Transitional and marginal zone B cells have a high proportion of unmasked CD22: implications for BCR signaling

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Keywords: B lymphocyte, CD22, sialic acid, Siglec

Abstract

CD22, a B cell-specific member of the Siglec family, is an important inhibitor of B cell signaling. The first Ig-like domain of CD22 specifically binds to α2,6-linked sialic acids. Through these interactions CD22 can mediate adhesion to other cells in trans, but can also bind endogenous ligands on the B cell surface in cis. Cis binding of CD22 to sialylated ligands enhances the efficiency of inhibition and thereby reduces the BCR signaling strength. In this study we used a newly developed oligomeric streptavidin-based sialylated probe as an artificial CD22 ligand. We found that CD22 is bound to ligands in cis on most B cells. However, there is a proportion of B cells with unbound (unmasked) CD22. The subpopulation with unmasked CD22 is 2-fold increased in transitional and marginal zone B cells in the spleen and on B1 cells in the peritoneum, when compared to mature B cells. Also, B cells with unmasked CD22 have an activated phenotype. Unmasking of CD22 could be functionally involved in lowering the signaling threshold on developmental checkpoints such as transitional B cells and during B cell activation or could be a consequence of such activation processes.

Introduction

CD22, a B cell-specific trans-membrane protein, is a coreceptor and negative regulator of the BCR. Inhibition of BCR signaling by CD22 occurs through recruitment and activation of Src homology 2 domain-containing protein tyrosine phosphatase-1 (SHP-1) (1–3). Accordingly, B cells of CD22-deficient mice are hyper-responsive to BCR stimulation (4–7). This is most evident by increased Ca2+ mobilization. CD22 belongs to the Siglec family of adhesion receptors. The Siglecs, which are primarily expressed on hematopoietic cells, are characterized by their binding to sialic acids in specific linkages (8). CD22 has high specificity for sialic acids in α2,6 linkage (2,6Sia) (9,10). The CD22 ligand, 2,6Sia, is expressed abundantly as an N-linked sugar on glycoproteins of many cells, among them lymphocytes and cytokine-activated endothelial cells (11,12).

CD22, as most other Siglecs, is bound to ligands in cis, i.e. to ligands on the B cell surface (the ‘masked’ state). This is deduced from experiments in which human or mouse B cells were stained with a polyacrylamide (PAA)-based 2,6Sia carrying glycoconjugate as synthetic ligand for CD22 (13–15). This synthetic ligand could not bind to CD22 on most B cells, unless they were pre-treated with sialidase to remove the cis ligands (‘unmasked’). The terminal sialoside structure recognized by CD22 is synthesized in vivo by the α2,6 sialyl-transferase ST6GalI. Expression of this enzyme is tightly controlled with high levels seen in B cells (16–18). With a newly developed mouse CD22-specific probe, Neu5Gc-α2,6Gal-PAA, we have recently shown that CD22 is constitutively unmasked on B cells of ST6GalI knockout mice (15).

CD22 is also involved in trans interactions to other cells. This can most clearly be demonstrated when CD22 is transfected into heterologous cells, such as COS cells or CHO cells which do not express high levels of ST6GalI and hence do not carry 2,6Sia on the same cellular surface. Expressed on the surface...
of these cells, CD22 can mediate adhesion to other 2,6Sia-carrying cells (9,12). A role for CD22 as an adhesion molecule in bone marrow homing of recirculating B cells has been described. We found that bone marrow sinusoidal endothelium cells specifically express 2,6Sia on their surface which can act as ligand for CD22 when recirculating B cells home to the bone marrow (19). Trans interactions of CD22 to 2,6Sia on target cells can also influence B cell signaling (20).

By solving the crystal structure of sialoadhesin (Siglec-1) and site-directed mutagenesis of the ligand binding site of both sialoadhesin and CD22, it was found that the primary molecular contacts of the two Siglecs occur with the sialic acid moiety of the carbohydrate (21,22). The affinity of CD22 for sialic acid is very low (10^{-4} M) (23). CD22 can bind to a number of sialylated proteins on the cellular surface, among them prominently CD45, as was demonstrated by CD22-Fc binding and protein precipitation (24). However, a recent surface plasmon resonance study showed that the affinity of CD22 for native CD45, another sialylated protein or a synthetic 2,6Sia-carrying glycoconjugate did not differ greatly (23). This means that the protein backbone of the glycan does not contribute to ligand binding of CD22, and it is only the presence and density of 2,6Sia that determines binding.

Recently, two sets of experiments have demonstrated that cis-interactions of CD22 on the B cell surface control signaling. In one approach, a CD22 protein with a mutated Fig. 1. A subpopulation of splenic B cells carries unmasked CD22. Splenocytes of C57BL/6 or CD22±/± mice were either treated with sialidase at 37°C, or left untreated at 37°C, and then stained with B220 and NeuGcc2,6Gal-SAAP (upper four panels) or the linker-SAAP control conjugate (lower two panels). The numbers give the percentage of B220+ cells positive for SAAP binding (± SD from four experiments).
2,6Sia-binding domain was expressed in a B cell line (25); in another approach, we used a CD22-specific sialic acid analog which inhibited ligand binding with high affinity (26). In both cases CD22 was less tyrosine phosphorylated, recruited less SHP-1 protein and B cell Ca\textsuperscript{2+} mobilization was increased after BCR stimulation. Thus, ligand binding in \textit{cis} directly affects CD22 tyrosine phosphorylation and signal inhibition. These studies directly imply that B cell subpopulations in which CD22 is not ligand bound should show differences in BCR signaling, compared to B cells where CD22 is bound. To identify and characterize B cell populations with unmasked CD22, a new streptavidin-based NeuGc\textsubscript{α}2,6Gal probe was utilized. This new synthetic ligand for murine CD22 was used to analyze B cells with unmasked CD22 in detail.

**Methods**

**Mice**

C57BL/6 and CD22-deficient mice (4) which are on a pure (100%) C57BL/6 background, were bred and housed in the animal facilities of our institute. All mice used were aged 6–10 weeks.

**Antibodies**

Antibodies used in flow cytometry were anti-mouse B220–phycocerythrin (PE) (clone RA3-6B2; PharMingen), anti-mouse CD21–biotin (clone 7E9), anti-mouse IgM–PE [goat (Fab)\textsubscript{2}; Medac] anti-mouse CD22–FITC (clone Cy34.1; PharMingen), anti-mouse IgD–biotin (clone 11-26C), anti-mouse CD23–PE (clone B3-B4; PharMingen), anti-mouse B7-2–PE (clone GL1; PharMingen), anti-mouse CD69–biotin (clone H1.2F3; PharMingen), anti-mouse CD5–PE (clone 53-7.3; PharMingen) and anti-mouse MHC-II (I-A\textsuperscript{b}, clone 25-9-17; PharMingen). Biotinylated antibodies were detected using streptavidin–CyChrome (PharMingen). CD22\textsubscript{21,3}–Fc was purified from transfected COS cells (a generous gift from A. van der Merwe and P. Crocker). CD22–Fc was detected with donkey anti-human IgG–FITC (Jackson).

**Preparation of NeuGc\textsubscript{α}2,6Gal–SAAP–FITC**

The synthesis and coupling of the sialoside NeuGc\textsubscript{α}2,6Gal\textsubscript{b}1,4GlcNAc–biotin was described in detail.
elsewhere (27). Some of the NeuGcα2,6Galβ1,4GlcNAc±biotin sialoside was provided by the Consortium for Functional Glycomics. To generate NeuGcα2,6Gal±SAAP±FITC, streptavidin±alkaline phosphatase (SAAP) was coupled to FITC. First, 250 μl SAAP (0.5 mg/ml; Sigma) was dialyzed against borate buffer, pH 9 for 24 h, then 6.25 μl FITC (4 mg/ml in DMSO; Molecular Probes) was added and the coupling reaction was allowed to proceed for 4 h in the dark. The FITC-labeled conjugate was dialyzed against PBS, 0.01% NaN₃, pH 9 for 48 h. To generate NeuGcα2,6Gal±SAAP–FITC, 30 μl SAAP–FITC (0.5 mg/ml) was incubated with 15 μl of 55 μM of the sialoside NeuGcα2,6Galβ1,4GlcNac, biotinylated using Sulfo-NHS-LC–biotin (Pierce) for 30 min at room temperature. This ratio of biotinylated sialoside and SAAP–FITC represents a 16-fold molar excess (double the amount of biotin-binding sites on dimeric streptavidin). For the control compound (linker–SAAP–FITC), Sulfo-NHS-LC–biotin was hydrolyzed in diethanolamine and then bound to SAAP–FITC in the same molar ratio as the sialoside. Both SAAP conjugates were extensively dialyzed, and stored with 1% BSA and 0.1% NaN₃ at −80°C.

Cell preparation, treatment and flow cytometry

For the analysis of splenic lymphocytes, single-cell suspensions of total splenic tissue were prepared. Peritoneal cells were isolated by peritoneal lavage with 5 ml of RPMI medium containing 5% FCS. Cells from bone marrow were obtained by flushing both femurs with 1 ml of RPMI medium using a syringe. Prior to FACS analysis, all cells were depleted of erythrocytes by lysis with hypotonic Gey’s solution. Sialidase treatment was performed with 0.2 U/ml neuraminidase from A. ureafaciens (Roche) (for 1 h at 37°C in PBS/0.1% BSA). Then 1–5 × 10⁶ erythrocyte-depleted cells were stained with various combinations of antibodies (PharMingen and Becton Dickinson) as indicated. Staining with NeuGcα2,6Gal–SAAP–FITC or linker–SAAP–FITC was performed by incubating the cells with the probe for 45 min on ice. For three-color staining using biotinylated antibodies, as well as the sialoside–SAAP–FITC, the bound biotinylated antibody was well saturated with streptavidin–CyChrome, before the NeuGcα2,6Gal–SAAP–FITC was added. Various control stainings were performed to exclude that free streptavidin binding sites on the sialoside–SAAP–FITC may cross-react with the biotin on biotinylated antibodies. Flow cytometry was performed on a FACSCalibur cytometer (Becton Dickinson). The data were analyzed using CellQuest software (Becton Dickinson).

In vitro stimulation

Mouse splenocytes were prepared as described above and plated in 24-well plates at 1 × 10⁶ cells/ml in PRMI medium.
with 5% FCS. The cells were stimulated with goat F(ab)\textsubscript{2} anti-IgM (10 µg/ml) (Jackson and Dianova) plus 100 U/ml IL-4, lipopolysaccharide (20 µg/ml) (Calbiochem) or anti-CD40 (5 µg/ml) (PharMingen and Becton Dickinson) for different time points. After the stimulation, cells were harvested, washed, stained with labeled antibodies and analyzed by flow cytometry, as described above.

**Results**

Previous studies using fluorescence microscopy with mouse cells had indicated that B cell subsets exist with unmasked CD22 (14). These studies were conducted with a sialoside–PAA-based probe containing the NeuAcalpha2,6Gal linkage. Yet, when the same probe was used in FACS analysis, no unmasked cells were observed (not shown). However, while both murine and human CD22 have a strong preference for the sialoside sequence Siaalpha2,6Galbeta1,4GlcNAc, they differ in their preference for the N-acetylmuraminic acid (NeuAc) and N-glycolylmuraminic acid (NeuGc). Human CD22 binds both NeuAc as well as NeuGc, while murine CD22 strongly prefers the NeuGc form (28,29). We have previously developed a synthetic probe with NeuGcalpha2,6Galbeta1,4GlcNAc coupled to PAA (NeuGcalpha2,6Gal–PAA) that was highly specific for murine CD22 (15). However, this probe also did not detect unmasked cells on FACS analysis. In contrast, using a new probe format containing the NeuGcalpha2,6Gal–SAAP sequence, unmasked cells were detected (B. E. Collins et al., unpublished). As described in Methods, this probe format is comprised of biotinylated sialoside adsorbed to commercial SAAP, consisting of streptavidin coupled to alkaline phosphatase in a molar ratio of 2:1. While initially developed for ELISA assays for Siglec specificity, by coupling FITC to SAAP prior to binding of the sialoside, the probe was found to be suitable for flow cytometry. Figure 1 shows the binding of this probe to mouse B cells from the spleen. NeuGcalpha2,6Gal–SAAP stained B cells specifically when they were pre-treated with sialidase. In contrast, most of B220\textsuperscript{+} B cells are not stained without sialidase treatment. There is, however, a significant fraction of untreated B cells of C57BL/6 mice (~10%) which can be stained with the sialoside–SAAP probe. The specificity of these stainings are demonstrated by two controls. First, when B cells from CD22-deficient mice were used, the NeuGcalpha2,6Gal–SAAP conjugate stained only a minor fraction

**Fig. 4.** The expression level of CD22 and 2,6Sia in various splenic B cell compartments. Three-color staining was done with anti-CD21 and anti-IgM, and B cell populations gated as shown in Fig. 2. The third color was CD22 (A) or CD22–Fc stain for 2,6Sia on the surface (B). m: mean fluorescence intensity of the indicated stainings.

**Fig. 5.** Marginal zone B cells show a high subpopulation with unmasked CD22. In three-color staining of C57BL/6 splenocytes, two B cell populations were gated as indicated (MZ = marginal zone B cells, M = mainly mature B cells) and the staining with NeuGcalpha2,6Gal–SAAP is shown in histograms. The numbers give the percentage of NeuGcalpha2,6Gal–SAAP\textsuperscript{+} cells (± SD from three experiments). **P < 0.01, Student’s t-test; tested for significant difference to mature B cells.**
of both untreated and sialidase-treated cells (~2–3%). Secondly, the linker without sialoside coupled to SAAP (linker-SAAP) gives a similar background staining of ~2–3% of all B220+ B cells, both before and after sialidase treatment (Fig. 1). This demonstrates that the NeuGcα2,6Gal sialoside has a high specificity for CD22. In contrast to the previously used NeuGcα2,6Gal–PAA probe, this synthetic ligand readily detects a population with unmasked CD22 in C57BL/6 mice.

We next wanted to analyze the subpopulation of splenic B cells with unmasked CD22 in more detail. Since B cells with CD22 molecules which are not ligand bound may show altered signaling, it was interesting to see whether these cells are enriched in certain developmental B cell stages, because B cell maturation depends on the BCR signaling capacity. When immature B cells enter the spleen they have the transitional phenotype 1 (T1 B cells) which is characterized by the expression pattern IgMhiIgDloCD21lo. They then enter the T2 stage (IgMhiIgDhiCD21hi). Finally, a maturation to follicular, mature B cells (IgMloIgDhiCD21int) takes place (30). Figures 2 and 3 show three-color staining of C57BL/6 mice with antibodies which separate these developmental stages. Populations corresponding to T1, T2 or mature cells were gated and the NeuGcα2,6Gal–SAAP binding analyzed. Cells with unmasked CD22 were found in all three populations. However, while within the mature B cell population there was a subpopulation of ~8–9% with unmasked CD22, the population with unmasked CD22 was increased to ~14% in transitional T1 and 20% in T2 cells (Figures 2 and 3). Also included in these gated populations are marginal zone B cells, which have overlapping surface markers with both T1 and T2 cells, and are characterized in more detail in Fig. 5. Negative controls using the linker–SAAP for staining of various B cell populations always gave a similar background staining of ~2% of B cells as in Fig. 1 (not shown).

It was important to exclude that the higher NeuGcα2,6Gal–SAAP binding in T1 and T2 cells was due to higher CD22 surface expression. Figure 4(A) shows that T2/marginal zone cells express a similar level of CD22 as mature B cells, while T1 cells express less. Thus probe binding to a higher fraction of T1 or T2 cells cannot be attributed to higher CD22 surface expression. One might expect that unmasking of CD22 correlates directly with down-modulation of 2,6Sia on the surface. This was analyzed by staining with CD22-Fc protein and detection with anti-human Ig antibody. The 2,6Sia expression level was 2-fold lower on T1 than on T2 and mature B cells (Fig. 4B). Thus the level of 2,6Sia on the B cell surface did not clearly correlate with unmasked CD22. The population of marginal zone B cells was analyzed separately with CD23/CD21 staining. In addition, marginal zone B cells (CD21hiCD23lo) contain a subpopulation with increased binding of
the NeuGcα2,6Gal–SAAP probe when compared to the CD21hiCD23lo cells, which are mainly mature B cells (Fig. 5).

The masking status of CD22 on B cells was also analyzed in other organs. In the bone marrow, the B cells which were stained with the NeuGcα2,6Gal–SAAP probe were mature IgDlo B cells. Similar to splenic mature B cells, ~10% mature bone marrow B cells carried unmasked CD22 (Fig. 6A). The other B cell population which had unmasked CD22 were transitional B cells of the bone marrow; however, no significant staining of immature B or pre-B cells was detected (not shown). B cells of the blood also contain a population with unmasked CD22 (Fig. 6B). When compared to a parallel staining of splenic B cells (as shown in Fig. 1), there was a higher subpopulation with unmasked CD22 in the blood (16.6% of B220+ cells in the blood versus 10% B220+ cells in the spleen). Finally, peritoneal B cells were analyzed. The peritoneum contains a population of CD5+ B1a cells in mice. A higher fraction of B cells with unmasked CD22 was detected on these B1a cells, when compared to the B2 population (Fig. 7).

Since unmasking of CD22 may be influenced by cellular activation processes, we studied whether those splenic B cells with unmasked CD22 have a different expression level of B cell activation markers. Figure 8(A) shows that B cells which could bind the NeuGcα2,6Gal–SAAP probe have an increased expression of the activation markers B7-2 (CD86), CD69 (not significantly increased) or MHC class II. Experiments were performed in which splenic B cells were stimulated in vitro with various stimuli to analyze whether this treatment leads to unmasking. One example of these experiments is shown in Fig. 8(B). Stimulation of splenic B cells with the shown stimuli for 48 h did not lead to any stronger CD22 unmasking, although the B cells were stimulated well, as demonstrated by B7-2 (CD86) up-regulation (Fig. 8B). Neither were significant changes in CD22 masking status detected at earlier (30 min, and 1, 2, 12 and 24 h) or later (72 h) time points during the cellular stimulation (not shown). This result is in contrast to a similar study on human peripheral blood B cells which demonstrated CD22 unmasking after in vitro stimulation (13).

Discussion

CD22 is an important negative regulator of B cell signaling. The binding of CD22 to sialylated ligands in cis controls the efficiency of the inhibition (25,26). Therefore it is important to determine to which degree CD22 is bound to endogenous ligands on B cells. This was analyzed in this study by use of a new probe which contained the natural ligand for CD22: NeuGcα2,6Galβ1,4GlcNAc (NeuGcα2,6Gal), coupled via a biotinylated linker to a dimeric SAAP conjugate. This oligomeric ligand stained specifically CD22 on B cells, if the B cells were pre-treated with sialidase. The specificity was demonstrated by the use of B cells of CD22−/− mice as a negative control. While most untreated B cells were not stained with the NeuGcα2,6Gal–SAAP probe, there was a subpopulation of ~10% of splenic B cells that could be stained. This staining was interpreted to result from a B cell population with unmasked CD22. The subpopulation with unmasked CD22 was enriched in immature transitional B cells (particularly in T2 cells) and on marginal zone B cells in the spleen. We could also detect subpopulations of B cells with unmasked CD22 in the bone marrow, blood and peritoneal cavity. Finally, the B cell subpopulation which bound the probe showed an activated phenotype.

We have previously used a synthetic CD22 ligand where the sialoside NeuGcα2,6Gal was coupled to a PAA platform (15). In contrast to the probe used in this study, the NeuGcα2,6Gal–PAA could not readily detect a substantial B cell population with unmasked CD22, although after sialidase treatment binding to almost 100% of the B cells was detected, similar to with the NeuGcα2,6Gal–SAAP probe. We think that these differences could be simply explained by a higher avidity of the NeuGcα2,6Gal–SAAP probe. In this case, the probe can better compete with cis glycoprotein ligands. Nevertheless, it is important to discuss whether the staining of a subpopulation of B cells with NeuGcα2,6Gal–SAAP really detects CD22. We think this is the case, because the probe does not stain CD22-deficient B cells, either before or after sialidase treatment. The weak background binding of CD22-deficient B cells is the same as the background staining of the linker–SAAP probe of wild-type B cells and can thus be attributed to unspecific binding of the SAAP backbone. It is relevant that another study...
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also detected unmasked CD22 in murine B cell subpopulations from the spleen, lymph node and bone marrow by use of a NeuGcα2,6Gal-PAA probe and fluorescence microscopy (14).

The binding of Siglecs to sialic acid-carrying cellular ligands in cis seems to be a general phenomenon within this family. The only exception may be sialoadhesin (Siglec-1), which due to its size (17 Ig-like domains) extends out of the cellular surface, away from sialylated transmembrane glycoproteins (8). Accordingly, B cells from the ST6Gall-deficient mouse line, which do not express 2,6Sia on the cellular surface, show completely unmasked CD22 (15,31). It is interesting to speculate how the level of cis binding controls the function of the Siglecs as inhibitory signaling receptors. The only example of Siglecs where this has been clearly demonstrated so far is CD22. When cis ligand binding of CD22 is disturbed, tyrosine phosphorylation of CD22 and SHP-1 recruitment are decreased, and inhibition of Ca2+ signaling is subsequently affected (25,26). This means that B cells with unmasked CD22 could potentially show less CD22-mediated inhibition and hence stronger BCR signaling. It has previously been shown that small changes of expression levels of CD22 can already cause a signaling phenotype. Even heterozygous loss of the CD22 gene (and resulting lower surface expression) can contribute to an autoimmune phenotype in BCR transgenic mice (32). Also, after in vitro stimulation of B cells with IL-4, lipopolysaccharide or anti-IgM, CD22 expression levels are characteristically up- or down-regulated respectively (33,34). This suggests that B cell signaling can be regulated by a modulation of the expression level of CD22.

We would like to suggest another model by which the B cell can regulate the strength of inhibition of BCR signaling through CD22. The level of inhibition can also be controlled by α2,6-linked sialylation on the B cell surface. Down-regulation of 2,6Sia on crucial protein ligands by up-regulation of a sialidase or down-regulation of a sialyltransferase may lead to unmasking of CD22, and hence a released inhibition and stronger BCR signaling. We would have expected to detect lower 2,6Sia by staining with CD22-Fc protein on those populations (such as T2 cells) which show a higher degree of unmasking. However, our analysis showed that T2 cells not only express a similar amount of CD22 protein on the surface as mature B cells, but are also stained similarly well with CD22-Fc. One way to explain this is that CD22-Fc may only bind well to accessible 2,6Sia moieties on the cellular surface, but may not detect subtle changes which may affect only certain crucial CD22 ligands. Alternatively, unmasking of CD22 may be the movement into another compartment on the plasma membrane, away from the compartment of the predominant ligand. Exclusion of lipid raft localization may be such a mechanism.

It is very intriguing that we have detected a significantly increased population of transitional T2 B cells with unmasked CD22 in the spleen. Transitional T2 cells are a crucial population as a developmental checkpoint for selection into the pool of mature, follicular or marginal zone B cells (30,35). Numerous knockout mice, deficient for various signaling proteins required for BCR signaling, which have a block in the transition from T2 to mature B cells, have exemplified that this transition is a process dependent on the BCR signal (36-39). Since B cells with unmasked CD22 would be expected to show stronger Ca2+ signaling, our findings suggest that these B cells are enriched in the pool of transitional B cells in order to allow a stronger B cell response. The blood contains a relatively high proportion of transitional T1 B cells, which are on their way from the bone marrow to the spleen (30). These may contribute to the higher population of B cells with unmasked CD22 in the blood. Marginal zone B cells are a specialized B cell population in the spleen. Marginal zone B cells are involved in specific immune responses to blood-borne particulate antigens (40). Also, for these cells, less inhibition by CD22 through unmasking would potentially lead to stronger signaling, which would distinguish them from follicular B cells. In future experiments we will address the question whether unmasking of CD22 truly correlates with lower SHP-1 recruitment and stronger Ca2+ signaling. Attempts to study this by comparing Ca2+ mobilization of NeuGcα2,6Gal-SAAP bound and unbound B cells have not revealed differences so far, but it is not clear how the bound sialoside conjugate affects CD22 function.

Obviously, unmasking of CD22 from endogenous ligands on the same cellular surface potentially allows interactions with ligands on other cells. This may be directly relevant for the function of CD22 as a homing receptor, as has been demonstrated in the bone marrow (19,41). Ligands on other cells such as endothelial cells would have to compete with B cell cis ligands and the unmasked CD22 may favor these trans interactions. One recent study showed that 2,6Sia expression together with a specific cellular antigen on target cells could depress B cell activation in a CD22-specific fashion (20). Also, the level of unmasked CD22 on the B cell could be crucial for these types of interactions.

Another interesting finding of this study was that B cells which can bind the NeuGcα2,6Gal-SAAP probe have an activated phenotype. This suggests that during B cell activation in vivo, 2,6Sia on the surface is down-regulated and CD22 gets unmasked, allowing a stronger B cell signaling. It can only be speculated whether CD22 unmasking is the result of cellular activation or plays a physiological role in this process in vivo. There is evidence that surface sialylation is down-modulated after cellular activation of lymphocytes (42) and that sialidases are activated (43,44). However, we were
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unable to demonstrate an increased rate of unmasked CD22 after in vitro stimulation of murine B cells. Anti-IgM treatments even reduced the amount of detectable unmasked CD22, but this treatment also leads to down-regulation of total CD22 (33). These results are in contrast to a report of human B cells which showed partial unmasking of CD22 when stimulated in vitro with anti-IgM and anti-CD40 (13). In the study with human B cells, a PAA carrier was used for the 2,6Sia, whereas here we used a streptavidin-based carrier. In previous experiments, however, we also used NeuGcα2,6Gal coupled to PAA for murine B cells and could also not demonstrate specific binding to CD22 after in vitro stimulation (15). Whether these differences reflect real differences of B cell activation between human and mouse B cells, or technical difficulties in detecting CD22 unmasking under our in vitro conditions, remains to be analyzed in future experiments. In summary, we have shown that a novel sialoside probe can be used to detect unmasked CD22 on subpopulations of murine B cells. The enrichment of unmasked CD22 on B cells such as transitional B cells and B cells with activated phenotype could potentially release the BCR from CD22 inhibition, and thereby lead to stronger B cell signaling.

Acknowledgements

We thank Carolin Dix for expert technical help. This work was funded by the Deutsche Forschungsgemeinschaft through SFB 465 (L. N.), and the National Institutes of Health grants GM25042 (B. E. C.) and GM60938 (J. C. P.). We thank the Consortium for Functional Glycomics and the National Institutes of Health grants GM25042 (B. E. C.) and GM60938 (J. C. P.). We thank the Consortium for Functional Glycomics for providing reagents (NIGMS U54-GM62116).

Abbreviations

2,6Sia sialic acids in α2,6 linkage
PAA polyacrylamide
NeuGcα2,6Gal NeuGcα2,6Galβ1,4GlcNAc
SAAP streptavidin–alkaline phosphatase
NeuAc N-acetylenuraminic acid
NeuGc N-glycolylenuraminic acid
PE phycoerythrin
T1 B cell transitional T1 B cell
T2 B cell transitional T2 B cell

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