Regulation of B cell development and B cell signalling by CD22 and its ligands α2,6-linked sialic acids

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Abstract
CD22 is an inhibitory co-receptor of B cell receptor (BCR)-mediated signalling which binds specifically to glycan ligands containing α2,6-linked sialic acids. This interaction modulates the CD22 activity by an unknown mechanism. Mice deficient for ST6GalI, the enzyme that generates α2,6-linked sialic acids, show an immunodeficient and opposing phenotype to CD22-deficient mice. By generating mice double-deficient for this receptor/ligand pair, we analysed its influence on B cell maturation and signalling. Both ST6GalI-deficient and ST6GalI-CD22-deficient mice showed normal B cell development, but an impaired marginal zone B cell population in the spleen. Both types of mutant mice also showed a reduced population of bone marrow recirculating B cells, a defect previously detected in CD22+/− mice. In adoptive transfer experiments, a migration defect of wild-type B cells to the bone marrow of ST6GalI-deficient mice was found. This suggests a direct involvement of CD22 and its ligands 2,6Sia in a homing process of recirculating B cells to the bone marrow. Interestingly, defective B cell Ca2+ signalling and proliferation of ST6GalI−/− mice was rescued in ST6GalI−/−CD22-deficient mice. This points to a new mechanism of BCR signal regulation by CD22 and its ligand.

Introduction
CD22 is an important inhibitory co-receptor on B cells (1). CD22 modulates B cell receptor (BCR) signalling by recruitment of the tyrosine phosphatase SHP-1 to its intracellular inhibitory ITIM motifs (2). Activation of SHP-1 regulates the strength of the BCR-induced Ca2+ signal. Regulation of Ca2+ signalling occurs both by dephosphorylation of intracellular SHP-1 substrates which are important for triggering of Ca2+ signals (3, 4), as well as by an SHP-1 dependent activation of the Ca2+ plasma membrane pump PMCA-4, which controls termination of the signal (5). In this manner, CD22 is thought to control the signalling threshold of B cells, preventing B cell over-stimulation. Accordingly, CD22-deficient mice show higher BCR-induced Ca2+ signalling, and their B cells show basal activation, such as higher expression of activation markers, and increased sensitivity to apoptosis (6–9). Dependent on the genetic background, CD22-deficient mice develop high-affinity autoantibodies (10, 11). Also, CD22 mice show characteristic changes in B cell maturation, such as a higher proportion of mature, follicular cells (4) and a reduced number of marginal zone (MZ) B cells in the spleen (12), thought to be direct consequences of increased signalling.

As a member of the sialic acid-binding Ig-like lectin (Siglec) family, CD22 binds specifically to its ligand, 2,6-linked sialic acid (2,6Sia) (13). 2,6Sia is a common N-linked terminal carbohydrate which is expressed on several glycoproteins in the serum and also on the surface of several cell types, among them lymphocytes (14, 15). By binding to 2,6Sia, CD22 can induce cell adhesion if the ligand is expressed on target cells (16, 17). This trans-interaction has been implicated in CD22-dependent recirculation of IgD+ B cells to the bone marrow (6). A specific endothelial expression of 2,6Sia has been detected in the sinusoids of the bone marrow, in support of this mechanism (18). However, B cells also express 2,6Sia on the surface and it has been shown that on most B cells CD22 is bound to endogenous ligands on the plasma membrane in cis (19–21). Recently, it was tested how cis ligand binding of CD22 regulates CD22 signalling. Our group developed sialic acid analogues which blocked CD22 ligand binding on human
B cell development and signalling by CD22

B cells (22). Another group used a genetic approach to mutate the ligand-binding domain of CD22 on a mouse B cell line (23). Both studies came to the conclusion that blocking of the ligand-binding function of CD22 leads to reduced CD22 tyrosine phosphorylation, reduced SHP-1 recruitment and increased Ca\(^{2+}\) mobilization in B cells. In another study, a CD22-knockin mouse with a mutated CD22 ligand-binding domain was generated (24). This mouse, although resembling in many aspects a CD22-deficient mouse, did not show increased B cell Ca\(^{2+}\) signalling, in contrast to the previous studies done in B cell lines.

The sialyltransferase ST6GalI is the only enzyme which can generate the Sia\(_{2-6}\)Gal\(_{1-4}\)GlcNAc-terminus, the α2,6-linked sialic acid which occurs on various N glycans (25). This trisaccharide is the preferred ligand for CD22 (17, 26). The ST6GalI enzyme is expressed in haematopoietic cells, as well as in liver cells (27, 28). ST6GalI gene transcription is regulated by multiple promoters and can be induced by cytokines (29). ST6GalI-deficient (ST6GalI\(^{-/-}\) mice) were generated which completely lacked 2,6Sia, the ligand for CD22, on the surface of lymphocytes (30). In contrast to CD22\(^{-/-}\) mice, ST6GalI\(^{-/-}\) mice showed a defect in IgD\(^{+}\) recirculating B cells, a defect previously detected also in CD22\(^{-/-}\) mice. In IgM\(^{-/-}\) mice, both ST6GalI\(^{-/-}\) and CD22 \(\times\) ST6GalI\(^{-/-}\) mice showed a defect in IgD\(^{+}\) recirculating B cells, a defect previously detected also in CD22\(^{-/-}\) mice. Also, both ST6GalI\(^{-/-}\) and CD22 \(\times\) ST6GalI\(^{-/-}\) mice showed reduced numbers of MZ B cells in the spleen. Ca\(^{2+}\) signalling defects and proliferation defects of ST6GalI-deficient mice were rescued in CD22 \(\times\) ST6GalI\(^{-/-}\) mice. These findings are discussed in conjunction with the regulation of B cell functions by CD22 and its ligand, CD22- and CD22-independent functions of the ST6GalI-deficient mice, ST6GalI\(^{-/-}\) mice and CD22 \(\times\) ST6GalI\(^{-/-}\) mice. These mice were obtained by flushing both femurs with 1 ml of RPMI medium. The suspension was then pelleted and red blood cells were removed by erythrocyte lysis.

Flow cytometry

B cell populations were assessed using flow cytometric analysis. Briefly, a single cell suspension was prepared as described above. Approximately 1.5 \(\times\) 10\(^{6}\) cells were stained, following blockade of the FcγRII receptor by the 2.4G2 antibody. The antibodies used for surface staining were FITC-conjugated B220, IgD, cd II MHC (I-Ab, clone 25-9-17; BD Biosciences, Heidelberg, Germany), CD1d, CD21, PE-conjugated B220 (clone RA3-6B2; BD Biosciences), IgM (goat (Fab\(_{2}\); Medac, Hamburg, Germany), CD23 (clone B3-84, BD Biosciences), CyChrome-conjugated B220 (our own hybridoma collection), biotinylated Thy 1 (BD Biosciences), IgM (29.11), CD21 (clone 7E9), IgD (clone 11-26c) and CyChrome–streptavidin conjugates (BD Biosciences).

The cells were analysed by three-colour flow cytometry using an FACScanCellar system and Cell QuestSoftware (BD Biosciences).

Calcium measurement

Cells (5 \(\times\) 10\(^{5}\)) were resuspended in RPMI 1640 supplemented with 1% FCS and loaded with 4.5 \(\mu\)M Indo-1 plus 0.003% pluronic F-127 (Invitrogen, Karsruhe, Germany) at 37°C for 45 min. The baseline Ca\(^{2+}\) concentration (proportional to the FLS to FL4 ratio) was recorded at 37°C and then the cells were stimulated with 8 and 20 \(\mu\)g ml\(^{-1}\) anti-IgM (B7.6). B cells were gated as Mac1- and CD5-negative and the response was analysed in this gate. Increases in intracellular Ca\(^{2+}\) were recorded in real time with use of FAVSvantage (BD Biosciences). Data were analysed using FlowJo software and displayed as overlay histograms of relative fluorescence intensity over time.

Proliferation assay

Mouse splenic B cells were prepared as described above. Cells were resuspended in RPMI 1640 medium supplemented with 5% FCS to obtain 5 \(\times\) 10\(^{6}\) cells ml\(^{-1}\) and cultured (0.2 ml per well, flat bottomed 96 well plates) for 48 h in the presence of goat F(ab\(_{2}\)) anti-IgM (Dianova, Hamburg, Germany) (1 \(\mu\)g ml\(^{-1}\) and 10 \(\mu\)g ml\(^{-1}\)), IL-4 (BD Biosciences) (200 U ml\(^{-1}\)), CD 40 (BD Biosciences) (1 \(\mu\)g ml\(^{-1}\)) and LPS (Calbiochem, Darmstadt, Germany) (5 \(\mu\)g ml\(^{-1}\)). [\(^{3}\)H]-thymidine ([\(^{3}\)H]Tdr, 0.5 \(\mu\)Ci per well) was added for the last 6 h of co-culture. Cells were harvested and radioactivity was measured in a liquid scintillation counter as indicator of cell proliferation.

Elispot Assay

An enzyme-linked immunoassay (ELISPOT) assay was carried out as described (18). Bone marrow and spleen cells from ST6GalI-deficient mice and age-matched control mice (six mice per group) were prepared. Plates were coated with goat anti-mouse IgM or with goat anti-mouse IgG (Southern Biotechnology, Birmingham, AL, USA). After overnight incubation of cells plated at varying concentrations, they were washed off, and the spots were revealed with alkaline
phosphatase-conjugated goat anti-mouse IgM or IgG antibodies (Southern Biotechnology).

Adoptive transfer of CFSE-labelled cells

Splenocytes were harvested from donor C57BL/6 mice as described above. For complement-mediated lysis of T cells, splenocytes were stained with rat IgM anti-mouse CD4 and rat IgM anti-CD8 antibodies on ice. Then cells were washed and incubated at 37°C for 45 min with rabbit baby complement (Cedarlane). Efficiency of T cell lysis was checked by flow cytometry. Labelling with 0.5 μM carboxyfluorescein succinimidyl ester (CFSE, Molecular Probes Inc.) was done for 5 min at RT, then cells were washed in supplemented RPMI 1640 containing 10% FCS. The labelled cells were incubated for an additional 30 min at RT in RPMI 1640 containing 10% FCS. Finally, cells were washed twice with PBS and 1 x 10⁷ cells were resuspended in 150 μL PBS and injected intravenously (i.v.) (1 x 10⁷ cells per mouse). One day following B cell transfer, mice were sacrificed. Bone marrow cells and total splenocytes were stained with anti-B220-PE and analysed for percentage of CFSE-labelled cells by flow cytometry.

Results

In order to investigate in detail B cell development in ST6Gal−/− mice and to study it in ST6Gal × CD22 double-deficient mice, expression of various cell surface molecules from wild-type, CD22−/−, ST6Gal−/− and ST6Gal × CD22−/− bone marrow cells were compared. Anti-B220 versus anti-IgM staining showed a normal number of B220loIgM− pro/pre-B cells in ST6Gal−/− and ST6Gal × CD22−/− mice (Fig. 1A). Also the immature population (B220lo IgMlo) was not significantly changed in both types of mice, although ST6Gal−/− mice showed lower numbers of immature B cells. ST6Gal−/− mice showed a significant reduction of transitional B cells (B220hi IgMlo), which was not seen in double-deficient animals. Notably, the population of recirculating, mature B cells (B220hi IgMhi) was reduced in all three types of gene-deficient animals. As the reduction of these cells in ST6Gal−/− mice did not reach significance (Fig. 1A). The majority of mature B cells of the bone marrow are thought to have matured in the periphery and recirculated back to the bone marrow. These cells are characterized by expressing high levels of IgD, therefore this marker was analysed as well. By this analysis all three groups of gene-deficient mice show a significant reduction of IgD+ cells in the bone marrow (Fig. 1B).

In the spleen, there were normal numbers of T cells and B cells detected in all four groups of mice (not shown). B cell maturation in the spleen was analysed by anti-IgM/anti-IgD stainings. By these stainings, transitional type 1 (T1) (together with MZ B cells), transitional type 2 (T2) and mature B cell (M) populations can be separated. Although there were slight changes, none of them were significant (Fig. 2A). Analyses with anti-CD21/anti-IgM antibodies gave similar results (data not shown). Since CD22−/− mice have an MZ B cells defect (12), we further analysed this population. Both ST6Gal−/− and ST6Gal × CD22−/− mice showed a similar MZ B cell defect as CD22−/− mice, when MZ B cells were identified as the B220loIgMlo/IgD− population (Fig. 2B). Identification of MZ B cells as CD21hi CD23lo cells gave similar results (data not shown). It has been previously reported that CD22−/− mice express an upregulated level of the activation marker MHC class II (6). A similar upregulation was seen in ST6Gal × CD22−/− mice (Fig. 2C). ST6Gal−/− mice expressed elevated MHC class II, but the expression level was variable and this increase was not significant. We also determined the expression levels of other activation markers. The level of CD69 was similar in all four types of mice (mfi: control, 12.6 ± 2.3; ST6Gal−/−, 16.8 ± 2.1; CD22−/−, 13.6 ± 3.1; ST6Gal × CD22−/− mice, 18.0 ± 2.9), as was the level of CD86 (mfi: control, 8.7 ± 0.7; ST6Gal−/−, 10.9 ± 2.1; CD22−/−, 10.1 ± 1.4; ST6Gal × CD22−/− mice, 11.6 ± 0.9). Finally, the population of B1a cells in the peritoneal cavity of double-deficient mice was normal (Fig. 2D).
cavity was analysed. We did not see any significant differences in the B1a cell population (IgM+ CD5lo), nor in the population which are mainly B2 cells (IgM+ CD5hi/C255hi) in all three types of mutant mice, when compared with controls (total cell numbers of control mice: B1a = 5.1 ± 3.5 × 10^5, B2 = 5.1 ± 2.6 × 10^5; ST6GalI−/−: B1a = 4.6 ± 2.1 × 10^5, B2 = 3.6 ± 1.4 × 10^5; CD22−/−: B1a = 4.9 ± 3.9 × 10^5, B2 = 6 ± 4.1 × 10^5; ST6Gal × CD22−/−: B1a = 4.8 ± 3.9 × 10^5, B2 = 4 ± 3.2 × 10^5; n = 5).

The deficiency of CD22 and that of ST6GalI affect BCR-induced Ca^{2+} signalling in an opposite manner (6–9, 30), therefore it was important to study Ca^{2+} mobilization in double-knockout mice. Splenic B cells from single- and double-deficient mice were stimulated with anti-IgM at two different concentrations and the Ca^{2+} mobilization was measured. At the lower anti-IgM concentration, ST6GalI−/− B cells showed the described impaired Ca^{2+} mobilization and in CD22-deficient B cells Ca^{2+} mobilization was augmented. In splenic B cells of ST6Gal × CD22 double-deficient mice, the Ca^{2+} response was also augmented, similar to CD22-deficient B cells (Fig. 3).

ST6GalI−/− B cells are known to have impaired proliferative responses towards various stimuli, while CD22−/− B cells only have a defect in anti-IgM-induced proliferation, whereas other stimuli induce normal or increased proliferation (6–9, 30). The proliferation response was measured by stimulating purified splenic B cells of control, CD22−/−, ST6GalI−/− and ST6Gal × CD22−/− mice with various reagents. B cells from all mutant mice showed proliferation defects, compared with control mice, when stimulated with anti-IgM (Fig. 4A). As reported earlier (30), proliferation of ST6GalI-deficient B lymphocytes in response to anti-CD-40 and IL-4 was significantly reduced (Fig. 4B). In contrast, LPS-induced proliferation was equivalent to the control mice. CD22-deficient B cells show a normal proliferation in response to LPS, however, a hyper-proliferative response towards IL-4, as reported earlier.

B cells from ST6Gal × CD22−/− mice showed proliferative responses to IL-4, anti-CD40 and LPS which were indistinguishable from CD22−/− mice, suggesting that the CD22 deficiency was dominant.
The bone marrow is known to be a major site of Ig secretion by long-lived plasma cells. In CD22⁻/⁻ mice, a lack of recirculating B cells in the bone marrow was accompanied by a reduction of plasma cells in this organ (18). This was attributed to a homing defect of both cell types in CD22-deficient mice. To address the question of whether this is also the case in ST6Gal-deficient mice, we determined the plasma cell numbers in the bone marrow and spleen of ST6GalI-/- mice and control mice. In the spleen of ST6Gal⁻/⁻ mice, both IgM- and IgG-secreting cells were reduced. In the bone marrow, IgM-secreting cells were found in normal numbers in ST6Gal⁻/⁻ mice, while IgG-secreting cells were reduced (Fig. 5). Since ST6Gal⁻/⁻ mice show reduced T-dependent responses (30), the reduction of IgG-secreting plasma cells in both organs may result directly from this immunodeficiency.

The decrease of recirculating B cells in the bone marrow of ST6Gal⁻/⁻ mice suggested a homing defect of recirculating B cells, similar as in CD22⁻/⁻ mice (6, 18). In order to analyse whether migration of mature B cells is impaired, an adoptive transfer experiment was performed. Splenic B cells from wild-type mice were purified, labelled with CFSE and injected intravenously into wild-type control or ST6Gal⁻/⁻ mice. Since α2,6Sia is expressed on endothelium of sinusoids in the bone marrow in normal mice, we expect the ST6Gal⁻/⁻ mice to lack this expression pattern. Twenty-four h after adoptive transfer, both groups of recipient mice were sacrificed and analysed by flow cytometry. While a similar number of CFSE-labelled cells was found in the spleen of both recipients, ST6Gal⁻/⁻ mice showed a clear reduction of CSFE-labelled B cells in the bone marrow (Fig. 6). Similar results were obtained when the recipient mice were analysed 48 h after adoptive transfer (not shown). This suggests a direct migration defect of wild-type cells to the bone marrow of ST6Gal⁻/⁻ recipients and therefore supports the model of a CD22-driven homing mechanism.

Discussion

In this study, we have shown a normal early B cell development in CD22⁻/⁻, ST6Gal⁻/⁻ and CD22 × ST6Gal⁻/⁻ mice, but defects in splenic MZ B cells numbers in all three mutant mice. A common defect of all three types of mutant mice was also a reduction of recirculating B cells in the bone marrow, suggesting a direct effect of CD22 and also 2.6Sia ligands on this population. Accordingly, a migration defect of wild-type B cells into the bone marrow of ST6Gal⁻/⁻ mice was found. IgG-secreting plasma cells were reduced both in the spleen and
bone marrow of ST6Gal\(^{-/-}\) mice. The Ca\(^{2+}\) and proliferation defects of ST6Gal\(^{-/-}\) mice were not detected in CD22 \times ST6Gal\(^{-/-}\) mice. In contrast, the double-deficient mice showed the same increased signalling phenotype as CD22-deficient mice.

As described previously, early B cell development in the bone marrow was not affected in both CD22\(^{-/-}\) and ST6Gal\(^{-/-}\) mice (6–9, 30). Similarly, as expected, CD22\(^{-/-}\)ST6Gal\(^{-/-}\) mice also showed no impairment of early B cell development. Pro-B and pre-B cell development in the bone marrow of all three types of mutant mice was analysed by several other surface markers which did not reveal any difference (data not shown). This suggests that at the important checkpoint of the transition of large pre-B to small pre-B cells, at which the pre-BCR is expressed, both the co-receptor CD22, as well as its ligand 2,6Sia play no important role. In the periphery, the main B cell population affected by the ST6GalI deficiency are MZ B cells in the spleen, which has not been described before.

MZ B cells are a special splenic B cell population residing outside the follicles, separated from the follicle by the marginal sinus (31). MZ B cells are in first contact with blood-born bacterial antigens and are involved in an early T-independent B cells response. MZ B cells are thought to be a separated splenic B cell lineage, which is derived from the same immature precursor cells as follicular mature B cells. CD22-deficient mice show a MZ defect, which we explained by an impaired MZ B cell development due to increased signalling (12). There are several examples of gene-deficient mice where changes in B cell signalling affect the lineage decision between follicular and MZ B cells (32). CD22-knockin mice with a mutated CD22 ligand-binding domain also show reduced MZ B cell numbers (24). This could also point to a role of CD22–ligand interactions in the regulation of MZ B cell numbers. An impairment in 2,6Sia–CD22 interactions could potentially also explain the MZ B cell defect of ST6Gal\(^{-/-}\) mice. This explanation is, however, purely speculative. In contrast to the bone marrow, we could previously not detect 2,6Sia expression in the spleen on cells other than follicular lymphocytes (18). For a mechanism of trans-interactions of CD22 involved in migration of B cells into the MZ one would expect to find 2,6Sia expression, e.g. on endothelial cells of the marginal sinus. The impairment of BCR signalling found in ST6Gal\(^{-/-}\) mice cannot easily explain the MZ defect because mice with impaired BCR signalling show rather normal or increased MZ B cell numbers (32).

An important finding of this study was the defect of IgD\(^+\), recirculating B cells in the bone marrow of all three types of gene-deficient mice. This defect was first discovered in CD22\(^{-/-}\) mice (6). We then found 2,6Sia to be expressed specifically on bone marrow sinusoidal endothelium. Both B cell transfer experiments, as well as blocking experiments by i.v. injection of CD22-Fc proteins strongly suggested...
a CD22-dependent homing mechanism of recirculating B cells to the bone marrow (18). Since ST6Gal−/− mice do not express any cell surface 2,6Sia, they are expected to be also defective in 2,6Sia expression on endothelial cells of the bone marrow. Therefore, the results we describe here was the expected finding: also ST6Gal−/− mice, with a defective CD22 ligand expression, show the same recirculation defect as CD22−/− mice, where the receptor is not present. This result is in contrast to the first publication of ST6Gal−/− mice, in which no reduction of the B220+ IgM+ population in the bone marrow was shown (30). As expected, double-deficient mice show the same phenotype. These studies were extended to short-term migration experiments. We show that wild-type splenic B cells do not migrate well to CD22 ligand-deficient bone marrow. This is complemental to our previous findings, that receptor (CD22)-deficient B cells do not migrate well to wild-type bone marrow, which was studied by a very similar cell transfer system (6). Both studies together strongly suggest a CD22/CD22 ligand-dependent homing mechanism for recirculating B cells. Mechanistically, we cannot distinguish whether the defects of CD22-deficient or ST6Gal-deficient mice are due to impaired entry or to impaired retention in the bone marrow. It should be noted, that the population of IgD+ mature B cells was found in normal numbers in the periphery (the spleen) in all three types of mutant mice. Therefore, the reduction of IgD+ B cells in the bone marrow is not due to a block of maturation or decreased survival of these cells in the periphery. CD22-knockin mice with a mutated ligand-binding domain of CD22 also showed the defect of IgD+ B cells in the bone marrow, although cell transfer experiments could not reveal differences in the migration properties of CD22-knockin and wild-type B cells (24).

In the bone marrow of CD22−/− mice lower plasma cell numbers were found (18). The correlation of lower plasma cell numbers and of lower recirculating B cells suggested a similar CD22-dependent homing mechanism for both cell types. In order to address this question also in ST6Gal-deficient mice, plasma cell numbers were analysed. For IgG-secreting plasma cells, a defect was found both in bone marrow and spleen. Since ST6Gal−/− mice have an impaired T-dependent immune response (30), this IgG-secretion impairment in both organs may result from this defect. Therefore we cannot conclude that plasma cell homing to the bone marrow is specifically affected by the ST6Gal-mutation. Further experiments with studies on antigen-specific plasma cell responses and cell transfer experiments are needed to clarify this point.

One main purpose of generating CD22 × ST6Gal−/− mice was to study the regulation of signalling by CD22 and its ligands 2,6Sia. Both the defects in Ca2+ responses, as well as the proliferation defects of ST6Gal−/− mice were rescued in double-deficient mice. This was not true for anti-IgM driven proliferation, where all three mutant mice showed similar defects when compared with control mice. The anti-IgM triggered proliferation defect of CD22−/− and ST6Gal−/− mice has been described before (6, 30). But CD22 × ST6Gal−/− mice showed proliferative responses to anti-CD40, IL-4 and LPS, as well as anti-IgM triggered Ca2+ responses which were increased to the same level as in CD22−/− mice.

The Ca2+ signalling and proliferation B cell defect of ST6Gal−/− mice has previously been interpreted as a more general defect, not necessarily caused by the lack of CD22 binding (30). Optimal crosslinking of the BCR by anti-IgM antibodies could be prevented by the lack of 2,6Sia. Also, potentially receptors other than CD22 could bind to 2,6Sia, and a lack of this interaction could contribute to the signalling defect of ST6Gal−/− B cells. However, if the CD22 deficiency is crossed to the ST6Gal deficiency, double-deficient B cells show increased signalling. This result shows that the phenotype of the CD22 deficiency can override the signalling defect of the ST6Gal deficiency. One interpretation of these data is that CD22 is directly involved and required for the Ca2+ defect of ST6Gal−/− mice. Such an augmented negative regulatory effect could for instance be explained by increased association of CD22 to the BCR in ST6Gal−/− mice. This interpretation is confirmed by a recent independent study where a very similar phenotype of ST6Gal × CD22-deficient mice is described (33). This study shows a higher co-localization of CD22 with the BCR in clathrin-rich microdomains of B cells lacking ST6Gal, suggesting stronger inhibition by CD22 in these cells.

These new data are in contrast to cell line studies as well as studies in primary human B cells which showed that cis ligand interactions are positively influencing the level of CD22 tyrosine phosphorylation and the suppression of Ca2+ responses (22, 23). Also, the CD22-knockin mouse with a mutated CD22 ligand-binding domain did not show a Ca2+ defect (24). Clearly, further experiments are needed to resolve these apparent contradictory results.

Further insights into the mechanism of how CD22 ligand binding on the B cell surface regulates Ca2+ signalling may come from biochemical studies identifying the 2,6Sia-carrying ligand proteins in cis. A recent report showed that CD22 is mainly engaged in homomultimeric complexes on the B cell surface (34). Photo-activatable sialic acid analogues were fed into B cell lines and activated on the B cell surface to cause covalent crosslinking to a sialic acid-binding receptor. Subsequent biochemical analysis showed that the main sialic acid carrying cis ligands of CD22 are neighbouring CD22 molecules. No other cis ligands were detected by this approach. Thus, lack of 2,6Sia on the B cell surface would affect mainly the homotypic interactions of CD22 and therefore would be expected to affect the same signalling pathway. However, how this oligomerization affects association to IgM and the regulation of inhibitory signalling is still unclear. It could be that CD22–CD22 interactions regulate subcellular compartments, such as the IgM and CD22 recruitment to lipid rafts. Differential recruitment of IgM and CD22 into lipid rafts has been described (35, 36).

IgM has previously been shown to bind to CD22 (37, 38) and also CD45 can bind to CD22 in cis (39). However, both interactions are not affected by a point mutation in the first Ig domain of CD22, which abolishes ligand binding. This is in agreement with our own studies done in human B cell lines where pretreatment of cells with sialic acid analogues (known to interrupt ligand binding) did not affect association of CD22 with both IgM and with CD45 (S. Ghosh, R. Brossmer, and L. Nitschke, unpublished results).

In summary, this study shows important B cell maturation defects in ST6Gal−/− mice. In particular, the bone marrow steady state and migration defect of recirculating B cells in
both CD22^−/− and in ST6Gal^−/− mice points to a likely regulation of homing by this receptor/ligand pair. The signaling studies of CD22 × ST6Gal-double-deficient mice showed a rescue of defective signalling in ST6Gal^−/− mice by the CD22 deficiency since both Ca^2+ responses, as well as most proliferative responses were elevated to the same level as in CD22-deficient mice. Although both the inhibitory receptor CD22 and its ligand 2,6Sia clearly regulate BCR signalling, analysis of the exact mechanism still needs additional experimental work.

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Abbreviations

BCR  B cell receptor
CFSE  carboxyfluorescein succinimidyl ester
MZ  marginal zone

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