The specificity of antibody recognition of the ABO blood group trisaccharide antigens has been explored by crystal structure analysis and mutation methods. The crystal structure of the Fv corresponding to the anti-blood group A antibody AC1001 has been determined to 2.2-Å resolution and reveals a binding pocket that is complementary to the blood group A-trisaccharide antigen. The effect of mutating specific residues lining this pocket on binding to the A and B blood group trisaccharide antigens was investigated through a panel of single point mutations and through a phage library of mutations in complementarity determining region H3. Both approaches gave several mutants with improved affinity for antigen. Surface plasmon resonance indicated up to 8-fold enhancement in affinity for the A-pentaasaccharide with no observable binding to the blood group B antigen. This is the first example of single point mutations in a carbohydrate-binding antibody resulting in significant increases in binding affinity without loss of specificity.

The affinity of anti-carbohydrate antibodies for their antigens is commonly observed to be 3–5 orders of magnitude lower than affinities of anti-protein or anti-peptide antibodies for their antigens, yet there is no clear mechanism to explain this phenomenon. One means of exploring this question is to attempt to generate mutant anti-carbohydrate antibodies with higher affinities, either by design of site-directed mutants or by randomizing selected codons in a phage library. However, the production of antibodies with improved affinities that maintain antigen specificity has proven challenging (1–4). For example, phage-display techniques have been reported to yield higher binding mutant scFvs, the resulting antibodies often contain multiple mutations and have rarely yielded dramatic increases in affinity for carbohydrate antigens.

Some of the differences in the behavior of anti-carbohydrate and anti-protein antibodies stem from the fact that these antigens are processed differently by the immune system. Unlike protein antigens, carbohydrate antigens are generally T-cell independent, therefore effectively limiting the B cell response to the production of IgM antibodies. The absence of somatic mutation of the rearranged antibody genes leads to the expression of essentially the germline sequences of these proteins, consequently limiting their affinity. This can be clearly seen in studies (8, 9) that compared the free and hapten-bound structures of an anti-peptide antibody 48G7 with its germline predecessor. The germline antibody was shown to bind the antigen with low affinity by an induced fit mechanism, whereas the mature 48G7 antibody possessed a combining site highly complementary to the hapten, leading to a 30,000-fold increase in binding affinity. Many of the amino acid residues mutated in the maturation process were not observed to directly contact the antigen but induced structural changes in the binding domains that resulted in improved binding. This pattern has been seen in other systems (10, 11) and is consistent with global analyses of the relative contributions of germline and somatic factors to antibody affinity (12, 13).

More recently, an in vitro scanning saturation mutagenesis has been described (14) and utilized to examine the changes in specificity associated with changing all important residues in the antigen-binding site of a digoxin-specific scFv (15). Interestingly, 86% of all mutants retained binding activity, and many of the mutants displayed measurable binding to digoxin.
analogues, indicating that the mutations resulted in changes in the specificity of the antibody fragment.

In a program to explore the concomitant effects of mutation on binding affinity and specificity of anti-carbohydrate antibodies, the ABO blood group antigens were selected as ideal targets. Anti-blood group A and anti-blood group B antibodies are present in persons lacking the corresponding antigen, and the immune response from a mismatched blood transfusion is rapid and can lead to severe complications and death (16). Remarkably, this means that the anti-A and anti-B blood group antibodies are able to unambiguously differentiate between trisaccharide antigens, which differ only in the substitution of an acetamido for a hydroxyl group. As a control of specificity, any mutation in an anti-A or anti-B blood group antibody that yielded higher affinity for its native antigen would be required to show no cross-reactivity with the other antigen.

A single-chain Fv (AC1001 scFv) was produced based on the sequence of the anti-blood group A IgM hybridoma AC1001 (17). This type of recombinant polypeptide links the carboxyl terminus of the variable light chain (VL) to the amino terminus of the variable heavy chain (VH) by a peptide linker of sufficient length to bridge this distance (18). The use of scFv avoids the heterogeneous products of proteolysis of the whole antibody as well as problems of association between VH and VL domains in heterogeneous products of proteolysis of the whole antibody as well as problems of association between VH and VL domains in recombinant protein. Following proteolysis to the Fv, the protein was crystallized (19), and its structure was determined. Appropriate mutants were designed into a scFv dimer form based on the three-dimensional structure as well as a phage library of CDR H3 mutants. The binding kinetics of the selected products were determined using surface plasmon resonance, and some of the scFv mutants showed up to a 30-fold increase in antigen binding with no loss of specificity as compared with the wild-type scFv.

**EXPERIMENTAL PROCEDURES**

**Structural Analysis by X-ray Crystallography**—Crystals of the anti-blood group A Fv were obtained, and the crystallographic data were collected as reported (19); data to 2.2 Å were processed using programs in the CCP4 Suite (20). Molecular replacement, refinements, and map calculations were done using the program X-PLOR (21). The model was visualized and adjusted using the programs FRODO (22) and SETOR (23). The molecular surface of the anti-A Fv was generated using MS (24), and the A-trisaccharide antigen was modeled into the Fv-binding site using SETOR. The Fv crystallized in the space group P2_1_2_1_2_1 with cell dimensions a = 51.7, b = 58.0, and c = 83.6 Å. Data were collected at ambient temperatures on an Enraf-Nonius FAST detector and reduced to a 2.2 Å resolution with a few reflections merging R-factor of 0.100. The 24,752 observed reflections were reduced to 12,348 unique reflections (Table I).

**Structure Determination and Refinement**—The VH and VH dimer of the antibody YsT9.1 (25) was used as a probe in molecular replacement experiments. Reflections in the resolution range 0.06–2.2 Å were used in the rotation and translation function searches. All subsequent structure refinements were done using the X-PLOR program with data in the 6.0–2.2 Å range. The YsT9.1 Fv probe was adjusted using rigid body refinement, and then the domain sequences were changed to those of AC1001 (17). Electron density maps were calculated and then displayed using the program FRODO. Refinement consisted of several rounds of manual intervention alternating with positional refinement and overall B-factor refinement. The overall fit of the model to the electron density was good with only a few instances of discontinuous or ambiguous electron density. The final model consists of 1720 protein atoms and 62 water molecules and refined to a final R-factor of 0.183 (Table I). Coordinates have been deposited to the Protein Data Bank under accession code 1UVS.

**Generation and site-directed Mutagenesis**—Generation of the wild-type anti-blood group A scFv gene construct (TGA scFv) has been reported previously (19, 26) except that a shorter peptide linker (RADAA) was used to join the VH and VL domains. PCR mutagenesis was used to construct the site-directed mutant clones listed in Table II. Oligodeoxynucleotides containing the appropriate mutations were synthesized on an automated DNA synthesizer model 394 (Applied Biosystems Inc.). The crude DNA oligonucleotides were piggybacked onto an automated DNA/RNA synthesizer model 387 (Applied Biosystems Inc.) to ensure that the desired mutation was present. Expression of the wild type and mutant scFvs was carried out in E. coli strain TG1 as reported previously (19, 28).

**Production and Screening of an H3 Phage Library**—A library was constructed in which residues 100–103 (Kabat numbering) of the heavy chain were completely randomized, giving 1.6 × 10^10 possible sequences. Randomization was generated by PCR amplification of the scFv gene from framework 1 of VH, framework 4 of VH, using a reverse primer that introduced an ApoLI site for cloning into fd-tetGIIID (27) and a degenerate forward primer. A second PCR step added a NotI site for inserting the library in fd-tetGIIID. The library was ligated into fd-tetGIIID and electropropated into E. coli TG1, giving 2.3 × 10^10 transformants. The transformed cells were plated on 2x YT (29) containing 20 μg/ml tetracycline and incubated overnight at 32 °C. The cells were washed off the plates with 2x YT with tetracycline, and following centrifugation, phage were isolated from the supernatant (30).

For panning, Immuno MaxiSorp™ microtiter plates (Nunc) were coated overnight at 4 °C with 50 μg/ml of BSA-A-trisaccharide (Chemibiod). Wells were washed three times with PBS and then blocked with 5% BSA in PBS for 1 h at room temperature. The BSA was removed, and 10-μl phage in 200 μl of BSA/PBS were added per well. Following a 1-h incubation at room temperature, the wells were washed three times with PBS, 10 times with 0.1% Tween 20 in PBS, and finally twice with PBS. Phage were eluted with 0.1 M sodium acetate, pH 2.8, and neutralized with 2 mM Tris, pH 9.5. Eluted phage were incubated with log phase E. coli, grown in LB (29), for 30 min at 37 °C followed by 30 min at room temperature. The cells were then pelleted, and phage were isolated as described above. Three rounds of panning were performed.

**BIACORE Analysis**—The binding kinetics for the interaction of the purified scFv fragments with the A antigen were determined by surface plasmon resonance using BIACORE 2000 or BIACORE 3000 biosensor systems (Biacore AB). Single-chain Fv binding to immobilized BSA-trisaccharide and BSA-B-trisaccharide (Chemibiod) was analyzed using BSA surfaces as a reference. For the analysis of oligosaccharide binding to immobilized scFv in the BIACORE 3000, anti-blood group A scFv specific for an unmutated polysaccharide antigen (31) were used as the active and reference surfaces, respectively. Immobilizations were done on research grade CM5 sensor chips in 10 mM sodium acetate, pH 4.5, using the amine coupling kit supplied by the manufacturer. All measurements were performed at 25 °C in 10 mM HEPES, pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005% P20 at a flow rate of 20 μl/min. Surfaces were regenerated by washing with analysis buffer.

The binding constants were calculated from the association rate and dissociation rate constants where possible or by Scatchard analysis of equilibrium binding. A comparison of the response on the active and control surfaces allowed for the subtraction of bulk effects associated with buffer changes and high analyte concentrations and for the calculation of specific binding. Prior to analysis, all scFv preparations were passed through a Superdex 75 column equilibrated in the BIACORE running buffer. The dimer peaks were collected for binding studies.
RESULTS

Antibody Structure—The structure of the anti-blood group A Fv shows that the CDRs of the VL and VH chains adopt canonical conformations, except CDR H3 for which canonical structures are undetermined. Of these, CDR L1, L2, L3, and H3 interact to form a pronounced pocket on the variable surface of the antibody (Fig. 1). The pocket is V-shaped and 2.8 Å wide at its deepest point, increasing to a width of 4.7 Å at the surface. The antigen-binding site is 11 Å deep and can therefore accommodate the terminal GalNAc of the trisaccharide antigen.

Model of the Antibody-Antigen Complex—The trisaccharide blood group A antigen was manually modeled into this pocket using a conservative approach in that only small movements of glycosidic and side chain dihedral angles were permitted. Overall, the orientation of the model (Fig. 2) was selected to correspond to the shape of the observed binding pocket, and the antigen conformation was based on the structure of the trisaccharide observed in the *Dolichos biflorus* lectin (32). The model was used as a predictive tool only, to identify residues that were likely to strongly affect trisaccharide recognition. A number of potential complementary interactions were observed, such as between the Asn-L34 and Tyr-L36 side chains and the hydroxyl groups 3 and 4 of the GalNAc residue of the antigen. The N-acetyl group of the GalNAc was within hydrogen-bonding distance of the side chains of Gln-L89 and Tyr-L36 in the model. Although the object of this modeling study was only to suggest a set of residues for mutation, it also suggested a reasonable basis for differentiation between the A and B antigens in which the hydroxyl group of the latter would be unable to bridge the distance necessary for hydrogen-bonding these groups.

Production of Mutant scFv Dimers—The designed mutations were introduced by site-directed mutagenesis into an scFv species with a short linker between the VL and VH domains to create dimeric species whose higher avidity would aid panning and surface plasmon resonance analysis. The same species was used as the template for a phage library construction.

Site-directed Mutants—With a dissociation constant of 2.9 μM (19), the monovalent scFv-trisaccharide interaction is near the lower limit of the affinity range of BIACORE measurements using the antibody fragment as the analyte or injected molecule. At the surface densities of the BSA-trisaccharide employed, the scFv binding was entirely bivalent. For mutants with measurable dissociation rates, the binding data fit well to a 1:1 interaction model (Fig. 3A), indicating that the binding was homogeneous and therefore all bivalent. The rate constants, presented in Table II, for the binding to trisaccharide antigen were derived in this way. Most mutants, including

| scFv | Kinetic analysis | Equilibrium analysis | pentasaccharide<sup>a</sup> | trisaccharide<sup>a</sup> |
|------|-----------------|---------------------|---------------------------|--------------------------|
|      | $k_a$  | $k_d$  | $K_D$     | $k_d$  | $K_D$     | $K_D$ | $K_D$ |
| WT   | $10^4$ M/s<sup>a</sup> | $10^{-2}$ s<sup>a</sup> | $10^{-6}$ M   | $10^{-6}$ M | $10^{-6}$ M | $10^{-6}$ M |
| L46/LN<sup>b</sup> | 26     | 6.9       | 20         | 20         | 20         | 20     |
| L46/LM | 18     |           |            |            |            |        |
| N102H,G | 22     |           |            |            |            |        |
| L103H,I | 2.6    | 4.8       | 1.9        | 1.5        | 0.3       | 1.9    | 11     |
| L103H,I | 4.8    | 4.0       | 0.8        | 0.86       | 0.3       | 1.1    | 6.5    |
| L46/LN/L103H,I | 2.7    | 5.4       | 2.0        | 2.8        | 2.8        |        |
| L46/LM/L103H,I | 3.1    | 6.4       | 2.1        | 1.4        | 1.4        |        |
| N102H/Q/L103H,I | 3.1    | 3.8       | 1.2        | 1.2        | 1.2        |        |
| L46/LM/N102H/Q/L103H,I | 2.4    | 4.3       | 1.8        | 1.4        | 1.4        |        |
| L46/LN/N102H/Q/L103H,I | 2.9    | 3.7       | 1.3        | 1.5        | 1.5        |        |

<sup>a</sup> Molecule (analyte) injected over the sensor chip.

<sup>b</sup> The one-letter code for amino acids is used with the L and H indicating light and heavy chain, respectively.

Fig. 1. Variable surface of the anti-blood group A scFv showing a pronounced pocket. The molecular surface of the antibody is shown in red, and the alpha carbon chains of CDRL1, L2, L3, and H3 are shown in black. A deep pocket is located at the antigen-binding site and is defined by CDRs L1, L2, L3, and H3.
L(103H)V, were tested for binding to BSA-B-trisaccharide, and affinity was observed. In all instances, $K_D$ values were derived from Scatchard analyses of equilibrium binding, but only the higher affinity mutants gave data that allowed for the determination of $K_D$ values from rate constants (Table II). The $K_D$ for the bivalent binding of wild-type scFv dimer to trisaccharide antigen was determined to be 26 nM as compared with a value of 290 nM for the binding of Fv monomer to the same antigen (19). The L(46L)/M, L(46L)/N, and N(102H)/Q mutations resulted in only marginal improvements in affinity. However, the effects of mutations at residue Leu-H103 were quite dramatic. The L(103H)I and L(103H)V mutations increased the $K_D$ for bivalent binding by 15- and 31-fold, respectively, relative to the wild type. The L(103H)I mutation in combination with mutations at positions L46 and H102 resulted in marginal improvements in some instances. Improved binding was primarily attributable to slower dissociation rates with a faster association rate also contributing to the superior performance of L(103H)I (Table II).

The true affinities of wild-type and mutant scFvs for trisaccharide and pentasaccharide antigen were determined by binding free monovalent antigen to immobilized scFv (Table II). This method gave a $K_D$ of 200 nM for the wild-type scFv-trisaccharide interaction, which is in good agreement with the previously reported value of 290 nM for the binding of Fv to immobilized BSA-A-trisaccharide (19). The affinity of wild-type scFv for the pentasaccharide antigen was 3-fold higher, Fig. 4. Relative to the wild type, the L(103H)I mutation improved the affinity for trisaccharide and pentasaccharide by ~2- and 3-fold, respectively. As with scFv dimer binding to immobilized BSA-A-trisaccharide, the L(103H)V mutation led to further 2-fold improvements. A comparison of the $K_D$ values for bivalent scFv binding with BSA-A-trisaccharide versus monovalent trisaccharide binding to scFv indicated that the avidity gain...
afforded by bivalency was ~75-fold for the L(103H)I and L(103H)IV mutants. It should be pointed out that the anomeric configuration of the galactose is locked in the glycoconjugate but not in the free trisaccharide.

**Phage Library Mutants**—After three rounds of panning, 21 clones were randomly picked, and all had the scFv insert with sequencing revealing 19 different sequences. All 19 clones were ligated into an expression vector with 11 giving soluble product yields that permitted BIACORE analysis. The sequences and antigen binding activities of these mutants are presented and compared with those of the wild-type scFv and the L(103H)IV mutant in Table III. The sequences of all mutants were very dissimilar to the wild-type sequence with nine clones showing only 25% sequence identity with wild type and the remaining two showing none.

Several of the mutants were observed to be superior to the best site-directed mutant, L(103H)IV, in terms of bivalent binding to BSA-A-trisaccharide. However, there was not always good correlation between binding of scFv to BSA-A-trisaccharide and free oligosaccharide binding to scFv. For example, whereas SGNK was one of the better mutants in terms of pentasaccharide binding to BSA-A-trisaccharide, its affinity for pentasaccharide was only marginally better than that of the wild-type scFv. Mutant HANK displayed enhanced affinity for BSA-A-trisaccharide and pentasaccharide but not trisaccharide. However, the highest affinity mutant in terms of pentasaccharide binding (KGPT) also had higher affinity for trisaccharide. A closer look at the affinities shows that the Leu-Ile-Val progression at position H103 is coupled with a decrease in flexibility of the side chain, indicating a potential entropic component in binding. Typically, higher entropy in the conformation of the binding site residues would be expected to be unfavorable as a thermodynamic penalty; would be incurred if antigen binding required residue immobilization. The reduced flexibility of the L(103H)I and L(103H)IV mutants thus provides a potential explanation for the increase in binding. Significantly, these scFv mutants did not display detectable binding to the B antigen, demonstrating that the increase in the affinity of the mutant for the blood group A antigen is accompanied by retention of the fine specificity of the native antibody, consistent with the model which suggests that those residues do not contact the acetamido/hydroxyl groups.

An attempt was made to produce mutants with even higher affinity by combining the single point mutations into double and triple point mutations. Surprisingly, the L(46L)N/L(103H)I and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I all displayed a slight increase in binding affinity over the L(103H)I single mutant, indicating that their respective contributions to binding were not cooperative. In contrast, mutants N(102H)Q/L(103H)I and L(46L)N/N(102H)Q/L(103H)I all display a slight increase in binding affinity over the L(103H)I.

**TABLE III**

| scFv           | Kinetic analysis | Equilibrium analysis | pentasaccharide | trisaccharide |
|----------------|------------------|----------------------|-----------------|---------------|
|                | $k_a$           | $k_d$               | $K_D$           | $k_a$         | $k_d$               | $K_D$           | $k_a$         | $k_d$               | $K_D$           |
| YGNL$^a$ (WT)$^b$ | 4.8             | 4.0                 | 0.82            | 26            | 0.86               | 0.3             | 1.1             | 6.5             |
| YGNV L(103H)IV | 5.0             | 4.0                 | 0.95            | 6.9           | 0.6                | 7.5             | 0.6             | 7.5             |
| LGTE           | 0.79            | 7.0                 | 8.9             | 5.8           | 0.6                | 5.1             | 0.6             | 5.1             |
| SLDK           | 1.1             | 8.3                 | 7.7             | 7.3           | 0.6                | 1.5             | 0.6             | 1.5             |
| HANK           | 2.7             | 5.0                 | 1.8             | 1.4           | 0.5                | 2.5             | 0.5             | 2.5             |
| YRHE           | 1.8             | 1.9                 | 1.1             | 1.2           | 0.6                | 3.1             | 0.6             | 3.1             |
| KGPT           | 4.7             | 5.5                 | 1.2             | 1.3           | 0.5                | 0.86            | 0.5             | 0.86            |
| SGNK           | 1.2             | 1.1                 | 0.87            | 1.2           | 0.6                | 2.7             | 0.6             | 2.7             |
| SANK           | 3.0             | 2.0                 | 0.66            | 0.66          | 0.7                | 5.5             | 0.7             | 5.5             |
| SANC           | 5.3             | 3.1                 | 0.58            | 0.58          | 0.5                | 2.8             | 0.5             | 2.8             |
| YKRT           | 5.5             | 3.0                 | 0.55            | 0.55          | 0.5                | 1.9             | 0.5             | 1.9             |
| HGRG           | 4.2             | 1.8                 | 0.43            | 0.46          | 0.4                | 1.3             | 0.4             | 1.3             |

$^a$ Molecule (analyte) injected over the sensor chip.  
$^b$ The wild type and mutant sequences are designates according to the amino acid residues at heavy chain positions 100–103 and using the one-letter amino acid codes.  
$^c$ Data also presented in Table II.

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**DISCUSSION**

Based on a model of the blood group A-trisaccharide with the AC1001 Fv (Fig. 2), amino acid mutations were proposed that would alter polarity, surface complementarity, and side chain aliphatic character to yield antibody fragments with potentially improved affinity for the trisaccharide epitope. The increase in binding affinity for the N(102H)Q, L(46L)M, and L(46L)N mutants is small (1.2-, 1.4-, and 1.6-fold, respectively, Table II). Much larger increases in affinity were obtained when the leucine at position H103 was mutated to isoleucine, L(103H)I, or valine, L(103H)IV, residues; the resulting increases in binding affinities for the Fv mutants were, respectively, 15- and 31-fold that of the wild-type antibody fragment. Although Leu-H103 lines the binding site, its side chain would probably not contact the trisaccharide antigen, and a conservative mutation at this position would not be expected to greatly alter interactions with the antigen. A closer look at the affinities shows that the Leu-Ile-Val progression at position H103 is coupled with a decrease in flexibility of the side chain, indicating a potential entropic component in binding. Typically, higher entropy in the conformation of the binding site residues would be expected to be unfavorable as a thermodynamic penalty; would be incurred if antigen binding required residue immobilization. The reduced flexibility of the L(103H)I and L(103H)IV mutants thus provides a potential explanation for the increase in binding. Significantly, these scFv mutants did not display detectable binding to the B antigen, demonstrating that the increase in the affinity of the mutant for the blood group A antigen is accompanied by retention of the fine specificity of the native antibody, consistent with the model which suggests that those residues do not contact the acetamido/hydroxyl groups.

An attempt was made to produce mutants with even higher affinity by combining the single point mutations into double and triple point mutations. Surprisingly, the L(46L)N/L(103H)I and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I, and L(46L)N/L(103H)I all displayed a slight increase in binding affinity over the L(103H)I single mutant, indicating that their respective contributions to binding were not cooperative. In contrast, mutants N(102H)Q/L(103H)I and L(46L)N/N(102H)Q/L(103H)I all display a slight increase in binding affinity over the L(103H)I.

Panning the phage library against BSA-A-trisaccharide selected for mutants with enhanced binding to this glycoconjugate, but this did not always result in improved affinity for free pentasaccharide or trisaccharide. These discrepancies are likely due to the locked anomeric configuration of galactose in the glycoconjugate and the pentasaccharide but not in the trisaccharide. Some of the phage mutants may have been selected for improved contacts with these features of the glycoconjugate. There is a great wealth of literature available dealing with the use of phage-display libraries to generate antibody fragments of desired specificity, and so it is significant that in these experiments, much higher increases in affinities were obtained using a rational design approach.

Both the site-directed and phage mutation results from AC1001 Fv are in marked contrast to those we obtained previously with another anti-carbohydrate antibody (1). The anti-*Salmonella* carbohydrate antibody, Se155–4, was extensively mutated in its CDR H3 by replacing each of the four native
residues with all 19 other amino acids, and none of the mutants showed any improvement in oligosaccharide binding (1).

To explore questions of affinity and specificity mimics in vitro the process of affinity maturation that antibodies can undergo when challenged with certain classes of antigen. Reducing the conformational flexibility of the antigen-binding site by the somatic mutation of a limited number of residues has undergone little if any somatic mutation.

The process of mutating a limited number of amino acids in order to explore questions of affinity and specificity mimics in vitro the process of affinity maturation that antibodies can undergo when challenged with certain classes of antigen. Reducing the conformational flexibility of the antigen-binding site by the somatic mutation of a limited number of residues has been shown to be a key factor in affinity maturation (34) and antibody affinity (35). This concept extends beyond the paratope as the free and peptide-bound structures of antibody 48G7 and its germline predecessor (8) showed that none of the nine mutated amino acid residues in the mature antibody directly contact the antigen but still cooperate in reducing entropic effects. Work is continuing to define residues that will distinguish between the A and the B trisaccharide antigens and on obtaining crystals of the mutant scFvs in complex with antigen.

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