Characterization of an Ig V\textsubscript{H} Idiotope That Results in Specific Homophilic Binding and Increased Avidity for Antigen

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mAb against G\textsubscript{D3} ganglioside demonstrate homophilic binding in which soluble anti-G\textsubscript{D3} mAb bind, through the G\textsubscript{D3} binding site, to a V\textsubscript{H} idiotope (designated Id\textsubscript{HOM}) on solid phase anti-G\textsubscript{D3} mAb. In this way, homophilic binding provides a mechanism for amplifying the binding of mAb to cell surface G\textsubscript{D3}. We show that serine 52a, within CDR2, is required for Id\textsubscript{HOM} expression, homophilic binding, and high avidity binding to cell surface G\textsubscript{D3}. Computer modeling based on the crystal structure of anti-G\textsubscript{D3} mAb R24 showed serine 52a situated at the mouth of the G\textsubscript{D3} binding pocket, but not directly involved with G\textsubscript{D3} binding. Substitutions at position 52a predicted to maintain the G\textsubscript{D3} binding pocket (e.g., threonine) resulted in the loss of Id\textsubscript{HOM} expression and homophilic binding and markedly decreased binding to cell surface G\textsubscript{D3}, but maintained low avidity G\textsubscript{D3} binding as measured by ELISA. All other substitutions at position 52a were predicted to significantly distort the binding pocket and resulted in the loss of both homophilic binding and any detectable avidity for G\textsubscript{D3}. We have structurally defined Id\textsubscript{HOM} and conclude that this idiotope is not required for the G\textsubscript{D3} binding pocket, but that the idiotope is necessary for homophilic binding, which is required for high avidity binding to cell surface G\textsubscript{D3}. We speculate that selection of certain V\textsubscript{H} genes may result in the expression of idiotopes that allow homophilic binding, and this may represent a general mechanism for increasing the avidity of Abs against T cell-independent Ags. The Journal of Immunology, 1996, 157: 1582-1588.

Abbreviation used in this paper: CDR, complementarity-determining regions.

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Site-directed mutagenesis

ChbR24-pdHL2.4, an expression vector that encodes for the coexpression of R24 light and heavy chain variable region genes with the constant regions of human IgG1x (8) was provided by Dr. Alan Jarvis (Repligen Corp., Cambridge, MA). Site-directed mutagenesis was performed in one of two ways. In the first method, a Muta-Gen Phagemid In Vitro Mutagenesis kit (New England BioLabs, Beverly, MA) was used. DNA encoding the V_{H}, region of chbR24 was isolated from the chbR24-pdHL2.4 expression vector as a Hindlll/Xhol restriction fragment, ligated into pBluescript II (Stratagene, La Jolla, CA), and cloned in Escherichia coli. Single stranded DNA was obtained, and in vitro mutagenesis was performed using a set of oligomers designed to introduce amino acid substitutions at specific codons within CDR2 of V_{H}. Each mutagenized V_{H} was sequenced by the disoxy chain termination method using Sequenase version 2.0 DNA (US. Biochemical Corp. Cleveland, OH). The mutagenized V_{H} was isolated by Hindlll/Xhol digestion and religated into the pdHL2.4 expression vector. The V_{H} insert was then sequenced to confirm the presence of the appropriate V_{H} mutation.

The second method of site-directed mutagenesis used the overlap extension PCR method (12). For the primary PCR reactions, complimentary strands of chbR24 V_{H} DNA were used as template. Two separate PCR products were generated with the desired mutations incorporated into overlapping 5' and 3' ends, respectively, by appropriately designed primers. These primers were also designed to introduce a Hindlll or Xhol site, respectively, into the opposite end of each PCR product. The two overlapping PCR products served as the template for the second PCR reaction to generate the full-length V_{H} segment containing the desired mutations. All PCR reactions were performed in a thermal cycler (Perkin-Elmer Corp., Norwalk, CT) using the following program: 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 30 cycles. The mutagenized V_{H} was inserted into the pCRII vector (Invitrogen, San Diego, CA) and sequenced to confirm the mutation. A Hindlll/Xhol restriction fragment containing the mutagenized V_{H} was then subcloned into the chbR24-pdHL2.4 vector. After each subcloning, the mutagenized V_{H} was sequenced to confirm that no further mutational changes had occurred.

Cell culture and transfection

Transfection of the various chbR24-pdHL2.4 constructs was accomplished by protoplast fusion with Sp2/0.Ag414 myeloma cells. Protoplasts were prepared from cultures of recombinant E. coli by pelleting the cells and resuspending them in 2.5 ml of cold 20% sucrose in 50 mM Tris-HCl, pH 8.0, followed by the addition of 0.5 ml of lysosyme (5 mg/ml) in 250 mM Tris-HCl, pH 8.0, and 1 ml of 250 mM EDTA, pH 8.0. After incubation on ice for 5 min, an additional 1 ml of Tris buffer was added, and the mixture was incubated at 37°C for 10 min. After formation of protoplasts, 20 ml of 10% sucrose in DMEM were added, and 15 min later, the protoplast suspension was used. For each fusion, 5 x 10^6 Sp2/0 cells were fused with 2 x 10^6 protoplasts in 1 ml of 50% Polyethylene glycol 1450 (Sigma Chemical Co., St. Louis, MO) for 90 s at room temperature. The fused cells were washed three times with DMEM containing 1% FCS, 100 U/ml of penicillin, and 100 U/ml of streptomycin, and then were resuspended in 10 ml of RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 1% nonessential amino acids, 100 U/ml of penicillin, 100 U/ml of streptomycin, and 100 μg/ml of kanamycin. The cells were then plated at 100 μl/well in 96-well plates and cultured at 37°C in 5% CO_{2}. After 24 h, an additional 100 μl of RPMI 1640 containing 100 μM methotrexate was added to prevent the growth of nonfused Sp2/0 cells. The culture supernatants were screened for secretion of human IgG using a capture ELISA in which supernatants were added to 96-well plates coated with goat anti-human IgG and then incubated overnight at 4°C. mAb R24 (10 μg/ml) was added to each well for 1 h. After washing, bound R24 was detected using an alkaline phosphatase-conjugated goat anti-mouse IgG3 second Ab. The plate was developed by adding nitrophenyl phosphate in a diethanolamine buffer. Absorbance at 405 nm was determined using a plate reader. Binding of mAb to purified G_{O} was measured by ELISA as previously described (13).

Immuo-TLC was performed with purified gangliosides as well as mixed melanoma ganglioside preparations, essentially as previously described (14), except that bound mAb was visualized using a peroxidase-conjugated goat anti-human IgG second Ab.

Flow cytometry

B16/728 cells were incubated on ice for 1 h in RPMI 1640 supplemented with 10% FCS containing 100 μg/ml of mAb. Cells were washed three times with PBS/1% BSA/0.05% Tween-20, and the pellet was resuspended in RPMI 1640 containing FITC-conjugated anti-human IgG (Tago, Burlingame, CA) at a 1/20 dilution. After 1 h incubation, the cells were again washed, resuspended in 0.5% paraformaldehyde, and analyzed using an EPICS Profile II fluorescence-activated flow cytometer (Coulter Corp., Miami, FL).

X-ray crystallography and computer modeling of R24 Fab fragments

The three-dimensional structure of the R24 Fab was determined by x-ray crystallography, the details of which will be published separately. A conservative approach was taken in modeling G_{O} into the observed binding pocket of R24. No side chains were permitted to move more than 0.5 Å, and no main chain atoms were moved. Modeling was conducted with the program SCOR (15). The model of the two sialic acid residues of G_{O} was generated from the program GEGOP (unpublished). Docking was performed by allowing conformational freedom to carbohydrate side chains. The model of G_{D} R24 that resulted contains several hydrogen bonds between the carbohydrate and Fab, and possesses a high degree of complementarity.

Results

Sequence analysis of variable regions of mAb that recognize G_{D} ganglioside

We have previously shown that the V_{H} regions of anti-G_{D3} mouse mAb R24, C5, K9, 11A, and MB3.6 all express the Id_{HOM} for homophilic binding (5). HJM1, a human anti-G_{D3} IgM, does not express this idiotope, but can bind to Id_{HOM} expressed on murine anti-G_{D3} mAb (data not shown). To characterize and map Id_{HOM}, V_{H} regions of these anti-G_{D3} mAb were sequenced and analyzed (Fig. 1). The first observation was that four of five mouse mAb directed against G_{D3} (R24, C5, 11A, and MB3.6) use a V_{H} segment from the most J_{H} proximal gene family, V7183 (16), and all five use a joining region from the J_{2} gene family (17). Identification of the diversity segment was possible only for R24 and C5, and both use a member of the VQ52 family. The sequence of an additional mouse anti-G_{D3} mAb, designated KM871, has been reported previously (19), and we noted that this mAb also uses the V7183 and J_{2} gene families to generate V_{H}. Thus, five of six sequenced mouse mAb against G_{D3} use the same V and J gene families to form V_{H} regions. These observations suggest that the
murine immune response to G\textsubscript{D3} is relatively stereotypic and that to produce high avidity Abs against G\textsubscript{D3}, mouse B lymphocytes use a restricted set of VDJ gene families to form the V\textsubscript{H} region.

Homophilic binding by mouse anti-G\textsubscript{D3} mAb is specific for anti-G\textsubscript{D3} mAb, since they do not bind to mAb specific for other Ags. This observation led us to hypothesize that the Id\textsubscript{HOM} is expressed within a V\textsubscript{H} CDR. A comparison of the V\textsubscript{H} CDR amino acid sequences among the mAb that express Id\textsubscript{HOM} indicated that the CDR1 and CDR3 sequences were highly variable and suggested no candidate idiotopes. The CDR2 sequence of R24, however, contains an eight-amino acid palindromic sequence starting at amino acid 51, of which the first six amino acids are conserved in mAb, since they do not bind to mAb specific for other Ags. The effect of V\textsubscript{H} CDR2 substitutions on binding to G\textsubscript{D3} was measured by both ELISA and flow cytometry. Substitutions at position 52a resulted in the loss of Id\textsubscript{HOM} but preserved the avidity of the R24 Ab as assessed by immuno-TLC against a mixture of melanoma gangliosides (data not shown). Results from experiments with variants Q61 and H61 demonstrate that substitutions at position 61 had no apparent effect on binding to G\textsubscript{D3}. Effects of CD24 V\textsubscript{H} substitutions on binding to G\textsubscript{D3} were measured by both ELISA and flow cytometry. Substitutions at position 52a generally resulted in complete loss of binding to G\textsubscript{D3}, as measured by ELISA (Table I), suggesting that this residue is involved in the G\textsubscript{D3} binding site. However, a serine/threonine substitution at position 52a, represented by variant LT52, which is identified as an essential residue for homophilic binding (Fig. 3), and did not alter the specificity of the Ab as assessed by immuno-TLC against a mixture of melanoma gangliosides (data not shown). Results from experiments with variants Q61 and H61 demonstrate that substitutions at position 61 had no apparent effect on binding to G\textsubscript{D3}.

Effects of CDR2 V\textsubscript{H} substitutions on chR24 binding to G\textsubscript{D3} ganglioside

The effect of V\textsubscript{H} CDR2 substitutions on binding to G\textsubscript{D3} was measured by both ELISA and flow cytometry. Substitutions at position 52a generally resulted in complete loss of binding to G\textsubscript{D3}, as measured by ELISA (Table I). Results from experiments with variants Q61 and H61 demonstrate that substitutions at position 61 had no apparent effect on binding to G\textsubscript{D3}, indicating that this residue is not critical for either binding to G\textsubscript{D3} or expression of Id\textsubscript{HOM}.

Effect of homophilic binding on G\textsubscript{D3} avidity

The observations that a serine/threonine substitution at position 52a resulted in the loss of Id\textsubscript{HOM} but preserved the G\textsubscript{D3} binding site suggested that Id\textsubscript{HOM} is near, but not equivalent to, the G\textsubscript{D3}

### Table 1. Effects of CDR2 substitutions at positions 52a and 61 in chR24 on expression of homophilic binding epitope and binding to G\textsubscript{D3}

<table>
<thead>
<tr>
<th>Chr24 Variant</th>
<th>Amino Acid</th>
<th>Expression of Id\textsubscript{HOM}</th>
<th>Binding to G\textsubscript{D3} by ELISA</th>
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<tr>
<td>R24 \textsuperscript{a}</td>
<td>S, D, 61</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LT52</td>
<td>T, D</td>
<td>-</td>
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<tr>
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</tr>
<tr>
<td>P52Q61</td>
<td>P, Q</td>
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\textsuperscript{a} R24 represents the native form of chR24 with no amino acid substitutions.
The cell surface for cell surface binding pocket into which the terminal sialic acid residue of G, is detected using alkaline phosphatase-conjugated Ab. H61, D52, and LT52 were coated onto ELISA plates. After blocking, decreasing concentrations of mouse R24 were added, and bound mouse R24 was detected using an alkaline phosphatase-conjugated goat anti-mouse IgG3 second Ab.

FIGURE 2. Expression of IdHoM by chR24 and selected chR24 variants. ChR24, labeled WT in the figure for wild-type or variants Q61, H61, D52, and LT52 were coated onto ELISA plates. After blocking, decreasing concentrations of mouse R24 were added, and bound mouse R24 was detected using an alkaline phosphatase-conjugated goat anti-mouse IgG3 second Ab.

FIGURE 3. Binding of chR24 and selected chR24 variants to G, ganglioside as measured by ELISA. ChR24, labeled WT in the figure for wild-type, or variants H61, Q61, P52, and LT52 were tested for binding to G,,-coated ELISA plates as described in Materials and Methods.

binding site. We were interested to determine how this substitution affected binding to cell surface G,. Substitutions at position 61 did not significantly affect binding to cell surface G, as measured by flow cytometry. On the other hand, LT52 demonstrated avidity for cell surface G, that was almost 2 orders of magnitude lower than that of native chR24, as measured by peak immunofluorescence (Fig. 4). This result contrasts with the ELISA results in which LT52 showed binding to G, equivalent to that of native chR24 (Fig. 3). This disparity is explained by the fact that there are additional constraints in mAb binding to cell surface G, (discussed below). We interpret these results as demonstrating that LT52 has decreased avidity for G, compared with native chR24, but this was not evident by ELISA due to the high sensitivity of this assay.

These results demonstrate that despite an intact G, binding site, homophilic binding is required for high avidity binding to cell surface G,. An alternative explanation is that the serine–threonine substitution at position 52a not only destroyed IdHoM but also sufficiently altered the G, binding site to result in decreased avidity for G,. To determine the relative position of ser52a with respect to the G, binding site, the structure of the Fab from R24 was determined by x-ray crystallography, which permitted us to model the G, binding site of R24.

Computer modeling of the G, binding site and IdHoM

The x-ray crystal structure of the Fab from R24 clearly showed a binding pocket into which the terminal sialic acid residue of G, could be docked (Fig. 5A). This model shows that histidine 35, tyrosine 50, and tyrosine 100c can participate in forming hydrogen bonds with oxygen molecules of G, and is consistent with previous R24 binding studies that showed that the terminal sialic acid of G, was critical for R24 binding (21). Ser52a is situated at the mouth of the G, binding pocket and does not interact with the terminal sialic acid residue of G,, although it is positioned to form hydrogen bonds with the second sialic acid residue. A threonine in place of serine at position 52a appears to alter the outer surface of this binding pocket while still allowing G, to bind (Fig. 5B). Other amino acid substitutions at position 52a, such as aspartic acid (Fig. 5C), would be expected to deform significantly the G, binding pocket, thus interfering with its ability to bind G,. This is consistent with the observation that substitution of any other amino acid, besides threonine, at position 52a resulted in the complete loss of avidity for G, (Table 1).

This model of the G, binding pocket is also consistent with the hypothesis that the decreased binding of LT52 to cell surface G, is not due to an altered G, binding site. The more likely explanation is that the intrinsic avidity of the G, binding pocket for G, is relatively low in the absence of homophilic binding and that homophilic binding increases the apparent avidity through enhanced multivalent binding. We conclude that homophilic binding is required for high avidity binding to cell surface G,.

Discussion

We reported previously that homophilic binding is a general characteristic of mouse anti-G, mAb and proposed a model in which the G, binding site of soluble mAb bound specifically to a V, idiotope (now termed IdHoM) on immobilized mAb (5). In the current report, studies with chR24 variants and computer modeling of the G, binding site provide further support for this model. IdHoM absolutely requires a serine at position 52a, since any substitution at this position destroyed the idiotope. Although IdHoM was not required for binding to G, by ELISA, most substitutions at position 52a destroyed both IdHoM and the G, binding site. This implies that IdHoM, as defined by ser52a, is near but not in the G, binding site. This was confirmed by the x-ray crystal structure of R24 Fab fragment, which revealed that the G, binding site is a pocket and predicted that amino acids within the pocket from V, CDR1 (His35), CDR2 (Tyr50), and CDR3 (Tyr100c) form...
FIGURE 5. Molecular model of C, binding to R24 and R24 variants at residue 52a based on the x-ray crystal structure of the unliganded R24 Fab fragment. A, Stereoview of the modelled fit of the two terminal sialic acid residues of C, (thick lines) docked into the binding pocket observed on the surface of R24. Histidine 35, tyrosine 50, and tyrosine 100c are positioned to form hydrogen bonds (dotted lines) with oxygen molecules on C,. Serine 52a contacts C, and is positioned to hydrogen bond with the second sialic acid residue of C,. B, A close-up stereoview of the environment surrounding serine 52a showing the minimal change in molecular surface and orientation of the hydroxyl group that would occur if the native serine (black) was replaced with threonine (red). C, A close-up stereoview of the change in molecular surface if aspartic acid (red) is substituted for serine 52a (black). Unlike substitution by threonine (B), this substitution causes a large distortion in the Ag binding surface and displaces the hydrogen-binding moiety to an extent that it can no longer form the original hydrogen-bonding pattern. Views B and C are rotated approximately 180 degrees about the vertical axis compared with view A.

H-bonds with G,. Ser52a is at the mouth of the pocket which, as predicted, maps Id,HOM near the G,D3 binding site, but separate from it. Modeling studies revealed that substitutions at position 52a, other than threonine, would be expected to significantly alter the G,D3 binding pocket. Thus, both immunologic and x-ray crystallographic studies support the idea that Id,HOM is a V, idiotope situated adjacent to the G,D3 binding site.

Provided that the G,D3 binding site of soluble R24 binds to Id,HOM, it is tempting to hypothesize that Id,HOM mimics G,D3 ganglioside. In this regard, it has been proposed that a subset of Abs carry an internal image of their own Ag and that these Abs play a key role in the regulation of the humoral immune response through an Id network (22). Alternatively, it is possible that Id,HOM represents a binding site for an alternative ligand yet to be identified. In support of this possibility is the finding that V, epitopes outside the traditional antigen binding site of other Ab molecules bind members of the Ig supergene family such as CD4 (3) and NCAM (4). In addition, binding sites for superantigens, such as staphylococcal protein A, have been mapped to framework and CDR2 regions of Ig and may play an important role in B cell regulation (1, 23). Thus, we do not rule out the possibility that Id,HOM serves as a binding site for other ligands. Although an alternative ligand that binds to Id,HOM has not been identified, we note that Id,HOM has significant homology with the third extracellular region of adenosine receptor A (24), a region that to date has no defined function.

The binding characteristics of the LT52 variant of chR24 demonstrate that homophilic binding is not required for binding to G,D3 measured by ELISA, but is required for high avidity binding to G,D3 on the cell surface. This may be due to the fact that on an ELISA plate, G,D3 is immobilized and at high density on a two-dimensional surface, conditions that decrease the degrees of freedom and allow low avidity interactions. In contrast, on the cell surface, G,D3 is mobile, and its accessibility to mAb may be hindered by membrane glycoproteins that are generally 10 to 100 times larger than G,D3. Thus, homophilic binding represents a mechanism for providing high avidity binding to cell surface G,D3.
which is crucial for complement-directed lysis and Ab-dependent cellular cytotoxicity, effector functions that anti-GD3 mAb mediate efficiently (13). This mechanism of high avidity binding could be especially important for Abs such as R24 that recognize nonprotein (so called T cell-independent) Ags. Somatic mutation, the classical mechanism for evolving a higher avidity Ab during the generation of a humoral immune response, requires T cell help that is not available in the case of a nonprotein Ag such as GD3. However, if the B cell uses a V<sub>H</sub> segment that contains a homophilic binding idotope, it may result in the formation of higher avidity Abs without V region maturation requiring T cell help.

This model predicts that high avidity mAb against other T-independent Ags may demonstrate homophilic binding. Indeed, V<sub>H</sub>-directed self-binding has been reported in the anti-phosphoryl choline mAb T15 and M603, in which an epitope within the V<sub>H</sub> CDR2 region was implicated by the finding that CDR2-derived peptides could inhibit self-binding (25). A second example is mAb 26-10 directed against the glycoside digoxin. While this mAb was not tested directly for homophilic binding, a serine residue at position 52 in V<sub>H</sub> CDR2 was found to be critical for high avidity binding to digoxin, even though ser52 did not contact Ag by x-ray crystallographic analysis (26). This finding raises the possibility that this ser52, like the ser52a of anti-GD3 mAb, provides an idotope for self-binding that results in higher avidity binding to Ag. These two examples of anti-phosphoryl choline and anti-digoxin mAb contain elements in common with our model of homophilic binding among anti-GD3 mAb and are consistent with the idea that a variety of mAb against nonprotein Ags can exhibit homophilic binding. There is evidence, however, that homophilic binding may also occur among Abs against protein Ags. Autoantibodies against thyroglobulin have been reported to express an idotope that not only allows self-binding, but also functions as an alternative binding site for thyroperoxidase (27). Taken together, these observations suggest that the V<sub>H</sub> repertoire may contain multiple mechanisms for generating self-binding idiotopes.

We observed that in five of six mouse anti-GD3 mAb sequenced, the V<sub>H</sub> region was formed by using a V<sub>H</sub> gene from the V7183 gene family. The mouse V<sub>H</sub> locus consists of 11 V<sub>H</sub> families, of which V7183, one of the smallest families, is the most I<sub>H</sub>-proximal. In mature mouse B cells, V<sub>H</sub> families are selected in proportion to the relative size of the gene family, indicating that V<sub>H</sub> gene family usage is largely random. This is indicated by the observation that the VJ558 family is the most heavily represented. In contrast, differentiating pre-B cells are biased toward using the most J<sub>H</sub>-proximal gene families, such as the V7183 and VQ52 families (28). The bias of mouse anti-GD3 mAb toward J<sub>H</sub>-proximal V<sub>H</sub> gene families strongly suggests that the murine Ab response to GD3 is the result of selection in a pre-B cell compartment rather than in mature B cells.

The bias of mouse anti-GD3 mAb toward J<sub>H</sub>-proximal V<sub>H</sub> gene families contrasts with the murine Ab response against other glycolipid Ags. The V<sub>H</sub> genes used in 46 mouse mAb against various ganglioside and sulfated glycolipid Ags other than GD3 were derived from the large V<sub>H</sub> gene families, consistent with random V<sub>H</sub> gene family selection rather than J<sub>H</sub>-proximal families (29). This suggests that the murine humoral immune response against non-GD3 gangliosides is produced by a mature B cell population and is consistent with the observation that Abs against these non-GD3 glycolipid Ags, such as GMAI, can develop spontaneously in older mice (30). Thus, the murine Ab response to GD3 appears to be qualitatively different from the Ab response to other ganglioside Ags, in that Abs against other gangliosides are produced by mature B cells selecting V<sub>H</sub> gene families at random.

There is evidence that early in human ontogeny, the V<sub>H</sub> gene usage is also biased toward J<sub>H</sub>-proximal gene families (31). Thus, it is tempting to hypothesize that the situation in humans mirrors that in the mouse; namely, that mature human B cells can generate Abs against various carbohydrate Ags other than GD3, but that it is the pre-B cell population that has a V<sub>H</sub> gene usage bias that favors the generation of high avidity Abs against GD3. Consistent with this hypothesis is the observation that in adults, Abs against a variety of ganglioside Ags, including GD2, GD3, and GD14, can arise naturally or can be induced by immunization (32-38), while high avidity Abs against GD3 do not develop spontaneously and have been relatively difficult to induce with immunization (35, 39).

In contrast with mouse mAb against GD3, human anti-GD3 mAb HM1 exhibits relatively low avidity GD3 binding, does not demonstrate homophilic binding, and cross-reacts with other gangliosides. Consistent with this difference is the observation that HM1 does not use a J<sub>H</sub>-proximal V<sub>H</sub> gene family and is probably the product of a mature human B cell.

This model of V<sub>H</sub> gene family usage suggests possible approaches to induce high avidity Abs against GD3, in patients. Circulating CD5<sup>-</sup> pre-B cells, which can be detected in adult peripheral blood, preferentially express J<sub>H</sub>-proximal V<sub>H</sub> genes (40). This pre-B cell compartment is known to require certain growth factors, such as IL-7 (41), and we speculate that strategies to expand and activate this subpopulation of B cells before immunization could result in high avidity Abs against GD3.

In summary, we have 1) defined the structural basis for homophilic binding among mouse anti-GD3 mAb, 2) generated a model for GD3 binding to R24 from the structure of the unliganded Fab that is consistent with immunologic data, and 3) demonstrated that homophilic binding is required for high avidity binding to cell surface GD3. These studies also suggest that the murine Ab response to GD3 results from activation of a pre-B cell compartment. This is in contrast to the Ab responses to other ganglioside Ags, which appear to rely primarily on mature B cells. If similar constraints apply to the anti-GD3 response in humans, strategies to activate pre-B cells that preferentially use J<sub>H</sub>-proximal V<sub>H</sub> gene families may be successful in inducing high avidity anti-GD3 Abs.

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References


