti-Id and show that the animal’s immune response includes Abs that bind to Ag. This approach is laborious and not necessarily predictive of the human immune response. A more direct structural and functional comparison of Ab binding to Ag and anti-Ids is possible in view of the advances in molecular biology techniques for manipulating and expressing functionally active Ab fragments (13–17).

The use of filamentous phage vectors for the expression of Fv, scFv, or Fab fragments in bacteria allows rapid examination of multiple clones with mutations in the V region sequence. We prepared libraries of Fab fragments with mutations in the CDRs4 of BR96, a mAb that binds the Leα carbohydrate tumor Ag (18, 19). These libraries were originally designed and created for the isolation of chimeric BR96 (chiBR96) (20) Fab fragments with increased affinity for tumor Ag (21). In this study, we probed the libraries with synthetic Leα (sLeα) (21) Ag and with an anti-Id to identify how mutations at a CDR position affect binding to each ligand. By identifying the residues critical for binding, the method provides a structural footprint that compares the binding sites of an anti-Id and Ag at a functional level. The identified residues were compared on the basis of x-ray coordinates of chiBR96 Fab complexed with Leα (22). Despite the proximity of the anti-Id epitope to the Ag binding site, this analysis clearly reveals several Ab residues that influence Ag binding differently from anti-Id binding.

Materials and Methods

Generation of BR96 anti-Id

BALB/c mice were immunized repeatedly with murine BR96 IgG3 and the isotype switch variants IgG1 and IgG2a (23). Fusions were performed with splenic cells from the animals and the P3×63-Ag.653 myeloma line (24). One cell line produced Ab that bound to BR96, but not to isotype-matched nonspecific Abs. The anti-Id is called 757.

Abbreviations used in this paper: CDR, complementarity-determining region; chiBR96, chimeric BR96; H1, H2, and H3, heavy chain complementarity-determining region 1, 2, and 3 loops, respectively; HRP, horseradish peroxidase; L1, L2, and L3, light chain complementarity-determining region 1, 2, and 3 loops, respectively; Leα, Lewis Y; nLeα, nonoate methyl ester derivative of Lewis Y; sLeα, synthetic Lewis Y tetrasaccharide hydrazide; sLeα-HSA, synthetic Lewis Y conjugated to human serum albumin.
Enzyme-linked immunosorbent assays

By ELISA, we assessed binding of 757 to chiBR96 once chiBR96 was bound to Ag. The assay was performed essentially as described (21, 25). Briefly, microtiter plates (Immunon II; Dynatech Labs., Chantilly, VA) were coated with sLe\(^x\) conjugated to human serum albumin (sLe\(^x\)-HSA) (Alberta Research Council (ARC), Edmonton, Alberta, Canada) or with H3396 tumor cell membranes overnight at 4°C. Binding of chiBR96 and chiBR96 Fab to Ag was detected with biotinylated 757, followed by avidin conjugated to horseradish peroxidase (HRP) (Vector Labs., Burlingame, CA). ChiBR96 Fab was derived proteolytically by papain digestion of chiBR96 Ig.

**Immunoblot**

ChiBR96 (20 ng) and chiBR96 Fab (20 ng) were applied to a 4 to 20% Tris-glycine gel (Novex, San Diego, CA) in sample buffer under nonreducing and reducing conditions. After electrophoresis, the Igs were transferred electrophoretically to a nitrocellulose membrane at 200 mA for 1 h in 0.1 M Tris-glycine, pH 8.3, 20% methanol buffer (26). Immunoblottting was performed essentially as described (27). After the blocking step, 757 (1 \(\mu\)g/ml) was applied to the nitrocellulose membrane overnight at 4°C. Binding of 757 to the Ig chains was detected with goat anti-human IgM (Fc specific) conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories, West Grove, PA).

**Construction of CDR libraries**

Molecular modeling of BR96 heavy and light chain V regions (28) showed the Ag binding site was better defined by canonical structure (29) than by sequence-defined hypervariable regions (30). Based upon the canonical definition, the following light chain regions were chosen for mutagenesis: Val50-Tyr57 in CDR1 (L1); Tyr54-Ser57 in CDR2 (L2); and Gly96-Phe101 in CDR3 (L3) (31). The residues in the heavy chain V region targeted for amino acid substitutions were Gly62-Asp63 in CDR1 (H1); Tyr50-Asp55 in CDR2 (H2); and Gly95-Arg100a in CDR3 (H3) (21, 31). The sequence-numbering system conforms to Kabat et al. (30).

The M13 phage BR96 Fab expression vector, IXBR96, was constructed as described (21). BR96 V\(_L\), V\(_H\), and \(\gamma\) sequences precede the human constant domain listed above (21). The single residue libraries had a membership of 32, with one mutation per phage, representing all 20 amino acids and one translation termination codon. The residues for individual CDR region libraries were H1 positions 26–33; H2 positions 50, 52–56, and 58; and H3 positions 95–99 and 100a. Libraries for positions 51, 57, and 100 were technically difficult to make, and because we were unsure they contained all mutant codons, we did not include them in this study.

Oligonucleotides were synthesized by \(\beta\)-cyanoethyl phosphoramidite chemistry on an ABI 394 DNA synthesizer (Applied Biosystems, Foster City, CA) and purified as described (21). Mutagenic oligonucleotides encoding random amino acid residues at specified positions were synthesized by incorporating the trinucleotide C/ANN (\(n = A, C, G, T\)). The oligonucleotides were constructed with 15 to 20 bases from the chiBR96 vector flanking the mutagenic site. Each set of oligonucleotide mixtures was phosphorilated and annealed to the appropriate CDR-modified IXBR96 templates. Uriddinylated ssDNA IXBR96 templates were prepared in the Escherichia coli due ung strain CJ236 (Bio-Rad, Hercules, CA), and site-directed mutagenesis reactions were performed according to the method of Kunke1 (32), as described by the reagent manufacturer (Bio-Rad). A portion of the extended, ligated mutagenesis reaction was electroeluted into E. coli strain DH10B (Life Technologies, Gaithersburg, MD) and titered onto a lawn of E. coli strain XL-1 Blue (Stratagene, San Diego, CA). Each library was amplified by infecting a 50-ml liquid culture of XL-1 Blue in 2 \(\times\) YT broth + 10 \(\mu\)g/ml tetracycline and then growing the infected strain for 5 h at 37°C. Bacteria were removed by centrifugation and the libraries were stored at 4°C. T4 polynucleotide kinase, T4 DNA ligase, and T4 DNA polymerase were purchased from Boehringer Mannheim Corp. (Indianapolis, IN).

**Filter lift analysis of CDR libraries**

Filter lifts from plated phage libraries were prepared (33) and analyzed as follows. Plaque lift assays probed with 2 \(\mu\)g/ml sLe\(^x\) hydrazide conjugated to horseradish peroxidase (sLe\(^x\)-HRP) were performed as described (21). Additional probes used included: goat anti-human \(\kappa\) light chain conjugated to alkaline phosphatase (Southern Biotechnology, Birmingham, AL); anti-decapeptide mAb S24.1 (4 \(\mu\)g/ml) (34); and purified 757 (1 \(\mu\)g/ml). The murine mAbs S24.1 and 757 were detected with a goat anti-murine IgG (Fc-specific) reagent conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories). Bromochlorodindolyl phosphate-nitro blue tetrazolium was the chromogen used for the alkaline phosphatase reactions (35). In this study, we did not distinguish between weak and strong reactions when enumerating plaques. If a plaque stained with a probe, the reaction was considered positive. Although weak staining may reflect poorer interaction with Ag or anti-Id, expression levels can also vary from assay to assay. A more objective assessment of the data was achieved by counting plaques that were positive independent of the intensity of the signal.

**DNA sequence analysis**

A representative subset of BR96 mutant clones from one single residue library was selected for DNA sequence analysis. Phage ssDNA was prepared, and the heavy chain CDR region of interest was sequenced by the dideoxynucleotide termination method (Sequenase Version 2; United States Biochemical Corp., Cleveland, OH).

**Structural analysis**

Computer graphic analysis was completed with the Insight II program (MSI, San Diego, CA) using the x-ray coordinates of chiBR96 Fab in complex with the noncovalent methyl ester derivative of Lewis Y (sLe\(^x\)) (pdb code "1CLY") (22). Color figures were produced with Insight II.

**Results**

**Generation of BR96 anti-Id 757**

The 757 anti-Id Ab recognizing BR96 was raised in syngeneic mice immunized multiple times with the original isotype of BR96, IgG3, and the IgG1 and IgG2a isotype switch variants. Because the syngeneic immune response was weak, 757 was the only BR96-specific anti-Id isolated. We have subsequently immunized BALB/c mice with a humanized form of BR96 and generated many anti-Id Abs with a variety of binding characteristics, including several that recognized chiBR96, although with lower affinity than 757, showing that in a different framework context the BR96 CDR regions are immunogenic.

**ELISA analysis of 757 binding to chiBR96**

The BR96 anti-Id was generated as a reagent for pharmacokinetic assays and immunohistochemistry to support clinical trials with chiBR96. Detection of a chimeric or human mAb in human sera and tissues requires a specific reagent, since commercially available reagents cannot distinguish between the therapeutic Ab and endogenous Abs. To demonstrate specificity of an anti-Id, a binding assay is often performed in which mAb and anti-Id are preincubated; then the solution is added to Ag, and binding of the mAb is measured (36). When this experiment was performed in an ELISA format with 757, the anti-Id did block binding of chiBR96 to Ag. Therefore, we knew that the anti-Id was not recognizing framework residues distal to the Ag binding site.

An anti-Id may block binding of the mAb to Ag, but still detect the mAb if the mAb is allowed to bind Ag first (36). To investigate further how the anti-Id and Ag binding sites were related, we performed an ELISA in which chiBR96 was first bound to Ag, followed by incubation with biotinylated 757. For this experiment,
both sLe\(^y\)-HSA and H3396 tumor cell membranes were used as the source of Ag. We also performed this assay with chiBR96 Fab to rule out the possibility that only one binding site of the IgG bound to Ag. ChiBR96 and its proteolytically derived Fab were incubated on sLe\(^y\)-HSA and H3396 tumor cell membranes, followed by incubation with biotinylated 757.

**Immunoblot analysis of 757 binding to chiBR96 heavy and light chain**

Heavy chain CDR residues generally contribute more to Ag binding than do light chains (37–39). For BR96, greater than 60% of the contacts with sLe\(^y\) are formed to heavy chain CDR residues (22). To investigate whether we could detect any interaction of 757 with heavy or light chain by immunoblot, we performed an experiment with nonreduced and reduced chiBR96 whole Ab and Fab. Detection by 757 was greatest with nonreduced Ab and Fab fragment (Fig. 2, *lanes* 1 and 2). When the whole Ab was reduced (Fig. 2, *lane* 4), no staining of the light chain was observed, and staining of the heavy chain was significantly less than observed with nonreduced Ab. Since the light chain of the reduced whole Ab was not stained by 757 (*lane* 4), we assume that the band visible in the reduced Fab fragment (*lane* 3) is also heavy chain. The two chains of the Fab fragment comigrate such that the two cannot be distinguished by immunoblot with specific reagents. While the anti-Id did not bind the \(\kappa\) light chain alone, the immunoblot does not eliminate the possibility that the interaction of heavy and light chain together is important for optimal binding, and that conformational epitopes are recognized. The reduced heavy chain was recognized by the anti-Id, indicating that either the heavy chain has greater involvement with 757 or that the heavy chain epitope has greater linear sequence preserved in an immunoblot.

**Identifying BR96 CDRs important to 757 binding using large libraries**

Because BR96 has a relatively low affinity for tumor Ag \((K_a = 10^7 \text{ M}^{-1})\) (18), we created filamentous phage libraries consisting of mutations within each of the BR96 CDRs to identify mutants with higher affinity (21). Each library consisted of \(10^6\) to \(10^8\) members, depending on the length of the CDR. Separate libraries of mutations were created for each H and L chain CDR and contained predominantly single, double, and triple mutations (21). For this study, we probed the CDR libraries separately to determine how mutations in each CDR of each chain affected binding of 757. If a CDR were particularly important for anti-Id binding, we expected most mutations to be detrimental so that a large proportion of the library would not bind to 757. In contrast, for CDRs that did not contribute significantly to binding, mutations in the CDR should have relatively little effect on binding to the anti-Id.

Each of the six CDR libraries was examined in three to four assays (1000–2000 plaques per assay) with an anti-human \(\kappa\) reagent and 757. The number of clones examined represents only a fraction of the total membership of each library, but it does provide a measure for the interaction of each CDR with the anti-Id. We
found that mutations in light chain CDRs did not reduce the number of clones binding the anti-Id compared with the number binding the anti-human \( \kappa \) reagent. The percentage of plaques in the three light chain libraries, L1, L2, and L3, that bound the anti-Id was slightly greater than the percentage that bound to the anti-human \( \kappa \) reagent (Table I). The higher percentage resulted, in part, because of a qualitative difference between the staining patterns provided by each reagent. The intensity of the staining reaction varied markedly for the plaque lifts probed with 757, which decreased the accuracy of enumeration. This variation suggests that light chain mutations may have affected the expression of correctly assembled Fab. In addition, mutations introducing stop codons in L3 eliminate the expression of Ck, but may not affect assembly of the light chain V region with the heavy chain.

The heavy chain CDRs were more critical to anti-Id binding (Table I). Binding of 757 to the H1 and H2 binding was almost entirely lost, with only 1 to 2% of plaques providing a positive signal. The H3 library had a greater percentage of plaques (25%) binding the anti-Id than observed with H1 and H2 (Table I).

**Effect of heavy chain single residue mutations to binding of 757 and sLe\(^{\alpha} \)**

Large libraries permit only a statistical view of how a CDR contributes to binding, since all mutations cannot be assessed. For the affinity maturation study of BR96 (21), we also prepared codon-based mutagenesis libraries for each individual residue within the three heavy chain CDRs. The smaller size of these libraries, 32 members representing all 20 amino acids at a given residue, allows complete screening of the library multiple times to ensure that all members have been examined. Because the inferences we draw from mAb reactivity for each library are statistical, it is important that, on the whole, the libraries contain the complete complement of 32 members.

To best demonstrate a library has the diversity of membership is to sequence clones and show that all of the mutagenic codons were incorporated. The purpose of this study was to compare and map residues that are critical to binding to Ag or anti-Id. Even though the library membership is only 32, to sequence the requisite number of clones to ensure all codons are represented is a very laborious effort that undermines the essential simplicity of this method, which we view as its major advantage.showing with 90% confidence that all residues are represented and is consistent with our statistical expectations.

From our experience with the libraries, we believe that they represent the theoretical membership. Growth, infection, and immunological characteristics serve as indicators that libraries are constructed correctly and have all or nearly all mutant codons represented. We performed plaque lifts with reagents that would bind to each of the C regions to show that V region genes had been inserted in frame at expected frequencies, and we were careful not to overinterpret the data quantitatively.

Each library was probed with sLe\(^{\alpha} \)-HRP and the anti-Id. We performed two to three plaque lift assays with 300 to 500 plaques per lift per CDR residue library. In addition, we probed each library with an anti-human \( \kappa \) reagent. Because clones making only light chain are detectable with the anti-human \( \kappa \) antisera, we included a second control probe, mAb 52.4 (34), that recognizes a decapeptide tag placed at the carboxyl end of CH1. Positive reactions with 52.4 indicate that the clones are expressing assembled Fab. Since the mutations are in the heavy chain CDRs, mutations that either introduce a stop codon in one of the CDRs or prevent appropriate assembly will not be detected by 52.4, and consequently are not included in the experiment. Therefore, the percentage of positive plaques for sLe\(^{\alpha} \) or 757 was calculated as follows:

\[
\frac{\text{(No. sLe}^{\alpha} \text{ or No. 757 positive plaques)}}{\text{No. 52.4 positive plaques}} \times 100
\]

As suggested from data with the larger, more complex CDR libraries, when residues in H1 and H2 were substituted, binding to the anti-Id was most affected. Less than 20% of the clones in CDR H1 libraries, residues 26, 28, and 31, and in H2 CDR libraries, residues 52a, 53, 54, and 55, bound 757 (Fig. 3). Of these residues, H2 positions 52a, 54, and to a lesser extent 55 were also nonpermissive to mutation for binding to sLe\(^{\alpha} \). In addition, mutations of H1 residue 29 and H2 residues 50 and 52, resulted in a loss of sLe\(^{\alpha} \) binding. Binding of sLe\(^{\alpha} \) was more affected than anti-Id binding by mutations in H3, primarily at residues 95 and 96, which had only about 30% of the clones binding to Ag.

**Structural analysis of BR96 residues critical to Ag and anti-Id binding**

To validate our scanning mutagenesis approach as a method to study the relationship between the binding sites for Ag and an anti-Id, we compared residues least permissive for anti-Id and for sLe\(^{\alpha} \) binding on the BR96 x-ray structure (Fig. 4). Residues were considered least permissive to change if less than 50% of the tested mutant clones bound to either ligand. The Ag binding site in BR96 has the shape of a deep pocket (22). The residue positions that most affected Ag binding, Tyr33, Tyr50, Gly95, and Leu96 (coded red in Fig. 4), are located deepest within the pocket of the Ag binding site. Those that impacted binding of the anti-Id only, Gly26, Phe27, and Gly53 (coded gold in Fig. 4), are located at the periphery of the pocket. Residues Phe29, Asp31, Tyr32, Ser52, Gln52A, Gly54, and Asp55 affected binding to both ligands (coded purple in Fig. 4). Gly26, Phe27, Phe29, which are critical determinants of the H1 canonical loop conformation, and Gly54, critical to the H2 loop conformation (29), were important to anti-Id binding. Mutations at two determinants, Phe29 and Gly54, were detrimental to Ag binding as well. Residues Asp31 and Gln52A are located at the interface between H1 and H2 and are important for anti-Id binding. In summary, mutations of residues in the H1 and H2 loops had greater impact on anti-Id binding, and those in loops H2 and H3 had greater effect on sLe\(^{\alpha} \) binding.

**Discussion**

The results of our study and those of Evans et al. (9) suggest that it is unlikely that an anti-Id to an anti-carbohydrate mAb would mimic Ag. Evans and colleagues solved the crystal structures for
an anti-*Brucella abortus* LPS Fab complexed to Ag and to its anti-Id Fab. They found that adequate contact was not made by the anti-Id in the deep and narrow anti-LPS binding site. BR96 represents a similar example.

The best example to date of an anti-Id that, to some extent, structurally mimics Ag is the anti-Id E5.2 to the anti-hen egg-white lysozyme mAb D1.3 (10). Furthermore, E5.2 induced an immune response in animals that included Abs to Ag (10). The complexity of the structure-function relationship between D1.3 and its ligands was illustrated in an alanine-scanning mutagenesis study (43). The contributing bond energies of the 13 amino acids common to binding of both the anti-Id E5.2 and hen egg white lysozyme Ag were measured (43). Dall’Acqua and colleagues (43) found that the functional epitopes (44) were formed by a subset of structural contact residues. Mapping by binding provides a different perspective than does a strictly structural analysis of Ag or anti-Id contact residues.

In this study, we used codon-based scanning mutagenesis to identify BR96 heavy chain CDR residues important for binding to Ag and anti-Id. Screening the BR96 CDR single residue libraries by binding indicated that the binding sites for sLe^\text{\^y}\ and anti-Id partially overlap. Residues least permissive to mutation for anti-Id binding were found in CDRs H1 and H2, while those nonpermissive for Ag binding were found in CDRs H2 and H3. Residues important for Ag binding map to the deep groove that forms the sLe^\text{\^y}\ binding site (22). In contrast, residues important for anti-Id binding are more exposed and accessible.

Mapping of BR96 residues nonpermissive to change illustrated that mutations disrupted both direct interactions, that is, contact with Ag, and indirect interactions. The four residues important only for Ag binding, Tyr33, Tyr50, Gly95, and Leu96, all contact nLe^\text{\^y}\ in the x-ray structure (22). Both tyrosine residues, 33 and 50, participate in the formation of the deep pocket binding site of BR96 (22). Indirect interactions include loop conformations, the interface between loops, and more distant interactions in which a change in one residue affected another’s contact with Ag. Residues Phe29 and Gly54 are critical determinants of the canonical loop conformations (29) of H1 and H2, respectively. Mutations of these residues are expected to significantly alter these loop conformations, and they did affect both sLe^\text{\^y}\ and anti-Id binding. Residues Gly26 and Phe27, also determinants of the H1 conformation, significantly reduced only anti-Id and not sLe^\text{\^y}\ binding. The importance of the H1 and H2 loops to anti-Id binding was also demonstrated by the sensitivity to changes at residues located at the interface of the two loops (Asp31 and Gln52A). Residues more distant from the Ag binding site that affected anti-Id as well as Ag binding included Asp55, which is greater than 10 Å of nLe^\text{\^y}\. A second residue, Ser52, packs against Tyr33, which does closely contact the bound Ag.

Results of this study demonstrated the importance of individual residues to binding of chiBR96 to its ligands. We observed that when either residue Gln52A or Gly54 in the H2 loop was mutated, binding to both sLe^\text{\^y}\ and anti-Id was lost. Mutations of residue Gly53 in the H2 loop had quite different effects on binding to each of the ligands. Approximately 10% of clones in the H53 library bound to the anti-Id. In contrast, virtually all mutations at this position had no effect on Ag binding. Furthermore, at position 53, a change from glycine to aspartic acid resulted in an increase in affinity to tumor Ag (21). We noted then that this amino acid change caused complete loss of anti-Id binding (unpublished observation).

Evaluating single residue libraries can be accomplished more rapidly than examining larger libraries, and the results may present a clearer indication of which residues should be the focus of further mutagenesis. Furthermore, we gain a more comprehensive view of the interactions between a mAb and its ligands by substituting residues with all possible amino acids, rather than a single amino acid as for alanine scanning. While a mutation of a single residue may eliminate all binding activity to a ligand, multiple mutations in CDRs may be necessary to enhance binding. We and others (20, 45) have reported that mutations improving Ab affinity for Ag are frequently additive. Single residue libraries offer a distinct advantage compared with larger, multimillion member libraries, because the smaller membership can be completely screened. After screening single residue libraries to identify mutations that affect binding, the individual mutations can often be combined into a molecule and the function reassessed.
graphic approaches and functional analysis by molecular genetics provides a comprehensive body of information to guide Ab engineering.

Acknowledgments

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References


FIGURE 4. Mapping of BR96 heavy chain residues important for sLeα and/or anti-Id binding. Residues were analyzed on the basis of the chiBR96 Fab-nLeα crystal structure (pdb code “1ICL” (22)). In A, the complex is shown in space-filling representation. For clarity, the BR96 C regions are omitted. The variable heavy and light chains are colored light blue and dark blue, respectively. The carbohydrate ligand nLeα is shown in green. Labels indicate the approximate positions of heavy chain CDR loops H1, H2, and H3. Residues for which no more than 50% of the tested mutant clones displayed binding are color coded (red, important for sLeα binding only; gold, important for anti-Id binding only; purple, important for both sLeα and anti-Id binding). In B, these residues are shown relative to the position of nLeα.

Just as one might alter the binding or specificity of a mAb, the strategy described in this study could also be applied to engineering the properties of an anti-Id. One application would be to distinguish an anti-Id with a desired property among a panel of anti-Lds. Similarity in the profile of mAb residues affecting binding of an anti-Id and Ag would indicate which anti-Id would most likely elicit an immune response similar to Ag in vivo. Those with overlapping or independent binding sites would be more useful for analytic reagents for clinical trials, particularly if free Ag exists in the blood. Understanding the contribution of CDR residues to ligand binding in detail is often critical to attempts to improve Ab function. The combination of structural analysis by crystallography and functional analysis by molecular genetics provides a comprehensive body of information to guide Ab engineering.


