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Copresentation of Antigen and Ligands of Siglec-G Induces B Cell Tolerance Independent of CD22

Fabian Pfrengle, Matthew S. Macauley, Norihiito Kawasaki, and James C. Paulson

Differentialization of self from nonself is indispensable for maintaining B cell tolerance in peripheral tissues. CD22 and Siglec-G (sialic acid–binding Ig-like lectin G) are two inhibitory coreceptors of the BCR that are implicated in maintenance of tolerance to self Ags. Enforced ligation of CD22 and the BCR by a nanoparticle displaying both Ag and CD22 ligands induces a tolerogenic circuit resulting in apoptosis of the Ag-reactive B cell. Whether Siglec-G also has this property has not been investigated in large part owing to the lack of a selective Siglec-G ligand. In this article, we report the development of a selective high-affinity ligand for Siglec-G and its application as a chemical tool to investigate the tolerogenic potential of Siglec-G. We find that liposomal nanoparticles decorated with Ag and Siglec-G ligand inhibit BCR signaling in both B1 and B2 B cells compared with liposomes displaying Ag alone. Not only is inhibition of B cell activation observed by ligating the BCR with Siglec-G, but robust tolerance toward T-independent and T-dependent Ags is also induced in mice. The ability of Siglec-G to inhibit B cell activation equally in both B1 and B2 subsets is consistent with our observation that Siglec-G is expressed at a relatively constant level throughout numerous B cell subsets. These results suggest that Siglec-G may contribute to maintenance of B cell tolerance toward self Ags in various B cell compartments. The Journal of Immunology, 2013, 191: 1724–1731.

The heactivation of B cells is initiated by engagement of their cognate Ag with the BCR, leading to a complex signal transduction cascade that can culminate in differentiation to Ab-secreting plasma cells (1). To avoid detrimental autoimmune responses, differentiation of self from nonself Ag is essential. This differential recognition can be aided by coreceptors that modulate BCR signaling to either promote an immune response to nonself or suppress a response to self (2). Two B cell inhibitory coreceptors are CD22 and Siglec-G, members of the siglec (sialic acid–binding Ig-like lectin) family, which recognize sialic acid–terminated glycans on glycoproteins and glycolipids (3). Knockout mice deficient in both siglecgs accumulate autoantibodies as they age, suggesting that they participate in maintaining B cell tolerance (4). However, the precise roles of these two siglecgs in regulating activation of B cells to self Ags are yet to be defined.

Receptors of the siglec family are differentially expressed on WBCs and regulate immune cell function (5). The expression of CD22 is primarily B cell restricted. In contrast, a limited number of studies suggest that Siglec-G is more broadly expressed on B cells and myeloid cells (6, 7). Whereas CD22 is conserved between mouse and human, Siglec-G is the murine ortholog of human Siglec-10. To date, no other siglecgs have been detected on murine B cells (8). Both CD22 and Siglec-G contain at least one ITIM on their cytoplasmic tail and are capable of inhibiting B cell receptor–mediated signaling (9). A leading model for CD22-mediated suppression of BCR signaling is that proximity to the BCR leads to phosphorylation of its ITIMs by Src kinases, which recruits inhibitory phosphatases such as SHP-1 to dampen BCR signaling (10, 11). Although Siglec-G has been less studied, Siglec-10 was shown to recruit SHP-1 in human B cells, suggesting that it may regulate BCR signaling by a mechanism similar to CD22 (12).

In addition to its function in B cells, Siglec-G has also been shown to play an important role in dendritic cells by discriminating danger-associated molecular patterns from pathogen-associated molecular patterns (PAMPs) (13). Much like the model for siglec-mediated inhibition in B cells, this inhibition is based on proximity to the receptor. Unlike PAMPs, danger-associated molecular patterns were shown to be capable of simultaneously binding TLR4 and CD24. By virtue of a stable interaction between Siglec-G and CD24, Siglec-G becomes recruited to the TLR, which leads to dampened activation, compared with stimulation by PAMPs that cannot bind CD24. Interestingly, disruption of the sialic acid–mediated interaction of CD24 and Siglec-G by microbial sialidases exacerbated inflammation in an intestinal perforation model of sepsis (14).

Insight into the role of Siglec-G on B cells has come from studying Siglec-G–deficient mice (6, 7). These mice show an expansion of their B1 cell population in the pleural and peritoneal cavities (15). B1 cells are responsible for the production of natural IgM and play an important role in the immune response to T-independent Ag such as bacterial polysaccharides (16). After stimulation of the BCR by anti-IgM, B1 cells isolated from the peritoneal cavity of Siglec-G–deficient mice show enhanced calcium signaling, whereas no significant difference was observed in splenic B2 cells (7). This observation suggested a dominant role for Siglec-G in B1 cells, which appeared to be in line with a slightly higher expression on B1 cells, as judged by Western blot analysis. Therefore, it is believed that
Siglec-G plays a dominant role in peritoneal B1 cells. Mice deficient in both Siglec-G and CD22 have an even higher number of B1 cells and, in contrast to the single knockout mice, develop spontaneous autoimmunity (4). Cumulatively, these mouse genetic studies strongly suggest that CD22 and Siglec-G act in a complementary way to suppress B cell responses to self Ags.

Recent studies have shown that a nanoparticle decorated with a B cell–reactive Ag and a high-affinity ligand for CD22 is able to induce Ag-specific tolerance in mice (17, 18). Copresentation of the Ag and CD22 ligand causes juxtaposition of CD22 with the BCR, leading to a tolerogenic circuit that culminates in apoptosis of the Ag-reactive B cell. On the basis of the specificity of the ligand for CD22, tolerance induction was mainly dependent on CD22, although one report showed partial dependence on Siglec-G (17). Thus, although several studies suggest that CD22 and Siglec-G cooperatively suppress B cell responses, an independent role for Siglec-G in B cell tolerance has not been demonstrated (4, 17, 18).

To examine the potential of Siglec-G to induce B cell tolerance, we have developed a high-affinity ligand for Siglec-G that does not cross-react with CD22 and show that siglec-engaging tolerance–inducing antigenic liposomes (STALs) displaying the Siglec-G ligand give rise to robust tolerance in mice toward both T–independent and T-dependent Ags. Analysis of the initial events following stimulation of B cells with liposomes indicates that both B1 and B2 cells are equally capable of being inhibited by recruitment of Siglec-G to the BCR. This latter finding is in accordance with our finding that Siglec-G is expressed at equivalent levels in both B cell subtypes, as assessed by using a newly developed anti–Siglec-G mAb. We also show that Siglec-G is expressed throughout B cell development, including pre–B cells before CD22 is expressed, suggesting that it may have a unique role in regulation of BCR signaling.

Materials and Methods

Animal studies

The Institutional Animal Care and Use Committee of The Scripps Research Institute (La Jolla, CA) approved all experimental procedures involving mice. CD22KO and Siglec-GKO mice, on a C57BL/6J background, were obtained from L. Nitschke (University of Erlangen, Erlangen, Germany) and Y. Liu (University of Michigan, Ann Arbor, MI), respectively. Wild-type (WT) C57BL/6J mice were obtained from The Jackson Laboratory (University of Michigan, Ann Arbor, MI), respectively. Wild-type (WT) C57BL/6J and C57BL/6J mice were obtained from The Jackson Laboratory rodent breeding colony. WT MD4 transgenic mice that express IgM<sub>a</sub> (C57BL/6J background) were obtained from The Jackson Laboratory and crossed onto a selection marker. Other cell lines expressing murine siglec in Chinese variable (Mev) mice were obtained from The Jackson Laboratory. The fol-

... was carried out by reaction of the respective compound with NHS-activated AF-647 (Invitrogen) to pegylated distearoylphosphoethanolamine (PEG2000-DSPE) conjugated with nitrophenol (NP) hapten, or Alexa 647 conjugated with Pasteurella multocida Oa-3-sialyllectose (PML-1) (PMST1), to yield the respective tri-...
100 nm, as described previously (18). All liposomes were composed of a 60:35:5 molar ratio of distearoyl phosphatidylcholine (Avanti Polar Lipids), cholesterol (Sigma-Aldrich), and the respective pegylated lipid. The total concentration of the liposomes, as defined by the molarity of the lipids, was 5 mM. Fluorescent liposomes for binding experiments contained 1% or 4% siglec ligand and 0.1% Alexa 647. Liposomes for functional assays contained 0.5% anti-IgM or 0.33–0.1% HEL and 1 or 4% siglec ligand.

**Liposome binding assays**

Murine splenocytes and siglec-expressing cells were incubated with the respective fluorescent liposomes for 40 min at 4°C in FACS buffer. Cells were washed, then stained with anti-CD19, and binding was assessed by flow cytometry.

**B cell purification**

B cells were purified from splenocytes by negative selection, using magnetic beads according to the manufacturer’s protocol (Milenyi Biotec). The purity of isolated cells was generally ≥ 99%.

**Ca²⁺ flux**

Following preparation of single-cell suspensions of splenocytes for cells from the peritoneal cavity, cells were loaded with Indo-1 (Invitrogen), as described previously (7). Cells were stimulated with liposomes, and Indo-1 fluorescence was monitored by flow cytometry, as described in our previous publication (18).

**B cell proliferation assay and Western blotting**

Purified IgMHEL B cells were stimulated with 5 μM liposomes for 3 min, and their activation was analyzed by Western blotting. For analysis of B cell proliferation, cells were first labeled with 1 μM CellTrace Violet (Invitrogen) prior to stimulation with liposomes. For all in vitro assays, the media consisted of RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM nonessential amino acid, 1 mM sodium pyruvate, and 50 μM 2-ME.

**Immunizations and ELISA**

Mice were injected i.p. or i.v. with liposomes displaying NP or HEL with or without siglec ligand. The liposomes were delivered in 200 μl PBS at 0.5 mM total lipid concentration. Whole blood (50 μl) was collected from mice via retro-orbital bleeds. NP- and HEL-specific Abs were determined by ELISA with NP(1)-BSA–coated (20 μg/ml) and HEL-coated (10 μg/ml) MaxiSorp plates (Nunc), as described previously (18). NP(1)-BSA was prepared as described earlier (24). The titer was defined as the endpoint titer, which was the dilution of serum that achieved an absorbance 2-fold above background.

**Results**

Siglec-G is expressed throughout B cell differentiation

To quantitatively investigate cell surface expression of Siglec-G on murine leukocytes, we developed a mAb toward the extracellular portion of Siglec-G. The Ab shows strong specificity for Siglec-G, as highlighted by its lack of binding to cells from Siglec-G–deficient mice (Fig. 1A). Among splenic leukocytes (Fig. 1B), Siglec-G is expressed at highest levels on B cells and, to a lesser extent, on dendritic cells and a subset of macrophages (Fig. 1B). Slight expression was also observed on neutrophils, and no expression was detected on T cells or NK cells. Of interest, evaluation of B cell subsets from the spleen, bone marrow, and the peritoneal cavity revealed a consistent expression of Siglec-G on all B cell subsets examined (Fig. 1C, 1D). Notably, significant Siglec-G expression appears early on in B cell development, as early as pre-pro and immature B cells. Siglec-G expression remains at a constant level on germinal center and memory B cells, whereas no expression is observed on plasma cells (Supplemental Fig. 1A). All subsets of splenic dendritic cells showed Siglec-G expression with the highest expression on CD11b⁺ myeloid dendritic cells (Supplemental Fig. 1B). In the liver, Siglec-G expression was found on B cells and macrophages (Supplemental Fig. 1C).

**Specificity of a newly developed high-affinity glycan ligand for Siglec-G**

To evaluate the independent roles of CD22 and Siglec-G as regulators of BCR signaling, we set out to evaluate the ability of Siglec-G to induce tolerance by enforced coligation to the BCR, using nanoparticles that expressed both an Ag and a siglec ligand, which we have described as STALs. To investigate the impact of the B cell siglecs on BCR signaling, selective ligands for either siglec were required. Previously, a high-affinity and selective glycan ligand for CD22 (6'-BPANeuGc; Fig. 2A) was described, but no equivalent ligand has been reported for Siglec-G (22). α2-6-Sialosides carrying a BPA group at the 9-position of the sialic acid (6'-BPANeuGc) show a 100-fold increase in affinity to CD22 compared with the natural, unsubstituted ligand. Because Siglec-G is known to be capable of binding α2-3-sialosides, whereas CD22 exhibits strict preference for α2-6-sialosides, we tested and fortuitously found that addition of the BPA substituent to an α2-3-sialosides results in a selective and high-affinity ligand for Siglec-G. Accordingly, the ligand 3'-BPANeuGc was chemozymatically synthesized and incorporated into fluorescent liposomes for assessing binding to siglec-expressing cells. These 3'-BPANeuGc liposomes showed excellent targeting to Siglec-G–expressing cells and did not bind to cells transfected with any of the other murine siglces (Fig. 2B). Furthermore, binding of the 3'-BPANeuGc liposomes to murine splenic B cells was completely dependent on Siglec-G, as no binding was detected in splenocytes from Siglec-G–deficient mice (Fig. 2C). Conversely, 6'-BPANeuGc liposomes bound exclusively to CD22-expressing cells (Fig. 2B).

**Juxtaposition of Siglec-G and the BCR leads to inhibited Ca²⁺-signaling in both, B1 and B2 B cells**

In previous studies, we and others (17, 18, 25) have demonstrated that enforced colocalization of the BCR and CD22, using nanoparticles displaying both a cognate Ag and CD22 ligands, leads to strong inhibition of B cell activation. With a Siglec-G–specific ligand in hand (3'-BPANeuGc), we set out to determine if the colocalization of BCR and Siglec-G also suppresses B cell activation, and ascertain the degree to which Siglec-G regulates BCR signaling in B1a cells and B2 cells. To this end, we prepared liposomes bearing an anti-IgM Fab fragment that serves as a surrogate Ag, either with or without the Siglec-G ligand (3'-BPANeuGc), to assess the influence of coligation of the BCR with Siglec-G. Stimulation of either B1a or B2 B cells with liposomes displaying anti-IgM alone led to strong B cell activation, as monitored by calcium flux. In contrast, stimulation with anti-IgM–containing liposomes that also displayed 3'-BPANeuGc resulted in substantial suppression of Ca²⁺ flux in both B cell populations (Fig. 3A, 3B). Although B cells from Siglec-G–deficient mice showed generally diminished responses after stimulation with the liposomes, there was no influence of the Siglec-G ligand on activation of the B cells from Siglec-G–deficient mice. Most importantly, the inhibitory activity of the Siglec-G ligand was also prevented by preincubation of the cells with the anti–Siglec-G mAb, demonstrating that the 3'-BPANeuGc ligand exhibits its inhibitory function exclusively through Siglec-G (Supplemental Fig. 2). Moreover, anti-IgM liposomes displaying the CD22-specific ligand (6'-BPANeuGc) suppressed activation, compared with liposomes displaying anti-IgM alone in a CD22-dependent manner in both B2 and B1a B cells (Fig. 3).

These results show that the 3'-BPANeuGc and 6'-BPANeuGc ligands are specific for Siglec-G and CD22, respectively, and that either siglec can independently suppress BCR activation when their ligands are incorporated into antigenic liposomes that enforce ligation of the siglec with the BCR.
Broad inhibition of B cell activation in an Shp-1–dependent manner

Although calcium flux is one hallmark of B cell activation, BCR signaling activates numerous signaling cascades that can initiate cell division and survival of B cells. To assess the impact of Siglec-G on the initial events in B cell activation, we analyzed cellular signaling pathways using phospho-specific Abs in conjunction with Western blotting. These experiments were carried out using splenic B cells from MD4 mice (26), which are reactive toward HEL. We first verified in HEL-specific B cells that liposomes displaying HEL and Siglec-G ligand suppress Ca\(^{2+}\) flux, compared with liposomes displaying HEL alone in a Siglec-G–dependent manner (Supplemental Fig. 3). Relative to liposomes displaying HEL alone, liposomes that additionally display the Siglec-G ligand suppressed phosphorylation (or degradation) of multiple BCR signaling events, including those that signal through MAPK, Erk, NF-κB, and Akt (Fig. 4A). The inhibition of B cell signaling we observe is Shp-1 dependent because the activation of B cells from Shp-1–deficient (moth-eaten) mice is not inhibited by the Siglec-G ligand (Fig. 4B). B cells from Shp-1–deficient mice contain a mixture of B1 and B2 cells. Therefore, the results suggest that Siglec-G exhibits its inhibitory function through Shp-1 in both B cell subpopulations. These results demonstrate that ligand-mediated recruitment of Siglec-G to the BCR strongly dampens both proximal and distal

FIGURE 1. Analysis of cell surface expression of Siglec-G on leukocytes. (A) The anti–Siglec-G Ab stains splenic B cells from WT but not Siglec- G–/– mice (anti-Siglec-G in red; isotype control in blue). (B) Siglec-G is expressed on B cells, dendritic cells, and a subset of macrophages from the spleen. (C) Siglec-G is expressed in equal amounts on CD5\(^{+}\) (B1a) and CD5\(^{-}\) (B1b+B2) B cells in the peritoneal cavity. Siglec-G is expressed at a constant level in B cell subsets of the spleen (D) and bone marrow (E).
signaling components in the BCR signaling cascade and that this inhibition is dependent on Shp-1.

Siglec-G ligands reduce B cell proliferation and survival in vitro

We next determined the longer-term fate of the HEL-reactive MD4 cells stimulated with STALs. Fluorescently labeled B cells were cultured in vitro with liposomes, and proliferation of the cells was monitored by fluorescence dilution. Cells incubated with liposomes displaying HEL alone underwent robust proliferation, as assessed at day 4 (Fig. 5A, 5B). Notably, all the cells exposed to liposomes displaying both HEL and 3\(^{-}\)BPA NeuGc were activated and divided, but there was a pronounced diminishment in proliferation. Moreover, a significant reduction of live cells relative to cells stimulated with HEL alone (Fig. 5B) was noted. These effects were dependent on Siglec-G, as no difference was observed when Siglec-G–deficient MD4 B cells were stimulated with liposomes displaying Ag and Siglec-G ligand. Therefore, coligation of the BCR and Siglec-G leads to inhibited B cell proliferation and decreased survival, compared with stimulation by ligation of the BCR alone.

Liposomes displaying Siglec-G ligand and Ag induce tolerance in vivo

Copresentation of Ag and CD22 ligands on nanoparticles has been documented to induce Ag-specific tolerance in mice (17, 18). On the basis of the ability of liposomes bearing the Siglec-G ligand to suppress BCR signaling in vitro, we set out to determine if Siglec-G could also independently induce tolerance in vivo. Accordingly, we investigated the ability of liposomal nanoparticles carrying the Siglec-G ligand to induce tolerance to exemplary T-independent and T-dependent Ags. The i.p. injection of immunogenic liposomes displaying the T-independent Ag NP resulted in reproducible production of IgM and IgG Abs, whereas liposomes that additionally displayed 3\(^{-}\)BPA NeuGc had greatly reduced anti-NP titers, and failed to produce Ab upon subsequent challenge with the immunogenic liposomes 2 and 4 wk later (Fig. 6A, Supp. Fig. 4A). Similar results were obtained after i.v. administration of the liposomes (Supp. Fig. 4B).

Similarly, i.v. administration of immunogenic liposomes displaying the T-dependent Ag HEL induced a strong IgG\(_1\) response, whereas only negligible amounts of anti-HEL Abs were produced after injection of liposomes with both HEL and 3\(^{-}\)BPA NeuGc (Fig. 6B). Subsequent challenges with HEL liposomes demonstrated that tolerance had been induced in the group of mice given STALs in the initial injection. The identical experiment was repeated in Siglec-G–deficient mice, and no significant difference in Ab titers between the two groups was observed, demonstrating that induction of tolerance is mediated by Siglec-G (Fig. 6B).

To assess the relevance of Siglec-G–mediated tolerance to its natural function on B cells, we investigated tolerance induction by
received a challenge with liposomes displaying Ag alone. Ab titers were liposomes displaying Ag and Siglec-G ligand. On days 14 and 28, all mice were injected at day 0 with either liposomes displaying Ag alone or
ance to NP in a Siglec-G–dependent manner (Fig. 6C). Therefore, recruitment of Siglec-G to the immunological synapse in B cells can induce tolerance in mice, and this may represent a natural tolerogenic mechanism that operates to maintain self-tolerance.

Discussion
In this article, we demonstrate that the two murine B cell siglecs, Siglec-G and CD22, can independently regulate BCR signaling, and can induce B cell tolerance when the Ag is presented in trans with siglec ligands that enforce ligation of the siglec with the BCR. An impediment for studying the role of trans ligands in regulating the function of Siglec-G has been the lack of a suitable high-affinity and selective ligand. In this study, we circumvent this need through the development of such a high-affinity glycan ligand for Siglec-G that does not cross-react with CD22 or any other murine siglec. Incorporation of this newly described Siglec-G ligand onto immunogenic liposomes clearly revealed that recruitment of Siglec-G to the immunological synapse inhibits B cell activation, as shown by calcium flux in an Shp-1–dependent manner, signaling components of the BCR, and proliferation. Therefore, we believe that enforced ligation of Siglec-G and the BCR, resulting from a membrane displaying both Ag and Siglec-G ligand, results in inhibition of B cell activation in a manner similar to that described previously for CD22.

In B cell proliferation assays, we noticed that not only was B cell activation inhibited by liposomes displaying Ag and 3′-BPANeuGc but also the number of live B cells was greatly reduced compared with B cells stimulated with Ag alone. This finding suggested that a tolerogenic circuit is induced, resembling the one we previously described by liposomes decorated with Ag and CD22 ligand. Indeed, STALs were able to induce robust tolerance in vivo toward both T-independent and T-dependent Ag. In contrast to WT mice, Siglec-G–deficient mice did not become tolerized, which clearly demonstrated the specificity of our observations.

Of interest, we discovered that antigenic liposomes displaying a natural α2-3-sialoside could also induce tolerance in a Siglec-G–dependent manner. We believe this may relate to a natural function for Siglec-G on B cells. Tolerization of B cells autoreactive for a cell surface Ag could be aided by Siglec-G, which will be recruited to the immunological synapse by a dense layer of sialic acid. The differential specificity of CD22 and Siglec-G is interesting in this regard. Outside the nervous system, there are two predominant linkages of sialic acid to underlying glycans: α2-3- and α2-6-sialosides. It is well known that the ratio of these two forms of sialic acid varies among different cell types, in large part owing to differential expression of the appropriate 2-6- and 2-3-sialyltransferases (27–30). For instance, hepatocytes and B cells exhibit a predominance of 2-3-sialosides, whereas Siglec-G clearly has the ability to bind α2-3-sialosides. Thus it will be of interest to determine if CD22 and Siglec-G act cooperatively to mediate peripheral B cell tolerance to autoreactive cell surface Ag through their combined ability to recognize a broader range of sialoside sequences than either siglec alone.

As a part of these studies, we used a recently developed platform for polyclonal stimulation of B cells, which are liposomes decorated with the Fab fragment of an anti-IgM–reactive Ab (18). These were initially used to allow us to investigate both B2 and B1a B cell subtypes, because peritoneal B1a cells from MD4 mice do not express the IgM transgene (31). B1a cells constitute the main population of B1 cells in the peritoneal cavity and can be
indistinguishable from B1b cells by their surface expression of CD5. On the basis of evidence that Siglec-G–deficient mice have more B1a B cells, Siglec-G is believed to play a significant role in the development of this B cell subset. In particular, the expansion in Siglec-G–deficient mice is believed to be the result of hyperreactivity of B1a cells to BCR stimulation (4, 7). In contrast, CD22–deficient mice show enhanced BCR-simulated B cell activation in B2 cells (32–33). Using the anti-IgM liposomes, we found that Siglec-G is capable of inhibiting B cell activation to a roughly equivalent extent in B1a cells from the peritoneal cavity and B2 cells from the spleen. This finding suggests that, besides its important function in B1a cells, Siglec-G is equally capable of inhibiting BCR signaling in splenic B2 cells upon encounter of its cognate Ag in the context of Siglec-G ligands. Whereas B1a and marginal zone B cells are thought to be the primary B cell subtypes responsible for immune responses toward T-independent type II Ags (36), follicular B2 cells are more adapted for T-dependent immune responses (37). Taken together with our finding that tolerance is induced toward both T-independent type-2 and T-dependent Ags, this observation suggests an important function for Siglec-G in maintaining tolerance toward sialylated Ags in various B cell compartments.

Consistent with the hypothesis of Siglec-G playing a role in various B cell compartments, we find equivalent cell surface expression of Siglec-G in B1a cells from the peritoneal cavity and B2 cells from the spleen. Furthermore, Siglec-G is developmentally expressed at constant levels throughout B cell development in the bone marrow, even before expression of IgM in immature B cells (Fig. 1). This observation is consistent with the suggestion that Siglec-G influences the fate of B cells during early development, as evidenced by the enhanced number of B1a cells in the peritoneal cavity of Siglec-G–deficient mice (38). In contrast to Siglec-G, CD22 is only weakly expressed in immature B cells before release into the periphery, and both CD22 expression and the Lyn/CD22/Shp-1 regulatory access are upregulated in mature peripheral B cells (9, 39–41). Thus, CD22 is not believed to play a role in central tolerance (39, 40, 42, 43). On the basis of the constant expression of Siglec-G in the earliest stages of B cell development, we believe that studies on its potential contribution to central B cell tolerance are warranted.

In summary, we describe antigenic liposomes decorated with bivalent sialoside ligands that can down-regulate BCR signaling in splenic B2 cells upon encounter of its cognate Ag in the context of Siglec-G ligands. We find that Siglec-G–deficient mice have more B1 cell population.

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Disclosures
The authors have no ﬁnancial conﬂicts of interest.

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