Localization of the Putative Sialic Acid-binding Site on the Immunoglobulin Superfamily Cell-surface Molecule CD22*

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B-lymphocyte antigen CD22 is a member of the recently described sialoadhesin family of immunoglobulin-like cell-surface glycoproteins that bind glycoconjugates terminating in sialic acid. One prominent ligand for CD22 is the highly glycosylated leukocyte surface protein CD45. Using surface plasmon resonance spectroscopy, we characterized the interaction of recombinant mouse CD22 with native CD45 purified from rat thymus (CD45-thy). By in situ desialylation and resialylation of immobilized CD45-thy, we show that mouse CD22 binds to the sialoconjugate NeuGcα2–6Galβ1–4GlcNAc carried on CD45-thy N-glycans. Previous studies have shown that the sialic acid-binding site lies within the two membrane-distal domains of CD22 (domains 1 and 2), which are V-set and C2-set immunoglobulin superfamily domains, respectively. To further localize the binding site, we have made 42 single amino acid substitutions throughout both domains. All 12 mutations that abrogated binding to CD45-thy without disrupting antibody binding were of residues within the GFCC'C' β-sheet of domain 1. These residues are predicted to form a contiguous binding site centered around an arginine residue in the F strand that is conserved in all members of the sialoadhesin family. Our results provide further evidence that immunoglobulin superfamily cell adhesion molecules use the GFCC'C' β-sheet of membrane-distal V-set domains to bind structurally diverse ligands, suggesting that this surface is favored for cell-cell recognition.

Immunoglobulin superfamily (IgSF) domains are probably the commonest domain type involved in cell-surface recognition, being present in ~40% of all proteins identified on the surface of leukocytes (1). One possible reason for this is that IgSF domains provide a stable, but versatile, recognition platform, capable of binding to structurally diverse ligands (2). Typically, IgSF cell adhesion molecules bind either to other IgSF molecules or to integrins (3, 4), but recent reports indicate that some IgSF cell adhesion molecules bind carbohydrate ligands (reviewed in Ref. 5). The best characterized of these lectin-like IgSF proteins are a group of homologous proteins (termed the sialoadhesin family) that bind carbohydrate structures terminating in sialic acid (5–7). Members of this family include the leukocyte proteins CD22, sialoadhesin, and CD33 as well as myelin-associated glycoprotein and Schwann cell myelin protein (5–7).

CD22 is expressed on a subpopulation of mature B-cells and has been implicated in cell adhesion as well as in modulating signaling through the B-cell antigen receptor (BCR) (reviewed in Ref. 6). CD22 associates loosely with the BCR (9, 10) and is tyrosine-phosphorylated following BCR ligation (11). This leads to association with and activation of the tyrosine phosphatase SHP (12), which can inhibit signaling through the BCR (13–15). The binding of anti-CD22 antibody-coated beads to B-cells decreases the activation threshold of the BCR, presumably by removing CD22 (and associated SHP) from the vicinity of the BCR (12). Together, these findings suggest that physiological interactions between CD22 and natural cell-surface ligands may function to modulate signaling through the BCR (12).

The extracellular region of mouse CD22 (18) consists of a single membrane-distal V-set IgSF domain (domain 1), followed by six C2-set IgSF domains (domains 2–7). However, two cDNA clones of human CD22 have been identified (CD22α (16) and CD22β (17)), one of which (CD22α) lacks the sequence encoding IgSF domains 3 and 4. Human CD22β (which is equivalent to mouse CD22 and is henceforth called CD22) binds with a low affinity (Kd ~ 30 μM at 4 °C (18)) to the sialylated glycoconjugate NeuGcα2–6Galβ1–4GlcNAc (19, 20). Although this structure is very common on N-glycans, recombinant CD22 appears to bind only to a limited number of lymphocyte cell-surface (19, 21, 22) and plasma (23) glycoproteins, suggesting that some of these molecules are preferred ligands. One prominent ligand is the large, abundant, and highly glycosylated leukocyte cell-surface glycoprotein CD45 (22, 24, 25), which carries multiple N-glycans terminating in α2–6-linked sialic acid (26).

With the exception of antibody-carbohydrate interactions, little is known about carbohydrate recognition by IgSF molecules (5) As a first step toward understanding the structural basis of sialic acid recognition, we undertook to identify the sialic acid-binding site on CD22. Previous studies on human (27) and mouse (22, 28) CD22 have shown that the sialic acid-binding site lies within domains 1 and 2. In the present study, we extend this work by making single amino acid substitutions...
of surface residues throughout domains 1 and 2 of mouse CD22. Our results suggest that the CD22 sialic acid-binding site is situated on the GFCC C β-sheet of domain 1 centered on an arginine residue in the F strand that appears to be essential for sialic acid recognition.

MATERIALS AND METHODS

Proteins, Lectins, and Monodonal Antibodies—Native CD45 (CD45-thy) and thy-1 were purified from rat thymus as described (29, 30). The purified proteins were precipitated in cold ethanol and dissolved in water (29, 30). Rat α,α-dicyclic glycoprotein (orosomucoid) was purchased from Boehringer (Mannheim, Germany). The purified mouse anti-human IgG monoclonal antibody (mAb) R10289 (34) was kindly provided by Professor R. J. Efferth and Dr. M. Goodall and is available from Recognition Systems (University of Birmingham Science Park, Birmingham, United Kingdom). The hydridoma CY34.1.2, which produces the mouse (IgG1) anti-mouse CD22 antibody CY34 (35), was obtained from the American Type Culture Collection (Rockville, MD).

Surface Plasmon Resonance Spectroscopy—All BIAcore experiments were performed on a BIAcore biosensor (Pharmacia Biosensor, Uppsala) at 25°C in the running buffer HBS, which contains 150 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, pH 7.4, and 0.005% Surfactant P-20 (Pharmacia Biosensor). Proteins were covalently coupled via amine groups onto the carboxymethylated dextran surface of CMS (research-grade) sensor chips (Pharmacia Biosensor) using the standard amine coupling kit (Pharmacia Biosensor) as recommended (36), with the following modifications. During coupling, CD45-thy and sCD45ABC-CHO were injected for 7 min at 20–40 μl/min at a flow rate of 100 μl/min. The anti-human Fc antibody was injected at 28 μl/min in 10 mM sodium acetate buffer, pH 4, respectively. Both proteins were regenerated by injecting 100 mM HCl for 3 min. The anti-human Fc antibody was injected at 28 μl/min in 10 mM sodium acetate buffer, pH 4, respectively. Both proteins were regenerated by injecting 100 mM HCl for 3 min.

RESULTS AND DISCUSSION

Mouse CD22 Binds to NeuGc on CD45 N-Glycans—Previous studies have shown that only the two NH₂-terminal domains of human (27) and mouse (22, 28) CD22 (domains 1 and 2) are required for sialic acid binding. However, we have found that a mouse CD22 construct containing domains 1 and 2, but lacking domain 3, was somewhat unstable (28). We therefore used a construct containing domains 1–3 of mouse CD22 fused to the Fc portion of human IgG1 (CD22Fc) (6). Using surface plasmon resonance spectroscopy, as implemented in the BIAcore instrument (43), we have shown that CD22Fc binds to native CD45 (CD45-thy) purified from rat thymus (28). To further characterize this interaction, we modified the sialylglycoconjugates present on CD45-thy and examined the effect on CD22Fc binding (Fig. 1).

When CD22Fc was injected over a sensor surface to which CD45-thy had been covalently immobilized, there was an increase in the response (measured in response units), which indicates binding of CD22Fc to CD45-thy (Fig. 1A). Following completion of the injection, the response decreased slowly, reflecting dissociation of bound CD22Fc (Fig. 1A). The remaining CD22Fc was eluted rapidly by the injection of 100 mM HCl (Fig. 1A, arrow). The lectins MAA and SNA (which are specific for α2–3- and α2–6-linked sialic acids, respectively) were also bound (Fig. 1A), indicating that CD45-thy carries both α2–3- and α2–6-linked sialic acids. Treatment of the immobilized CD45-thy with sialidase abolished CD22Fc binding and substantially decreased both MAA and SNA binding (Fig. 1B). When desialylated CD45 was α2–6-resialylated with Galβ1–4GlcNAc α2–6-sialyltransferase using NeuAc as substrate, SNA binding increased substantially, indicating successful α2–6-resialylation, but CD22Fc was still unable to bind (Fig. 1C). In contrast, CD22Fc binding was fully restored when CD45-thy was α2–3-resialylated using NeuGc as substrate (Fig. 1D). The specificity of the α2–6-resialylation is indicated by the increase in SNA (but not MAA) binding following resialylation (Fig. 1C, D).

These results indicate that mouse CD22 binds to α2–6-linked NeuGc carried on CD45-thy N-glycans. Further evidence that the sequence NeuGc –2–6Galβ1–4GlcNAc was both necessary and sufficient for CD22Fc binding was obtained in an experiment using scD45ABC-CHO. The lectin MAA bound unmodified scD45ABC-CHO, whereas SNA did not, indicating that scD45ABC-CHO contains no detectable α2–6-linked sialic acid (Fig. 1E, Untreated). Therefore, it is not surprising that CD22Fc did not bind unmodified scD45ABC-CHO (Fig. 1E, Untreated). However, CD22Fc did bind scD45ABC-CHO following α2–6-resialylation with NeuGc (Fig. 1E). In contrast, neither α2–6-resialylation with NeuAc nor α2–3-resialylation with NeuGc could restore CD22Fc binding (Fig. 1E). Taken together, these results establish that mouse CD22Fc binds to CD45-thy through an interaction with the structure NeuGc –2–6Galβ1–4GlcNAc carried on CD45-thy N-glycans. This is consistent with a recent analysis of the specificity of mouse CD22Fc using resialylated erythrocytes (44).

Analysis of the Sialic Acid Composition of CD45-thy—Since mouse CD22 requires α2–6-linked NeuGc for binding, biologically relevant ligands for CD22 should contain this sialic acid rather than NeuAc. Normal human tissues do not contain NeuGc, but this sialic acid is common in rodents (45, 46). However, the relative amount of NeuGc differs between cell types and is developmentally regulated (47–50). Previous stud-
ies of mouse lymphocytes found that NeuGc constituted 40–50% of the sialic acid in glycolipids (51, 52). However, no analysis of mouse or rat lymphocyte glycoproteins has been reported. We therefore analyzed the sialic acid composition of glycoproteins isolated from rat thymus (Table I). For comparison, we also studied rat serum protein and rat CD45 that had been expressed in CHO cells (Table I). This analysis revealed that most (>98.8%) of the sialic acid in the thymic proteins CD45-thy and thy-1 is NeuGc. In contrast, the serum protein α1-acid glycoprotein, which is synthesized by hepatocytes, contains mainly (>89%) NeuAc (Table I). NeuAc constituted 98% of the sialic acid in sCD45ABC-CHO (Table I), which is in agreement with other studies of glycoproteins expressed in CHO cells (53). Taken together, these results demonstrate that CD45-thy is a suitable ligand for murine CD22 since it contains abundant α2–6-linked NeuGc. In support of a physiological role for this interaction, Law et al. (22) recently demonstrated that CD45 is prominent among the glycoproteins that are immunoprecipitated from mouse B-cell lines using mouse CD22Fc.

Sequence Alignments and Mutagenesis Strategy—Two mouse CD22 alleles have been isolated from BALB/c and DBA/2J mice, respectively (54, 55). While sequencing the CD22 construct used in the present study (which originated from C57Bl mice (6)), it emerged that it encodes a third allele (Fig. 2, CD22 C57Bl). This allele is identical to the BALB/c allele in the region encoding domains 1–3, with the exception of the codons for residues 79 (Val instead of Cys, numbered from the initiation codon), 247 (Arg instead of Cys), and 250 (Arg instead of His), in which the DNA sequence is identical to the DBA/2J allele (Fig. 2). These changes result in the loss of an unusually

### Table I

| Protein                    | NeuAc | NeuGc |
|----------------------------|-------|-------|
| Rat thymus CD45           | ND*   | >98.8 |
| sCD45ABC-CHO              | 98.1  | 1.9   |
| Rat thymus thy-1          | 1     | 99    |
| Rat α2-acid glycoprotein  | 89    | 11    |

* ND, none detected. The detection limit was ~1.2%.

**Fig. 1.** Mouse CD22Fc binds NeuGcα2–6Galβ1–4GlcNAc carried on CD45 N-glycans. A–D, CD45-thy was covalently coupled to the BIAcore sensor surface. CD22Fc and the sialic acid-binding lectins MAA and SNA were then injected at 0.5 mg/ml for 4 min each (bars) over unmodified thymic CD45 (A), sialidase-treated CD45 (B), sialidase-treated CD45 resialylated with NeuAc using Galβ1–4GlcNAc α2–6-sialyltransferase (C), or sialidase-treated CD45 resialylated with NeuGc using Galβ1–4GlcNAc α2–6-sialyltransferase (D). Following each injection, bound protein was eluted with a 4-min injection of 100 mM HCl (arrows mark the beginning of these injections). E, sCD45ABC-CHO was coupled to the sensor surface. Mouse CD22Fc, MAA, and SNA were injected (0.5 mg/ml for 4 min) first over unmodified sCD45ABC-CHO and then after the indicated desialylation and resialylation steps. The binding response during each injection was measured 20 s after the injection (to eliminate the bulk phase effect) and is expressed as a percentage of the maximal response seen for each ligand during the experiment, which was 1380, 3640, and 7300 response units for CD22Fc, MAA, and SNA, respectively. ST6N, Galβ1–4GlcNAc α2–6-sialyltransferase; ST3N, Galβ1–3(4)GlcNAc α2–3-sialyltransferase.
positioned pair of cysteine residues that are present in the CD22 BALB/c allele, but not in any of the other sialoadhesin family members (56).

To aid in the selection of residues to mutate, domains 1 and 2 of CD22 were realigned with IgSF domain sequences for which there are structural data available (Fig. 2). Domain 1 of CD22 was aligned with the V-set domain (domain 1) of rat CD2 (57, 58) (Fig. 2), whereas domain 2 was aligned with domain 2 of VCAM-1 (59). CD22 residues in domains 1 and 2 could be assigned accurately to the structurally conserved B, C, E, and Fβ-strands (Fig. 2) by aligning residues characteristic of IgSF domains (2, 60, 61). In a similar manner, residues in CD22 domain 1 could be assigned to the beginning of the D strand and to the end of the G strand, and residues in domain 2 could be assigned to the A strand and to the end of the G strand (Fig. 2). In contrast, CD22 residues could not reliably be assigned to the C′ and C″ strands of domain 1 or to the C/D strand of domain 2 (Fig. 2). The assignment of residues to the loop regions was tentative except for the E–F loop, which is structurally conserved in V-set and C2-set IgSF domains (2, 60, 61).

The sialic acid-binding site on sialoadhesin has been definitively localized to its V-set domain (domain 1 (28)), but in the case of CD22, a contribution from domain 2 has not been ruled out (27, 28). To further localize the sialic acid-binding site on CD22, we mutated residues predicted to lie on the surface of domain 1 or 2. We introduced drastic changes rather than mutating to alanine because our primary aim was to delineate the structural binding site. It has been shown that alanine mutagenesis may only identify a fraction (25–40%) of the residues within the binding site (62, 63). We have previously used this approach of making drastic mutations to identify the interacting surfaces of the cell adhesion molecules CD2 and CD48 (42)2 and obtained results that agree well with structural studies (58, 64).

Identification of the Sialic Acid-binding Site on CD22—Mutant CD22Fc chimeras were expressed by transient transfection of COS-7 cells and then analyzed for ligand and antibody binding by surface plasmon resonance spectroscopy using the approach outlined schematically in Fig. 3A (upper left). TCS containing wild-type or mutant CD22Fc was injected over a sensor surface to which an anti-Fc mAb had been covalently coupled (Fig. 3A and B, long bars). The initial rapid increase is due to the high bulk refractive index of the injected TCS (“bulk phase effect”), whereas the slower, more sustained increase reflects the binding of CD22Fc to the anti-Fc mAb on the sensor surface (Fig. 3A and B, long bars). The contribution from the bulk phase effect ends when the injection of the TCS is completed and the flow of the running buffer resumes. The response then drops rapidly to a new, elevated baseline, the level of which is proportional to the mass of bound CD22Fc, with 1000 response units representing 1 ng/mm² of bound protein (43). The control protein BSA and CD45-thy (both at 26 mg/ml) were injected over the sensor surface both before (to control for a bulk phase effect) and after the binding of wild-type or mutant CD22Fc to the sensor surface. A substantially increased response is seen when CD45 is injected over immobilized wild-type CD22Fc to the sensor surface (Fig. 3, A and B, long bars). The contribution from the bulk phase effect ends when the injection of the TCS is completed and the flow of the running buffer resumes. The response then drops rapidly to a new, elevated baseline, the level of which is proportional to the mass of bound CD22Fc, with 1000 response units representing −1 ng/mm² of bound protein (43). The control protein BSA and CD45-thy (both at 26 μg/ml) were injected over the sensor surface both before (to control for a bulk phase effect) and after the binding of wild-type or mutant CD22Fc to the sensor surface. A substantially increased response is seen when CD45 is injected over immobilized wild-type CD22Fc, reflecting binding, whereas the response to the injection of BSA is unchanged (Fig. 3, A and B, Wild type). The mutant CD22Fc constructs were analyzed in the same way and compared with wild-type CD22Fc (Fig. 3).

Initially, nine mutations were made in each of domains 1 and 2. S. J. Davis, E. A. Davies, and P. A. van der Merwe, unpublished data.
Of these, only two mutations, both in domain 1, led to a decrease in CD45 binding (R130E and E140K) (Table II). Both mutants bound normally to mAb CY34 (Fig. 4A and Table II). The sequence alignment (Fig. 2) places Arg-130 and Glu-140 on adjacent F and G β-strands in domain 1 (Fig. 5). Interestingly, Arg-130 is one of only five residues in domain 1 (apart from residues characteristic of IgSF domains) that are completely conserved within the sialoadhesin family (indicated by † in Fig. 2) (2, 56, 60, 61), suggesting that it may play an important role in sialic acid recognition. To provide stronger evidence for this, we made the substitutions R130A and R130K, which are less likely to abrogate binding by introducing unfavorable effects. Both mutations abolished CD45 binding (Fig. 3B) without affecting the binding of mAb CY34, strongly suggesting that Arg-130 is critical for sialic acid recognition.

### Table II: Binding of CD22 mutants to CD45 and antibody CY34

| Mutant | CD45 binding | CY34 binding |
|--------|--------------|--------------|
| Domain 1 |              |              |
| E38K   | ++           | +            |
| R43E   | ++           | +            |
| K47E   | ++           | +            |
| K49E   | ++           | +            |
| D58K   | ++           | +            |
| N59H   | ++           | +            |
| L61D   | --           | +            |
| Q64K   | ++           | +            |
| N65H   | ++           | +            |
| Y66D   | --           | +            |
| E67R   | ++           | +            |
| K70E   | ++           | +            |
| K73E   | ++           | +            |
| K74E   | ++           | +            |
| T76K   | ++           | +            |
| K85E   | ++           | +            |
| K88E   | ++           | +            |
| K98E   | ++           | +            |
| Q99E   | ++           | +            |
| H117D  | ++           | +            |
| R120D  | +            | --           |
| S124R  | +            | --           |
| G128E  | +            | --           |
| R130A  | +            | --           |
| R130E  | +            | --           |
| K149D  | --           | +            |
| E160R  | +            | +            |
| T170R  | +            | +            |
| K185E  | +            | +            |
| S202D  | +            | +            |
| K212E  | +            | +            |
| K219E  | +            | +            |
| Q238E  | +            | --           |
| R250D  | +            | --           |

The 18 mutations made initially are in boldface. Not included in the analysis are six CD22 mutants that either were not expressed (R108E) or were expressed at very low levels (H30D, F63D, D181R, E189K, and K234D) and bound neither CY34 nor CD45, strongly suggesting that they were not correctly folded. The binding of mAb CY34 to the CD22 mutants was assayed as shown in Fig. 4. ++, binding indistinguishable from that of wild-type CD22; +, binding detected but clearly decreased (10–30% of wild-type level); --, no binding detected (<5% of wild-type level).

The binding site was further defined with 22 additional mutations in and around the GFCC'C' β-sheet of domain 1 (Fig. 2 and Table II). Of a total of 42 mutations made (Table II), 30 had little or no effect on CD45 binding (examples include K74E, R120D, K149D, and K185E (Fig. 3)), 10 completely abolished CD45 binding (examples include R130E, R130K, R130A, and W138R (Fig. 3)), and 2 substantially decreased, but did not abolish, CD45 binding (E140K and K73E (Fig. 3)). The partial effect of the latter mutants suggests that they lie on the periphery of the binding site. According to the alignment shown...
in Fig. 2, the mutations that abrogate CD45 binding fall within the GFCC'C' β-sheet and are predicted to form a well defined contiguous region centered around Arg-130 in the F strand (Figs. 2 and 5). The positioning of the F and C strand mutations is likely to be correct because the alignment of CD22 with CD2 in both these regions is excellent (Fig. 2), and these strands form part of the structurally conserved core of IgSF domains (60, 61). Because of a poor alignment with CD2, the positioning of the G and C' strand mutants is more tentative (Fig. 2). However, it is clear that residues in the F–G loop and/or the beginning of the G strand contribute to the CD45-binding site.

Our finding that none of the nine mutations in domain 2 affect CD45 binding (Fig. 3 and Table II) suggests that domain 2 does not contribute directly to sialic acid recognition. This is consistent with the observation that domain 1 of sialoadhesin is sufficient for sialic acid binding (28). Furthermore, mutagenesis of sialoadhesin (65) suggests that its sialic acid-binding site is also localized to the GFCC'C' β-sheet of domain 1, centered around the same conserved F strand arginine (Figs. 2 and 6). Taken together, these data suggest that sialic acid recognition by CD22 and sialoadhesin involves only domain 1. Prior observations that domain 1 of CD22 binds poorly (22) or not at all (27, 28) to ligand when expressed in the absence of domain 2 may be explained by an inability of domain 1 to fold correctly in the absence of domain 2. Support for this is provided by two lines of evidence that suggest that domains 1 and 2 of CD22 are intimately associated. First, the conserved cysteines present in domains 1 (A–B loop) and 2 (B–C loop) of sialoadhesin family members appear to form an interdomain disulfide bridge (66).3 Second, residues in both domains 1 and 2 contribute to the CY34 epitope (see below and Table II).

A potential source of artifact in the present study is the possibility that some or all of the mutants do not lie within the sialic acid-binding site, but instead disrupt the overall folded structure of CD22. While this possibility cannot be eliminated, several considerations suggest that this is unlikely. First, all mutants that did not bind CD45 still bound mAb CY34. Our mutagenesis studies suggest that CY34 binds to a “discontinuous” or “conformational” epitope on CD22 (see below), which requires the correct folding of domain 1 and 2. This is supported by our observation that several, widely spaced mutants that were expressed only at very low levels bound neither CD45 nor CY34 (see Footnote a to Table II). Second, the 12 mutations

3 A. May, E. Y. Jones, A. C. Willis, A. N. Birdlay, and P. R. Crocker, unpublished data.
that decrease CD45 binding (without affecting CY34 binding) lie within a single contiguous area, with the mutations that have a partial effect (K73E and E140K) situated on the edge of this area. And finally, mutations in the equivalent region of sialoadhesin also disrupt sialic acid binding without disrupting the binding of mAbs directed to this domain 1 (65).

The CY34 Epitope Includes Portions of Domains 1 and 2—
CY34 is an allele-specific mouse anti-mouse CD22 mAb (35) that has been reported to bind the CD22 BALB/c allele, but not the DBA/2J allele (55). The CD22 C57Bl allele identified in the present study also binds CY34 (28). Using truncation mutants, it has been shown that the CY34-binding site lies within domains 1 and 2 of CD22 (22, 28). Of the 42 mutants in domains 1 and 2, three (R120D, K149D, and K185E) abolished CY34 binding (Figs. 4A and 5 and Table II). None of these three mutations affected CD45 binding, suggesting that they do not disrupt the overall structure of CD22 (and Fig. 4A and Table II). The mutated residues are widely distributed in the primary sequence, with Arg-120 in the E–F loop of domain 1, Lys-149 at the junction of domains 1 and 2, and Lys-185 in the C strand of domain 2. Although distant in the primary sequence (Fig. 2), Arg-120, Lys-149, and Lys-185 are likely to lie in close proximity in the folded structure (see Fig. 5 for the predicted positions of Lys-149 and Arg-120). Thus, as with the majority of monoclonal antibodies (67, 68), CY34 binds a discontinuous (and therefore conformationally sensitive) epitope that includes portions of domains 1 and 2 and is some distance from the putative sialic acid-binding site (Fig. 5). In agreement with the latter, CD45 binding to immobilized CD22 is not inhibited by bound CY34, nor is CY34 binding inhibited by bound CD45 (Fig. 4B), demonstrating that their binding sites on CD22 do not overlap.

IgSF Molecules Involved in Cell-Cell Recognition Bind Structurally Diverse Ligands Using the Same β-Sheet—This analysis of CD22 and the accompanying study on sialoadhesin (65) suggest that both these proteins bind sialoglycoconjugates through the GFCC'C° β-sheet of their membrane-distal V-set

![Fig. 5. Approximate positions of mutations in domain 1 of mouse CD22 that disrupt CD45 and CY34 binding.](image)

![Fig. 6. Ligand-binding sites on the cell-cell recognition molecules CD22, sialoadhesin, VCAM-1, CD80, and CD2.](image)

Localization of the Sialic Acid-binding Site on CD22

Mutations in these positions lead to:

- **No effect**
- **Decreased sialic acid (CD45) binding**
- **Decreased mAb (CY34) binding**
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