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Galectin-1 and Galectin-8 Have Redundant Roles in Promoting Plasma Cell Formation

Chih-Ming Tsai,*† Chin-Huey Guan,‡ Hsiao-Wu Hsieh,§ Tsui-Ling Hsu,¶ Zhijay Tu,¶ Kuan-Jung Wu,‡ Chun-Hung Lin,‡,§ and Kuo-I Lin*†

Galectin (Gal) family members are a type of soluble lectin, and they play important roles in immunomodulation. Their redundant roles have been proposed. We previously found that Gal-1 promotes the formation of Ab-secreting plasma cells, but B cells from Gal-1-deficient and control animals produce comparable amounts of Abs. In the current study, we used synthetic sulfomodified N-acetyllactosamine (LacNac) analogs and short hairpin RNAs for Gal-8 to demonstrate a redundancy in the effects of Gal-1 and Gal-8 on plasma cell formation. Gal-1 and Gal-8 were both expressed during plasma cell differentiation, and both Gals promoted the formation of plasma cells. Gal-1 and Gal-8 bound better to mature B cells than to plasma cells, and the expression of glycosyltransferase enzymes changed during differentiation, with a decrease in mannosyl (α1-6)-glycoprotein β1,6-N-acetylgalcosaminyltransferase and N-acetylgalcosaminyltransferase-1 mRNAs in plasma cells. Synthetic sulfomodified Galβ1-3GlcNac disaccharides (type 1 LacNacs) selectively prevented Gal-8 binding, leading to a blockade of Ab production in Gal-1–deficient B cells. Furthermore, synthetic type 1 LacNacs that were able to block the binding of both Gals greatly reduced the effect of exogenously added recombinant Gal-1 and Gal-8 on promoting Ab production. These results reveal a novel role for Gal-8 in collaboration with Gal-1 in plasma cell formation, and suggest the possibility of using distinct LacNac ligands to modulate the function of Gals. The Journal of Immunology, 2011, 187: 1643–1652.

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Abbreviations used in this article: Blimp-1, B lymphocyte-induced maturation protein-1; C2GnT, β1,6-N-acetylgalcosaminyltransferase; CRD, carbohydrate-recognition domain; Gal, galectin; LacNac, N-acetyllactosamine; MAA, Maackia amurensis agglutinin; mGal, mouse Gal; Mgt5, mannosyl (α1,6-) glycoprotein β1,6-N-acetylgalcosaminyltransferase; rGal, recombinant Gal; RT-QPCR, quantitative RT-PCR; shRNA, short hairpin RNA; ST6Gal1, α-2,6-sialyltransferase 1; YFP, yellow fluorescent protein.

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plasma cells and that Gal-1 and Gal-8 seem to selectively recognize ligands on mature B cells. These findings provide novel insights into how Gals collaboratively modulate immune responses.

Materials and Methods

Cell lines and reagents

Splenic B cells from 12- to 16-wk-old C57BL/6 mice (National Laboratory Animal Center, Taiwan) and from 

**Flow cytometry**

B cells were harvested, washed in PBS once, and then suspended at a density of 100 cells/ml in PBS with 2% FBS. A total of 10^6 cells was used for each staining experiment. The following Abs were used: PE-conjugated anti-mouse CD138/syndecan-1 (BD Pharmingen; clone 281-2) and allophycocyanin-conjugated anti-mouse CD45R/B220 (BD Pharmingen; clone RA3-6B2). Biotin-conjugated Lycopersicon esculentum and Sambucus nigra lectins were purchased from EY Laboratories. Biotin-conjugated Maackia amurensis agglutinin (MAA) lectin was purchased from Vector Laboratories. Strep-tavidin–FITC–labeled secondary Ab was purchased from SeroTech and used at a 1:500 dilution. Stained cells were analyzed using a FACSQuant (BD Biosciences), and the results were analyzed by FlowJo software (BD Biosciences). The protocol to detect the binding of Gal-Fc fusion proteins and FITC-labeled Gals was described previously (9). For experiments that involved neuraminidase treatment, splenic B cells, adjusted to a density of 2 × 10^7 cells/ml with reaction buffer (50 mM sodium acetate [pH 5.5], 100 mM NaCl, 1 mM CaCl_2, 100 μg/ml BSA), were treated with Salmonella typhimurium α2-3 neuraminidase (50 μU/ml; New England Biolabs), Clostridium perfringens α2-6 neuraminidase (50 μU/ml; New England Biolabs), or buffer alone at 37˚C for 15 min, followed by PBS washes and incubation with Gal-1–FITC or Gal-8–FITC. B cell proliferation determined by staining with CFSE (Sigma-Aldrich) was performed, as previously reported (9).

**ELISA and Multiplex bead array assays**

Cell supernatants were harvested, and an ELISA was used to determine the amount of IgM or Gal-1, as described (9). The amount of Gal-8 was determined using a Gal-8 ELISA kit (USCNLIFE). The amounts of IL-6 and IL-10 were determined using the MILLIPLEX mouse cytokine/chemokine kit (Millipore), as described previously (24).

Production of Gal-Fc fusion proteins and bacterially expressed recombinant Gal proteins

Gal-1-Fc and Gal-8-Fc fusion proteins were produced in a Free-Style 293 expression system (Invitrogen), as described (9). Recombinant Gal (rGal)-1 and Gal-8 were produced in a bacterial expression system, following reported methods (25). Briefly, full-length human Gal-1 or murine Gal-8 cDNA was ligated to a pET-15b expression vector (Invitrogen), the resultant vector was transformed into Escherichia coli BL21 (DE3; Novagen), and expression was induced by incubation with 1 mM isopropyl β-D-thiogalactoside for 4 h at 37˚C. Bacteria were then lysed with P-Per extraction buffer (Pierce) with 0.1 M PMSF and 8 μM DTT. rGal-1 and rGal-8 were purified by lactose-Sepharose affinity chromatography and eluted with 0.1 M β-lactose. The 28-kDa dimeric Gal-1 was further isolated by a Superdex 75 gel filtration column (GE Healthcare). The expression of rGal-1 and rGal-8 was examined by 12% SDS-PAGE, followed by Coomassie blue staining. Endotoxin in bacterially expressed Gal-1 and Gal-8 was removed by Endo Trap Endotoxin Removal System (Lonza), and the endotoxin-free Gal-1 and Gal-8 were confirmed with the Limulus amebocyte lysate test using a kit from Lonza. rGal-1 and rGal-8 were then labeled with FITC using a Lightning-Link R-Fluorescein conjugation kit (Innova Biosciences).

**Synthesis of LacNac analogs**

The procedures for synthesis of sulfated LacNacs were mainly described in a publication (Z. Tu, H.-W. Hsieh, C.-M. Tsai, S.-G. Wang, K.-J. Wu, K.-I. Lin, and C.-H. Lin, submitted for publication), in which the efficient synthesis of fully protected Galβ1-3/4GlcNAc disaccharides was carried out. Subsequent deprotection and sulfation steps resulted in eight non- or 1H and 13C nuclear magnetic resonance, optical rotation, and mass spectrometric analyses. The detailed protocol will be available upon request.

**Statistical analysis**

A two-sided Student t test was used for all statistical testing; p values <0.05 were considered statistically significant.

**Results**

**Gals are expressed differentially during plasma cell differentiation**

Because Gal-1 is induced during the differentiation of mouse and human plasma cells, and because it can promote Ig production...
in vitro (9), we first sought to determine whether other Gals were involved in this process. Stimulation of purified splenic B cells with LPS mimics T cell-independent plasma cell differentiation and is characterized by IgM production and surface expression of B220<sup>low</sup>CD138<sup>+</sup>. Among the Gal family genes examined, Gal-1 mRNA increased after stimulation of splenic B cells with LPS (Fig. 1A). The time course for the increase in Gal-1 mRNA expression paralleled the increase in Blimp-1 mRNA expression, in agreement with our previous work (9). Levels of Gal-2, Gal-4, and Gal-9 were not detectable in LPS-stimulated B cells (Fig. 1A). Gal-3 mRNA expression was low at day 0 and decreased further during differentiation (Fig. 1A). Gal-8 mRNA expression was high on day 0 and decreased significantly after LPS stimulation (Fig. 1A).

In a parallel study, flow cytometry was used to isolate day 0 B cells, B220<sup>high</sup>CD138<sup>−</sup> undifferentiated B cells, and B220<sup>low</sup>CD138<sup>+</sup> plasma cells from LPS-stimulated splenic B cell cultures. RT-QPCR and immunoblot analysis were conducted with the isolated cell populations. Levels of Blimp-1 mRNA were used to confirm the identities of the sorted cell populations (Fig. 1B). Gal-1 mRNA (Fig. 1B) and protein (Fig. 1C) increased during differentiation, with the highest levels found in plasma cells. Gal-3 expression was reduced in plasma cells (Fig. 1B, 1C). Gal-8 mRNA decreased during differentiation and was lowest in plasma cells (Fig. 1B), but Gal-8 protein increased during differentiation (Fig. 1C). The accumulation of secreted Gal-1 as well as Gal-8 proteins in differentiated plasma cells was confirmed by ELISAs (Fig. 1D).

**Gal-1 and Gal-8, but not Gal-3, promote plasma cell formation**

Because the expression of Gal-1, Gal-3, and Gal-8 was dynamically changed during the differentiation of plasma cells from B cells, we wondered whether Gal-3 and Gal-8, like Gal-1 (9), influence the formation of plasma cells. Primary B cells were transduced with bicistronic retroviral vectors encoding YFP and FLAG-tagged Gal-1, Gal-3, or Gal-8. Gal expression was confirmed by immunoblot analysis using an Ab against FLAG (Fig. 2A). Ectopic expression of either Gal-1 or Gal-8 in LPS-stimulated mouse splenic B cells resulted in an increase in B220<sup>low</sup>CD138<sup>+</sup> plasma cells, as compared with cells that expressed only YFP (Fig. 2B). Ectopic expression of Gal-3 did not increase the proportion of B220<sup>low</sup>CD138<sup>+</sup> plasma cells (Fig. 2B). The YFP<sup>+</sup> cells were also sorted and analyzed for levels of secreted IgM in culture. Ectopic expression of Gal-1 or Gal-8 significantly increased IgM secretion (Fig. 2C). Gal-3, however, did not alter IgM production (Fig. 2C). This effect of Gal-1 and Gal-8 could be due to an extracellular effect, as ectopically expressed Gal-1 and Gal-8 were both produced in culture media (Fig. 2D). Furthermore, sorted cells were used to examine the expression of plasma cell-associated genes in cells that overexpressed Gal-8, relative to Gal-1 or YFP alone. Ectopic expression of Gal-8 significantly increased the mRNA levels of Blimp-1 and the spliced form of XBP-1 (XBP-1s), genes that are crucial for plasma cell differentiation and Ab secretion (26), respectively, in comparison with cells that overexpressed Gal-1 or YFP (Fig. 2E). We also examined the ratio of the secreted form (μs) to the membrane form (μm) of μ-chain mRNA and found that, like Gal-1, Gal-8 was able to enhance the splicing of the μ-chain mRNA (Fig. 2F). These data reveal a new role for Gal-8 and its underlying molecular effects in promoting plasma cell formation.

**Gal-1 and Gal-8 bind more effectively to mature B cells than to plasma cells**

Because both Gal-1 and Gal-8 promoted the formation of plasma cells, we sought to determine their binding profile during differentiation. Bacterially expressed rGal-1 and rGal-8 were purified, monitored (Fig. 3A), and labeled with FITC. Gal-1-FITC and Gal-8-FITC bound to mature B cells, and binding was dependent on galactoside recognition, because coincubation with lactose, but not sucrose, blocked the binding of Gal-1-FITC and Gal-8-FITC.

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**FIGURE 1.** Gal expression during plasma cell differentiation. A, RT-QPCR was used to determine mRNA levels for the indicated Gals relative to the level of the internal control, mL32, at days 0, 1, and 3 after LPS (5 μg/ml) stimulation of splenic B cells. B, RT-QPCR was used to determine mRNA levels of the indicated Gals relative to levels of the internal control, mL32, in cell populations that were sorted on the basis of staining for B220 and CD138. A representative flow cytometry dot plot is shown. C, Immunoblot analysis was used to examine protein expression of Gal-1, Gal-3, and Gal-8 in sorted cell populations. Actin was used as the loading control. D, ELISA was used to determine levels of secreted mGal-1 and mGal-8 in day 3 culture supernatants from control (−) and LPS-stimulated (+) splenic B cells used in the assay presented in A. The results shown in A, B, and D are represented as the mean ± SD (n = 3). *p < 0.05, **p < 0.01, ***p < 0.005.
to the cells (Fig. 3B). We then examined the ability of Gal-1 to compete with Gal-8 for binding to mature B cells, and vice versa. Splenic B cells were incubated with lactose to remove the bound Gal and incubated with Gal-1-FITC along with 0 (mock), 1, 3, or 10 μM unlabeled Gal-8, followed by flow cytometric analysis of Gal-1-FITC binding. The binding of Gal-1-FITC to B cells was blocked by Gal-8 in a dose-dependent manner (Fig. 3C, left panel). In the reciprocal experiment, Gal-1 did not alter the binding of Gal-8-FITC to mature B cells (Fig. 3C, right panel). These data suggest that the available glycan ligands for Gal-1 and Gal-8 on mature B cells may, at least in part, differ in terms of their sequences, quantity, or spatial accessibility. As an alternative approach to examine Gal binding, we generated Gal-1-Fc and Gal-8-Fc fusion proteins and validated the expression (Fig. 3A), and then analyzed their binding to specific cell populations by flow cytometry. After stimulation of splenic B cells with LPS, Gal-8-Fc bound less effectively to B220-lowCD138⁺ plasma cells than to B220-highCD138⁺ B cells (Fig. 3D). This was also true for Gal-1-Fc binding (Fig. 3D). Similar phenomena were observed when FITC-labeled Gal-1 or Gal-8 was used for staining (Fig. 4C).

To further understand why the binding of Gal-1 and Gal-8 decreases during the differentiation of plasma cells. In agreement with these findings, we found that the binding of L. esculentum, a tomato lectin that has a high affinity for GlcNAc and poly-LacNac (34), was lower on B220-highCD138⁺ plasma cells than on B220-highCD138⁻ B cells (Fig. 4B). In addition, the binding of MAA lectin, which recognizes the α-2,3 sialic acid linkages (35), was undistinguishable between B220-highCD138⁺ plasma cells and B220-highCD138⁻ B cells (Fig. 4B). However, the binding of S. nigra, a lectin that preferentially recognizes α-2,6 sialic acid linkages (35), was only slightly increased in B220-highCD138⁺ plasma cells (Fig. 4B), which could be because ST6Gal1 preferentially used N-glycans as its substrates, producing only a partial
repertoire for *S. nigra* binding. Interestingly, it appears that the α2-6 sialic acid linkages on B220lowCD138+ plasma cells prevented the binding of Gal-1 and Gal-8, because treating differentiating splenic B cells with α2-3,6 neuraminidase, which removes sialic acids from oligosaccharides that are either α2-3 or α2-6 linked (data not shown), but not with α2-3 neuraminidase, restored the binding of Gal-1-FITC and Gal-8-FITC to B220low CD138+ plasma cells (Fig. 4C).

All together, these data show that Gal-1 and Gal-8 share a similar binding profile to B cells and plasma cells, and that there is an association between Gal-1 and Gal-8 binding and glycosyltransferase expression.

**FIGURE 4.** The expression profile of glycosyltransferases and lectin binding to plasma cells and B cells. A, RT-QPCR analysis of the expression of the indicated glycosyltransferase genes in LPS-stimulated splenic B cell subsets that were sorted on the basis of staining for B220 and CD138. Results were normalized to mL32 and are represented as the mean ± SD (n = 3). *p < 0.05. B, *L. esculentum* (LEL), MAA, and *S. nigra* (SNA) binding to splenic B cells treated with LPS (5 μg/ml) for 3 d. Cells were stained with B220, CD138, and plant lectins, followed by flow cytometric analysis. The gray histogram represents B cells that were not stained. C, Splenic B cells were incubated with lactose (100 mM) for 30 min, followed by PBS washes. Cells were then incubated with Gal-1-FITC (left panel) or Gal-8-FITC (right panel) along with the indicated doses of unlabeled Gal-8 (left panel) or Gal-1 (right panel) as competitors. Binding was subsequently examined by flow cytometric analysis. The control histogram represents cells that were not stained. D, Splenic B cells were treated with LPS (5 μg/ml) for 3 d and stained for B220, CD138, Gal-1-Fc, and Gal-8-Fc, and binding was examined by flow cytometric analysis. The bolded histogram line represents staining of plasma cells, and the thin histogram line represents mature B cells. The gray histogram represents cells stained with isotype control Ab, FITC-conjugated anti-human IgG, only.

**Gal-1 and Gal-8 have selective ligands**

We next wished to determine whether Gal-1 and Gal-8 recognize different glycans. A comparison of glycans that bind Gal-1 and Gal-8 based on data from the Web site of the Consortium for Functional Glycomics revealed that nearly half of the Gal-1–binding ligands also bind to Gal-8 (Supplemental Table 1). Interestingly, compounds that have sulfomodification at the C3 of galactose of type 2 LacNAc appear to bind to Gal-1 better than to Gal-8 (Supplemental Table 1). This observation prompted a study into the effects of various sulfated LacNAc derivatives on the binding of Gal-1 and Gal-8 to B cells. We used a panel of synthetic LacNAc analogs that...
contain sulfomodification at galactose or GlcNAc of type 1 or type 2 LacNAc (Fig. 5A). For instance, a compound that has a mono-
sulfate modification at the C3 of galactose (3’SII) of type 2 LacNAc was named 3’SII; a compound that has a monosulfate modification at the C6 of GlcNAc (6SII) of type 1 LacNAc was named 6SII.

Several type 2 LacNAc analogs, including II, 6SII, and 3’SII, blocked the binding of Gal-1 to purified splenic B cells in a concentration-dependent manner (Fig. 5B, left panel); among these analogs, 3’SII was most effective. In contrast, none of the type 2 LacNAc analogs altered the binding of Gal-8 to B cells (Fig. 5C, left panel). Type 1 LacNAc analogs, including I, 6SI, and 6’SII, were less effective than type 2 LacNAc analogs at blocking the binding of Gal-1 to B cells (Fig. 5B, right panel). Interestingly, some type 1 LacNAc analogs, including I, 6SI, and 3’SII, can partially block the binding of Gal-8 to B cells (Fig. 5C, right panel); 6SI was most effective at blocking Gal-8 binding. The quantitative data also revealed that 6SI was most effective at inhibiting Gal-8 binding (Supplemental Fig. 1).

Gal-1 promotes the stimulus-mediated induction of Ig (9). We showed that B cells from Gal-1 knockout (Lgals1−/−) mice

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**FIGURE 5.** The effect of type 1 LacNAc (I) or type 2 LacNAc (II) analogs on the binding of Gal-1 and Gal-8 to B cells. *A.* The chemical structures of different sulfomodified type 1 (I) or type 2 (II) LacNAcs. *B.* Flow cytometry was used to examine the binding profiles of splenic B cells stained with Gal-1-FITC and treated with the indicated non- or monosulfated II or I analogs at different doses. *C.* Flow cytometry was used to examine the binding profiles of splenic B cells stained with Gal-8-FITC and treated with the indicated non- or monosulfated II or I analogs at different doses. The control histogram represents cells that were not stained. Results in *B* and *C* are representative of at least three independent experiments.
IgM production by LPS-stimulated Lgal-8 and LPS-stimulated splenic B cells from cells transduced with lentiviral vectors expressing mGal-8 levels in splenic B cells. Treatment with I did not change, and treatment with 6SI only compared with the corresponding compound-treated, LPS-

FIGURE 6. Both type 1 LacNAc analogs and siRNA against Gal-8 blocked plasma cell formation in Lgals1+/− B cells. A, Flow cytometric analysis of CD138 and B220 expression on Lgals1+/+ or Lgals1−/− splenic B cells treated for 3 d with LPS and 0.1 mM of the indicated type 1 LacNAc analogs. B, ELISA was used to measure IgM levels in cell culture supernatants harvested from day 3 cultures, as described in A. C, ELISA was used to measure mGal-8 levels in splenic B cells transduced with lentiviral vectors expressing control or Gal-8 shRNAs. D, shRNA-transduced and LPS-stimulated splenic B cells from Lgals1+/+ or Lgals1−/− mice were sorted on day 3 according to the expression of GFP and then were cultured for additional 2 d. Cell culture supernatants were then harvested for analysis of IgM levels by ELISA. Results in B–D are shown as the mean ± SD (n = 3). *p < 0.05, **p < 0.01, ***p < 0.005.

We also examined the effects of both Gal-1 and Gal-8 that resulted from their extracellular functions. Interestingly, as compared with the exogenously added rGal-1, which induces the formation of Ab-secreting plasma cells (9), exogenously added rGal-8 was even more potent for promoting the generation of Ab-secreting plasma cells (Fig. 7A, 7B). Particularly, it is noteworthy that rGal-1 and rGal-8 had an additive effect in this setting (Fig. 7A, 7B). This extracellular effect of Gal-1 and/or Gal-8 was further supported by the efficient inhibition of plasma cell formation and IgM production when cells were cotreated with 6SI, which blocked the binding of both rGal-1 and rGal-8, but not with 6′SI (Fig. 7C, 7D).

Discussion

Gals have been suspected of having redundant roles in the regulation of diverse biological processes because all Gals recognize galactose, with the exception of Gal-11, which lacks several key residues in the CRD for sugar binding (36). In the current study, we used synthetic sulfated LacNAc analogs and shRNAs against Gal-8 to reveal a redundancy in the roles of Gal-1 and Gal-8 in the formation of plasma cells.

Our previous study revealed a glycan-dependent effect of Gal-1 on the promotion of plasma cell differentiation (9). Lgals1−/− B cells were, however, able to differentiate into plasma cells normally, suggesting the involvement of other Gals. Indeed, we demonstrated in this work that Gal-1, Gal-3, and Gal-8 were expressed by B cells. The mRNA level of Gal-8 decreased during plasma cell differentiation, but the protein level of Gal-8 accumulated in plasma cells. The accumulation of Gal-8 protein suggests that the protein stability of Gal-8 increases during differentiation. In support of this possibility are results showing that the length of the linker peptide regulates the stability of certain Gals (37). Gals having long linker peptides, such as the short form of Gal-8 and Gal-9, are more susceptible to thrombin cleavage, whereas Gals having short linker peptides, such as the short form of Gal-8 and Gal-9, are resistant to thrombin cleavage (37). The Gal-8 mRNA detected in our cultures
was primarily in the short form (data not shown); therefore, we propose that Gal-8 accumulates in plasma cells because the Gal-8 protein produced during plasma cell differentiation is relatively resistant to proteolysis.

Gal-8 acts as an extracellular modulator to induce apoptosis in thymocytes (38), and it acts as a matrix protein that promotes cell adhesion by clustering cell surface integrin receptors (39). In the current study, we observed that ectopic expression of Gal-8 as well as treatment with rGal-8 in B cells promoted the generation of plasma cells. Furthermore, certain LacNAc derivatives had a stronger affinity for Gal-8 than did others, and shRNA against Gal-8 selectively diminished plasma cell production by \( Lgals1^{−/−} \) B cells, indicating that both Gal-1 and Gal-8 are involved in plasma cell formation. Indeed, both Gals were able to activate splenic B cell proliferation (Supplemental Fig. 3A), a crucial event prior to efficient plasma cell differentiation, and induce the production of IL-10 (Supplemental Fig. 3B), a plasma cell differentiation factor. Although both Gals are redundant in plasma cell formation, we speculated that each Gal may stimulate at least partly distinct signaling pathways because we observed that Gal-8, but not Gal-1, was able to induce Blimp-1 mRNA, and that Gal-8, but not Gal-1, significantly induced the production of plasma cell proliferative factor, IL-6, in this setting (Supplemental Fig. 3B). It will be important to further dissect the signaling pathways initiated by Gal-1 or Gal-8. Nevertheless, the redundancy of Gal-1 and Gal-8 seems to be unique to B cells because Gal-1 acts in T cells to modulate the balance between Th1, Th17, and Th2 immune responses. Indeed, \( Lgals1^{−/−} \) mice show exaggerated Th1 and Th17 responses after Ag challenge in vivo (35).

Our study showed that Gal-8 competed with Gal-1 for binding to mature B cell surfaces, but Gal-1 did not compete with Gal-8 for binding. It is unclear how Gal-8 ligands on mature B cell surfaces overlap with Gal-1 ligands. The glycan ligands recognized by Gal-8 have been determined by a glycan microarray (40). Gal-8 recognizes sulfated glycans, sialylated glycans, and poly-LacNAc structures. Notably, the N-terminal CRD of Gal-8 can bind to type 1 LacNAc, but the C-terminal CRD of Gal-8 has a higher binding affinity for poly-LacNAc structures (40). The differential biological activities of the N- versus C-terminal CRD of Gal-8 have been shown (41), including in killing \( E. coli \) strains (42), in activating platelets (43), and in modulating neutrophil function (44); however, which CRD of Gal-8 is responsible for its positive role in plasma cell formation remains to be determined. In the current study, sulfomodified Gals or GlcNAc on type 1 or type 2 LacNAcs were used as competitors to elucidate the binding ligands for Gal-1 and Gal-8 on mature B cell surfaces. Gal-1 had a strong binding affinity for type 2 and sulfated type 2 (6SII and 3’SII) LacNAcs, but less for type 1 LacNAcs. Gal-8 had a stronger binding affinity for the type 1 LacNAcs, 6SII and 3’SII. B cells express \( β_1,3\)-galactosyltransferase (45, 46), which is involved in the synthesis of the \( β_1,3 \) linkage structure of the type 1 chain (47). Whether the B cell surface expresses type 1 LacNAc or sulfated type 1 LacNAc

![FIGURE 7](http://www.jimmunol.org/)
remains to be examined. Nevertheless, our cell-based assays revealed ligands that may be preferentially recognized by Gal-1 and Gal-8. Further identification of the natural glycan ligands for Gal-1 and Gal-8 on B cells will be important to fully understand their mode of action in plasma cell differentiation.

Like Gal-1, Gal-8 had a stronger binding affinity for mature B cells than for plasma cells. One possible explanation for why Gal-1 and Gal-8 did not bind as strongly to plasma cells is related to a reduction in overall glycans and an increase in α2-6 sialic acid linkages in plasma cells. In fact, L. esculentum lectin showed decreased binding to plasma cells, suggesting that longer and more complex glycan structures might be expressed on activated mature B cells than on plasma cells. Our data also showed that Mgtα5 and C2GnT mRNA levels were significantly decreased in plasma cells, which supports the idea that expression of glycan structures for Gals binding was generally reduced. Furthermore, our observation that α2-3 neuraminidase, treatment restored the binding of Gal-1 and Gal-8 to plasma cells is also in agreement with the notion that α6-sialylated terminal LacNAc prevents Gal binding (14, 17). A second explanation is that protein receptors for Gal-1 and Gal-8 might be also downregulated during plasma cell development. In T cells, the protein receptors for Gal-1 include CD45, CD43, and CD7 (48), and interactions between Gal-1 and those receptors are associated with the expression of C2GnT and Mgtα5 (35). Gal-8 interacts with CD44 on synovial fluid cells to regulate autoimmune inflammation in rheumatoid arthritis (49). In addition, interaction of Gal-8 by Gal-3 was found to promote immunoglobulin production during plasma cell differentiation. J. Immunol. 181: 4570–4579.


