Human B-lymphocytes Express α2–6-Sialylated 6-Sulfo-N-acetyllactosamine Serving as a Preferred Ligand for CD22/Siglec-2*

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CD22/Siglec-2, an important inhibitory co-receptor on B-lymphocytes, is known to recognize α2–6-sialylated glycan as a specific ligand. Here we propose that the α2–6-sialylated and 6-GlcNAc-sulfated determinant serves as a preferred ligand for CD22 because the binding of a human B-cell line to CD22 was almost completely abrogated after incubating the cells with NaClO₃, an inhibitor of cellular sulfate metabolism, and was also significantly inhibited by a newly generated monoclonal antibody specific to the α2–6-sialylated 6-sulfo-N-acetyllactosamine (LacNAc) determinant (KN343, murine IgM). The α2–6-sialylated 6-sulfo-LacNAc determinant defined by the antibody was significantly expressed on a majority of normal human peripheral B-lymphocytes as well as follicular B-lymphocytes in peripheral lymph nodes. The determinant was also expressed in endothelial cells of high endothelial venules of secondary lymphoid tissues, including lymph nodes, tonsils, and intestine-associated lymphoid tissues, more strongly than on B-lymphocytes, suggesting a role for CD22 in B-cell interaction with blood vessels and trafficking. These results indicate that the α2–6-sialylated 6-sulfo-LacNAc determinant serves as an endogenous ligand for human CD22 and suggest the possibility that 6-GlcNAc sulfation as well as α2–6-sialylation may regulate CD22/Siglec-2 functions in humans.

CD22/Siglec-2 (sialic acid-binding immunoglobulin-like lectins) is an important inhibitory receptor on B-lymphocytes. It controls the signaling threshold of B-cell receptors, preventing their overactivation (1–4). It has inhibitory immunoreceptor tyrosine-based inhibitory motifs in its cytoplasmic domain and regulates B-cell receptor signaling by recruiting the tyrosine phosphatase SHP-1 (Src homology 2 domain-containing protein-tyrosine phosphatase-1) (5). It is known to also affect distribution and trafficking of B-lymphocytes, such as in the homing of IgD⁺ B-lymphocytes to the bone marrow (6). Disruption of CD22 is known to result in production of high-affinity autoantibodies, an increase in follicular mature B-lymphocytes, and a reduction in the number of marginal zone B-lymphocytes (7, 8).

CD22/Siglec-2 is known to specifically bind to its ligand, α2–6-sialylated glycan (9–12). B-lymphocytes themselves significantly express α2–6-sialylated glycan on their surface, which can serve as a cis-ligand for endogenous CD22, thus masking the ligand binding activity of CD22 on the majority of B-lymphocytes. CD22/Siglec-2 is proposed to exert trans-interaction with target cells expressing α2–6-sialylated glycan only when expression of the endogenous cis-ligand is suppressed or when the trans-ligand on target cells has a significantly higher binding activity than the cis-ligand (13, 14). For better understanding of CD22 function, it is important to know the diversity of α2–6-sialylated glycan and to find candidate glycans that preferentially bind to CD22.

In mice, it has long been known that CD22 prefers the α2–6-N-glycolylsialic acid terminus over the α2–6-N-acetylsialic acid terminus (15). Quite recently, it was proposed that the activity of CMP-NeuAc hydroxylase, which converts N-acetylsialic acid into N-glycolylsialic acid, plays a role in regulating CD22 activity (16). Another proposed candidate had been 9-O-acetylation of terminal sialic acid (17). Little is known in humans, however, about the heterogeneity of α2–6-sialylated glycans in terms of CD22 binding activity.

It has been reported that an organochemically synthesized α2–6-sialylated and 6-sulfated glycan serves as a good ligand, eventually better than simply α2–6-sialylated determinants, for recombinant human CD22-Fc in an in vitro assay system (18). Our recent preliminary experiments indicated that suppression of carbohydrate sulfation abrogates binding of cul-
asured human B-lymphoid cells to immobilized CD22 in a cellbased assay system (see “Results”). These findings collectively raised the possibility that some sulfated determinants also serve as preferred ligands for human CD22 at the cellular level. To address this issue, we tried to clarify the role of sulfation in the carbohydrate ligands for CD22 using a newly generated monoclonal antibody specific to the α2–6-sialylated 6-sulfo-N-acetyllactosamine (LacNAC)2 determinant.

EXPERIMENTAL PROCEDURES

Cells, Antibodies, and Reagents—A cultured human colon cancer cell line (SW480) and B-lymphoid cell line (Daudi) were obtained from the Tohoku University Cell Resource Center for Biomedical Research (Sendai, Japan). A human B-lymphoid cell clone derived from Namalwa cells, selected for high expression of 6-sulfated carbohydrate determinants and transfected with a gene for fucosyltransferase VII, was prepared as described previously (19).

Antibodies G152 and G72 (both murine IgM) directed against α2–3-sialylated and 6-sulfated glycans were prepared as described previously (20, 21). Antibody GL7 (rat IgM) specific to the nonsulfated α2–6-sialylated LacNAC determinant was obtained from ebioscience (San Diego, CA) (16, 22).

For generation of antibodies specific to the α2–6-sialylated 6-sulfo-LacNAC determinant, SW480 cells were transfected with expression vector pIREsneo containing the gene for human high endothelial cell GlcNAc-6-sulfotransferase (HEC-GlcNAC6ST), a GlcNACβ6-O-sulfotransferase (23), using Lipofectamine 2000 (Invitrogen) and screened in culture medium containing 400 μg/ml G418. BALB/c mice were intraperitoneally immunized with 2 × 107 SW480/HEC-GlcNAC6ST cells twice at 2-week intervals, and 3 days after the final immunization, and splenic cells were fused with mouse myeloma P3/X63-Ag8U1 according to the method described by Köhler and Milstein (24).

Recombinant sialidase S from Streptococcus pneumoniae specific to the α2–3-linked sialic acid terminus was obtained from Glyko Inc. (San Leandro, CA), and sialidase A from Arthrobacter ureafaciens, which cleaves the α2–3/6/8/9-linked sialic acid terminus, was obtained from Nacalai Tesque (Kyoto, Japan). The recombinant α2–6-sialyltransferase from Photobacterium damselae JT0160 and the α2–3-sialyltransferase from P. damselae JT-ISH-467 (used to recover α2–6- and α2–3-linked sialic acid termini on the sialidase A-treated cells) were obtained from JT Plant Innovation Center (Iwata, Japan).

Cells (1 × 106) were first fixed for 5 min in 0.5% paraformaldehyde and then sialidase A-treated for 1 h. After washings, the cells were reacted for 1 h at 30 °C in 1.0 ml of incubation mixture (pH 6.0) containing 10 μl of CMP-NeuAc (50 mg/ml; Sigma catalog no. C8271), 2 μl of alkaline phosphatase (35,106 units/ml; Calbiochem catalog no. 524572), 1 μl of α2–6-sialyltransferase (10 units/ml), or 1.5 μl of α2–3-sialyltransferase (7.5 units/ml).

2 The abbreviations used are: LacNAC, N-acetyllactosamine (Galβ1→4GlcNAc); HEC-GlcNAC6ST, high endothelial cell GlcNAC-6-sulfotransferase; ELISA, enzyme-linked immunosorbert assay; HEVs, high endothelial venules.

Enzyme-linked Immunosorbert Assay (ELISA) and Preparation of Pure Carbohydrate Determinants—ELISA was performed using synthetic carbohydrate determinants immobilized on the bottom of 96-well culture plates by a standard method described previously (20, 25, 26). Serial dilution of immobilized carbohydrate determinants started from 20 ng/well. Peroxidase-conjugated rabbit anti-rat IgM (μ-chain specific; Zymed Laboratories Inc., South San Francisco, CA) was used for GL7, and peroxidase-conjugated goat anti-mouse IgM (μ-chain specific; Cappel Laboratories, Malvern, PA) was used for other antibodies. The pure synthetic α2–6-sialylated 6-sulfo-LacNAC determinant was synthesized by established methods from three building blocks, a sialic acid donor (27), a lactosamine donor (28), and lactosyl cholestanol with a free hydroxyl residue at C–3 (29). Lactosyl cholestanol was coupled with the lactosamine donor using a catalytic amount of bis(cyclopentadienyl)hafnium(IV) dichloride-silver triflate (Cp2HfCl2-AgOTf) according to the method of Matsumoto et al. (30). The sialyl chloride donor was coupled to this by the procedure described by Kuhn et al. (27) and Helferich (31). The protective group at C-6 of GlcNAC was selectively removed and sulfated, followed by removal of all other protective groups, which gave the desired compound NeuAcα2–6Galβ1–4GlcNAcβ6-O-sulfate)1–3Galβ1–4Glc-cholesterol: 500-MHz 1H NMR (CDCl3/CD3OD/D2O; δ = 3.13; tetramethylsilane), δ = 2.023 (s, 3H, NAc), 2.032 (s, 3H, NAc), 2.710 (dd, 1H, J1a, J1b = 8.1, 9.0 Hz), 4.133 (d, 1H, J1, J3eq = 1.7 Hz), 4.244 (dd, 1H, J1, J3eq = 5.4 Hz, J3eq,6 = 11.0 Hz, H-3eeq), 3.250 (dd, 1H, J1, J3eq = 7.8 Hz, H-1a, H-1b, H-1c, or H-1d), 4.450 (d, 1H, J1, J3eq = 7.8 Hz, H-1a, H-1b, H-1c, or H-1d), and 4.740 (d, 1H, J1, J3eq = 7.8 Hz, H-1a, H-1b, H-1c, or H-1d). An isomeric compound, NeuAca2–3Galβ1–4GlcNAcβ6-O-sulfate)1–3Galβ1–4Glc-cholesterol, was synthesized by similar methods, and the non-sialylated compound, Galβ1–4GlcNAcβ6-O-sulfate)1–3Galβ1–4Glc-cholesterol, was prepared by sialidase A treatment. Synthetic sialyl paragloboside with an α2–6-sialic acid terminus (NeuAca2–6Galβ1–4GlcNAcβ1–3Galβ1–4Glc- ceramide) was obtained from Wako Pure Chemicals (Osaka, Japan).

CD22-mediated Cell Binding Assays—Recombinant human CD22-Fc was obtained from R&D Systems (Minneapolis, MN) and coated on the bottom of 24-well plates at a concentration of 20 μg/ml overnight at 4 °C. 2′,7′-bis(2-carboxyethyl)-5-(6-carboxyfluorescein) acetoxyethyl ester (BCECF-AM)-labeled Namalwa cells (1 × 106/0.5 ml/well) were added, and the plate was placed on a rotating platform for incubation under shear (90 rpm) for 20 min at room temperature. Where indicated, the cells were preincubated with the inhibitor antibody (25 μg/ml) before addition to 24-well plates. After non-binding cells were washed out three times with phosphate-buffered saline, they were lysed with 0.5% Nonidet P-40, and the attached cells were counted by measuring fluorescence intensity using an Arvo 1420 multilabel counter (Wallac, Gaithersburg, MD). Recombinant human E-selectin-Fc (R&D Systems) served as a control for sulfate-independent binding.

Monolayer cell adhesion experiments were performed as described previously (26, 32, 33). SW480/HEC-GlcNAC6ST cells (expressing the α2–6-sialylated 6-sulfo-LacNAC determi-
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RESULTS

Generation of Anti-α2–6-Sialylated 6-Sulfo-LacNAc Monoclonal Antibody KN343—As we did not have purified or synthetic α2–6-sialylated 6-sulfo-LacNAc for use as an immunogen for generation of monoclonal antibody at the initial stage of this study, we used human colon cancer cells (SW480) transfected with the gene for the GlcNAcβ6-6-sulfotransferase HEC-GlcNAc6ST (SW480/HEC-GlcNAc6ST cells) as an immunogen. The obtained hybridoma clones were initially screened for their reactivity with SW480/HEC-GlcNAc6ST cells, but not with parental SW480 cells, to ascertain their 6-sulfation dependence (Fig. 1A). Antibodies G152 and G72, which we previously raised against 6-sulfated determinants carrying α2–3-sialylated termini, served as positive controls. The 6-sulfation-dependent clones were further subjected to a second screening using SW480/HEC-GlcNAc6ST cells treated with sialidase S (specific to the NeuAcα2–3 terminus) or sialidase A (cleaves NeuAcα2–3/6/8 termini). A KN343 (murine IgM) clone was selected because it reacted with sialidase S-treated cells but not with sialidase A-treated cells, which is compatible with its specificity for the NeuAcα2–6-linked terminus (Fig. 1B). Antibodies G152 and G72, which are specific to the 6-sulfated determinants carrying NeuAcα2–3-linked termini, which should not react with either sialidase S- or sialidase A-treated cells, served as controls for the experimental conditions.

To ascertain the specificity of KN343 for the NeuAcα2–6-linked terminus, the cells were reacted with 2–6-sialyltransferase plus CMP-NeuAc after the sialidase A treatment. Antibody KN343 specifically reacted with the cells treated with 2–6-sialyltransferase plus CMP-NeuAc, but not with the those treated with 2–3-sialyltransferase plus CMP-NeuAc (Fig. 1B, lower two panels), indicating its specificity for the NeuAcα2–6-linked terminus. In contrast, antibody G152, which is specific to 6-sulfated determinants carrying NeuAcα2–3-linked termini, reacted only with the cells treated with α2–3-sialyltransferase plus CMP-NeuAc, but not with those treated with α2–6-sialyltransferase plus CMP-NeuAc.

To ascertain the specificity of antibody KN343 for α2–6-sialylated 6-sulfated determinants, its reactivity was tested using organochemically synthesized pure carbohydrate determinants in ELISA. As shown in Fig. 1C, the antibody specifically reacted with the pure α2–6-sialylated 6-sulfo-LacNAc determinant, but hardly at all with the non-sulfated α2–6-sialylated LacNAc determinant. At high concentrations, only a weak cross-reactivity was noted with the non-sulfated α2–6-sialylated determinant. This was in clear contrast to the specificity of antibody GL7, which was shown quite recently to be specific to the α2–6-sialylated determinant (16), reacting only with the non-sulfated α2–6-sialylated determinant and not with the α2–6-sialylated 6-sulfated determinant (Fig. 1C).

These findings established that KN343 is a specific antibody for the α2–6-sialylated 6-sulfo LacNAc determinant at both the ELISA and cellular levels. Sulfated determinants often give false-positive results in ELISAs because of their strong negative electric charges, and it should be borne in mind that this antibody shows the same specificity also at the cellular level.
Roles of Sulfate Residues in Carbohydrate Recognition by Recombinant CD22—A clone of Namalwa cells was found to most strongly express the \(\alpha_2-6\)-sialylated 6-sulfotransferase (HEC-GlcNAc6ST), but not with parental SW480 cells. Antibody G152, which is specific to the \(\alpha_2-3\)-sialylated 6-sulfated Lewis X determinant (20), served as a control. B, results of flow cytometry indicating that antibody KN343 recognizes cell-surface \(\alpha_2-6\)-sialylated determinants. The reactivity of antibody KN343 with a clone of Namalwa cells was lost after sialidase A treatment, but not after sialidase S treatment. Recovery of the reactivity was observed when the sialidase A-treated cells were incubated with \(\alpha_2-6\)-sialyltransferase, but not with \(\alpha_2-3\)-sialyltransferase. Antibody G152 again served as a control; its reactivity was lost after either sialidase A or S treatment and was recovered only when the sialidase-treated cells were incubated with \(\alpha_2-3\)-sialyltransferase. C, results of ELISA using pure carbohydrate determinants indicating glycan specificity of antibodies KN343 (upper panel) and GL7 (lower panel). Antibody KN343 specifically reacted with the \(\alpha_2-6\)-sialylated 6-sulfated LacNAc determinant, but not with other determinants. Antibody GL7, which is known to be reactive with non-sulfated \(\alpha_2-6\)-sialyl-LacNAc determinants (16), served as a control.

Roles of Sulfate Residues in Cell-Cell Adhesion Mediated by CD22—The roles of sulfate residues in glycan recognition by CD22 were further ascertained by monolayer cell adhesion assays. For this, Daudi cells strongly expressing CD22 (Fig. 3A) were employed for the adhesion experiments with parental SW480 or SW480/HEC-GlcNAc6ST cells. The sialidase-treated Daudi cells exhibited significant adhesion to SW480/HEC-GlcNAc6ST cells, which express the \(\alpha_2-6\)-sialylated 6-sulfated determinant, but did not adhere to parental SW480 cells, which do not express the determinant (Fig. 3B). The adhesion of Daudi cells was abrogated by sialidase or NaClO₃ treatment of SW480/HEC-GlcNAc6ST cells (Fig. 3B) or by the addition of antibody KN343 (Fig. 3C). Antibody GL7 exhibited only marginal inhibition of cell adhesion, suggesting the important role of sulfated residues in the adhesion.

It has been proposed that endogenous CD22 ligands mask the interaction of CD22 with \(\text{trans}-\)ligands (13, 35). As Daudi cells strongly express non-sulfated \(\alpha_2-6\)-sialylated determinants defined by GL7 as well as CD22 but only weakly express the \(\alpha_2-6\)-sialylated 6-sulfated determinants defined by KN343 (Fig. 3A), the cells were thought to be suitable for testing the role of endogenous sulfated ligands of CD22 on its interaction with \(\text{trans}-\)ligands expressed in SW480/HEC-GlcNAc6ST cells. As expected, sialidase treatment of Daudi...
cells markedly enhanced binding of the cells to SW480/HEC-GlcNAc6ST cells. The treatment of Daudi cells with 25 mM NaClO3 for 5 days was found to confer a similar enhancement of binding to SW480/HEC-GlcNAc6ST cells (Fig. 3D). The results obtained with parental SW480 cells lacking the determinant served as the control. Pretreatment of SW480/HEC-GlcNAc6ST cells included sialidase A treatment or a 7-day culture in the presence of 40 mM NaClO3 where indicated. C, inhibitory effects of antibodies on the adhesion of sialidase-treated Daudi cells to SW480/HEC-GlcNAc6ST cells. Blocking antibodies were used at a concentration of 20 μg/ml. D, effects of pretreatments of endogenous CD22 ligands in Daudi cells on their adhesion to SW480/HEC-GlcNAc6ST cells. Pretreatment of Daudi cells to modify their endogenous CD22 ligands included sialidase A treatment or a 5-day culture in the presence of 25 mM NaClO3 where indicated.

Distribution of the α2–6-Sialylated 6-Sulfo-LacNAc Determinant in Peripheral Blood Leukocytes—The α2–6-sialylated 6-sulfo-LacNAc determinant defined by antibody KN343 was expressed in subpopulations of lymphocytes, but was virtually undetectable in granulocytes and monocytes among normal human peripheral leukocytes (Fig. 4A). Granulocytes are known to express conventional non-sulfated α2–6-sialyl-LacNAc determinants (36), but our results suggest that they lack sulfated α2–6-sialyl determinants.

Among lymphocyte subsets (Fig. 4B), the α2–6-sialylated 6-sulfo-LacNAc determinant was significantly expressed in CD19+ B-lymphocytes (53 ± 78%) and CD56+ natural killer
cells (41 ~ 70%), but only weakly in CD3+ T-lymphocytes (4 ~ 6%). This was in clear contrast to the distribution of the α2–3-sialyl-6-sulfated determinants we characterized previously (34), which are preferentially expressed in CD3+CD4+CD45RO+ helper memory T-lymphocytes and CD56+ natural killer cells, but virtually never in CD19+B-lymphocytes. The non-sulfated α2–6-sialyl-LacNAc determinant defined by antibody GL7 was homogeneously expressed in virtually all CD19+B-lymphocytes.

Recombinant CD22-Fc was found to bind to CD19+B-lymphocytes. The binding was significantly inhibited by the addition of antibody KN343, but was hardly inhibited by antibody GL7 (Fig. 4C). The inhibitory effect of the simultaneous addition of antibodies KN343 and GL7 was not much different from that exerted by antibody KN343 alone (Fig. 4C). These findings again corroborate the importance of the α2–6-sialylated 6-sulfo-LacNAc determinant as the ligand for CD22.

Distribution of the α2–6-Sialylated 6-Sulfo-LacNAc Determinant in Human Lymphoid Tissues—In peripheral lymph nodes, the α2–6-sialylated 6-sulfo-LacNAc determinant was expressed in follicular B-lymphocytes (Fig. 5). The distribution pattern was similar to that of non-sulfated α2–6-sialyl determinants defined by GL7 (Fig. 5, A and B). The difference between sulfated and non-sulfated determinants was that the germinal center B-lymphocytes almost completely lacked the sulfated determinants (Fig. 5A), but weakly expressed the non-sulfated determinants (Fig. 5B).

The most striking difference between sulfated and non-sulfated α2–6-sialyl determinants in peripheral lymphoid tissues was that the sulfated determinants were strongly expressed in high endothelial venules (HEVs), whereas the non-sulfated determinants were not detectable (Fig. 5, A and B). HEVs in tonsils as well as in intestine-associated lymphoid tissues also strongly expressed the α2–6-sialylated 6-sulfo-LacNAc deter-
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manifest (Fig. 5, C and D), whereas blood vessels in the spleen and thymus did not express the determinants (E and F).

DISCUSSION

Human CD22/Siglec-2 has been known to specifically recognize α2–6-sialylated glycans. The reactivity of CD22 with α2–6-sialylated and 6-sulfated glycans was first noted in the printed covalent glycan microarray technique using synthetic oligosaccharide and recombinant CD22-Fc protein by Blixt et al. (18). Our results indicate that α2–6-sialylated 6-sulfated LacNAc determinants are really expressed in situ on human B-lymphocytes in peripheral blood as well as in lymphoid tissues such as peripheral lymph nodes and can confer significant binding of CD22 at the cellular level.

Previous glycan array assays (18) indicated that additional 6-GlcNAc sulfation of α2–6-sialylated glycan enhances CD22 binding relative to the corresponding non-sulfated glycan and suggested that α2–6-sialylated and 6-sulfated LacNAc glycan could be a preferred high affinity ligand for human CD22 (10, 18). In line with this, our results indicate the importance of 6-GlcNAc sulfation because NaClO4 treatment of the cells, which resulted in loss of 6-GlcNAc sulfation but retained α2–6-sialylation, almost totally abrogated binding to CD22. The strong inhibition of CD22 binding with antibody specific to the α2–6-sialylated 6-sulfated LacNAc determinant also serves to corroborate that additional 6-GlcNAc sulfation significantly enhances CD22 binding.

Endogenous CD22/Siglec-2 ligands present on B-lymphocytes are known to be α2–6-sialylated glycans, which mask the CD22 interaction with trans-ligands. Our results suggest that the α2–6-sialylated 6-sulfated LacNAc determinant can also serve as a cis-ligand for endogenous CD22 and participate in the masking. It has long been assumed that α2–6 sialyltransferases such as β-galactoside α2–6 sialyltransferase-1 (ST6Gal-1) regulate expression of CD22 ligands and therefore regulate the masking and unmasking of CD22/Siglec-2. Recently, however, it was shown that N-glycolylation of sialic acid by CMP-NeuAc hydroxylase significantly affects CD22 ligand activity in murine system, as glycans carrying NeuGcα2–6 termini are much preferred ligands for murine CD22 compared with glycans carrying NeuAcα2–6 termini (16). Because humans have no NeuGc due to deletion of exons in the CMP-NeuAc hydroxylase gene (37, 38), this regulatory mechanism is not applicable to humans. Instead, our results suggest a role for 6-O-sulfotransferases, which are known to be affected by various stimuli and depend on cellular activation status (34, 39–41), in the regulation of CD22 ligand activity, as well as α2–6-sialyltransferases. An unexpected finding of this study is that the α2–6-sialylated and 6-sulfated LacNAc determinant is strongly expressed in HEVs in lymphoid tissues, including peripheral lymph nodes, tonsils, and intestine-associated lymphoid tissues. Endothelial expression of the determinant seems to be HEV-specific because endothelial cells lining the blood vessels in the spleen and thymus did not express the determinant. Expression of the determinant in HEVs was much stronger than that in B-lymphocytes, judging from the results of immunohistochemical staining. This is not unusual given that 6-O-sulfotransferases HEC-GlcNAc6ST and GlcNAc6ST-1 are strongly expressed in HEVs (23, 42) and synthesize the α2–3-sialylated 6-sulfo-Lewis X determinant, which serves as a specific ligand for L-selectin (19, 20, 33, 43, 44). Our results thus suggest that these two 6-O-sulfotransferases also synthesize the α2–6-sialylated 6-sulfo-LacNAc determinant in HEVs. The physiological functions for the endothelial CD22 ligands remain to be clarified. It has long been suggested that CD22 may be involved in B-lymphocyte trafficking and homing, including that to the bone marrow (6, 35). The reduced number of B-lymphocytes in peripheral lymph nodes was noted in 6-O-sulfotransferase-deficient mice (44). The strong expression of the CD22 ligand α2–6-sialylated 6-sulfated LacNAc determinant in HEVs suggests its involvement in the homing and recruitment of peripheral B-lymphocytes.

REFERENCES

1. Nitschke, L. (2005) Curr. Opin. Immunol. 17, 290–297
2. Nitschke, L., and Tsubata, T. (2004) Trends Immunol. 25, 543–550
3. Sato, M., Adachi, T., and Tsubata, T. (2007) J. Immunol. 178, 2901–2907
4. Wakabayashi, C., Adachi, T., Wienands, J., and Tsubata, T. (2002) Science 298, 2392–2395
5. Doody, G. M., Justement, L. B., Delibrias, C. C., Matthews, R. J., Lin, J., Thomas, M. L., and Fearon, D. T. (1995) Science 269, 242–244
6. Nitschke, L., Lloyd, H., Ferguson, D. J., and Crocker, P. R. (1999) J. Exp. Med. 189, 1513–1518
7. O’Keefe, T. L., Williams, G. T., Davies, S. L., and Neuberger, M. S. (1996) Science 274, 798–801
8. O’Keefe, T. L., Williams, G. T., Batista, F. D., and Neuberger, M. S. (1999) J. Exp. Med. 189, 1307–1313
9. Varki, A., and Angata, T. (2006) Glycobiology 16, 1R–27R
10. Crocker, P. R. (2005) Curr. Opin. Pharmacol. 5, 431–437
11. Grewal, P. K., Boten, M., Ramirez, K., Collins, B. E., Saito, A., Green, R. S., Ohtsuki, K., Chui, D., and Marsh, J. D. (2006) Mol. Cell. Biol. 26, 4970–4981
12. Powell, L. D., Sgroi, D., Sjoberg, E. R., Stamenkovic, I., and Varki, A. (1993) J. Biol. Chem. 268, 7019–7027
13. Razi, N., and Varki, A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7469–7474
14. Collins, B. E., Blixt, O., Han, S., Duong, B., Li, H., Nathan, J. K., Bovin, N., and Paulson, J. C. (2006) J. Immunol. 177, 2994–3003
15. Van der Merwe, P. A., Crocker, P. R., Vinson, M., Barclay, A. N., Schauer, R., and Kelm, S. (1996) J. Biol. Chem. 271, 9273–9280
16. Naito, Y., Takematsu, H., Koyama, S., Miyake, S., Yamamoto, H., Fujinawa, R., Sugai, M., Okuno, Y., Tsujimoto, G., Yama, T., Hashimoto, Y., Itohara, S., Kawasaki, T., Suzuki, A., and Kozutsumi, Y. (2007) Mol. Cell. Biol. 27, 3008–3022
17. Sjoberg, E. R., Powell, L. D., Klein, A., and Varki, A. (1994) J. Cell Biol. 126, 549–562
18. Blixt, O., Head, S., Mondala, T., Scanlan, C., Huflit, M. E., Alvarez, R., Bryan, M. C., Fazio, F., Calareso, D., Stevens, J., Razi, N., Stevens, D. I., Skelhel, J. I., van Die, I., Burton, D. R., Wilson, I. A., Cummings, R., Bovin, N., Wong, C. H., and Paulson, J. C. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 17033–17038
19. Ohmori, K., Kanda, K., Mitsuoka, C., Kanamori, A., Kurata-Miura, K., Sasaki, K., Nishi, T., Tamatani, T., and Kannagi, R. (2000) Biochem. Biophys. Res. Commun. 278, 90–96
20. Mitsuoka, C., Sawada-Kasugai, M., Ando-Furui, K., Izawa, M., Nakanishi, H., Nakamura, S., Ishida, H., Kiso, M., and Kannagi, R. (1998) J. Biol. Chem. 273, 11225–11233
21. Uchimura, K., Muramatsu, H., Kadomatsu, K., Fan, Q. W., Kurosawa, N., Mitsuoka, C., Kannagi, R., Habuchi, O., and Muramatsu, T. (1998) J. Biol. Chem. 273, 22577–22583
22. Laszlo, G., Hatuock, K. S., Dickler, H. B., and Hodes, R. J. (1993) J. Immunol. 150, 5252–5262
23. Bistrup, A., Bhaktar, S., Lee, J. K., Below, Y. Y., Gunn, M. D., Zuo, F. R., Huang, C. C., Kannagi, R., Rosen, S. D., and Hemmerich, S. (1999) J. Cell Mol. Biol. 225, 22577–22583
24. Köhler, G., and Milstein, C. (1975) Nature 256, 495–497
25. Kannagi, R. (2000) Methods Enzymol. 312, 160–179
26. Miyazaki, K., Ohmori, K., Izawa, M., Koike, T., Kumamoto, K., Furukawa, K., Ando, T., Kiso, M., Yamaji, T., Hashimoto, Y., Suzuki, A., Yoshida, A., Takeuchi, M., and Kannagi, R. (2004) Cancer Res. 64, 4498–4505
27. Kuhn, R., Lutz, P., and MacDonald, D. L. (1966) Chem. Ber. 99, 611–617
28. Rosenbrook, J. W., Riley, D. A., and Larkey, P. A. (1985) Tetrahedron Lett. 26, 3–4
29. Sato, S., Nunomura, S., Nakano, T., Ito, Y., and Ogawa, T. (1988) Tetrahedron Lett. 29, 4097–4100
30. Matsumoto, T., Maeta, H., Suzuki, K., and Tsuchihashi, G.-I. (1988) Tetrahedron Lett. 29, 3567–3570
31. Helferich, B. (1952) Adv. Carbohydr. Chem. Biochem. 7, 209–245
32. Takada, A., Ohmori, K., Yoneda, T., Tsuyuoka, K., Hasegawa, A., Kiso, M., and Kannagi, R. (1993) Cancer Res. 53, 354–361
33. Kimura, N., Mitsuoka, C., Kanamori, A., Hiraiwa, N., Uchimura, K., Muramatsu, T., Tamatani, T., Kansas, G. S., and Kannagi, R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4530–4535
34. Ohmori, K., Fukui, F., Kiso, M., Imai, T., Yoshie, O., Hasegawa, H., Matsumoto, K., and Kannagi, R. (2006) Blood 107, 3197–3204
35. Hansaki, K., Varki, A., and Powell, L. D. (1995) J. Biol. Chem. 270, 7533–7542
36. Le Marer, N., and Skacel, P. O. (1999) J. Cell. Physiol. 179, 315–324
37. Chou, H. H., Takematsu, H., Diaz, S., Iber, J., Nickerson, E., Wright, K. L., Muchmore, E. A., Nelson, D. L., Warren, S. T., and Varki, A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11751–11756
38. Irie, A., Koyama, S., Kozutsumi, Y., Kawasaki, T., and Suzuki, A. (1998) J. Biol. Chem. 273, 15866–15871
39. Tjew, S. L., Brown, K. L., Kannagi, R., and Johnson, P. (2005) Glycobiology 15, 7C–13C
40. Uchimura, K., Kadomatsu, K., El Fasakhany, F. M., Singer, M. S., Izawa, M., Kannagi, R., Takeda, N., Rosen, S. D., and Muramatsu, T. (2004) J. Biol. Chem. 279, 35001–35008
41. Schakel, K., Kannagi, R., Kniep, B., Goto, Y., Mitsuoka, C., Zwirner, J., Soruri, A., von Kietzell, M., and Rieber, E. P. (2002) Immunity 17, 289–301
42. Uchimura, K., Muramatsu, H., Kaname, T., Ogawa, H., Yamakawa, T., Fan, Q. W., Mitsuoka, C., Kannagi, R., Habuchi, O., Yokoyama, I., Yamamura, K., Ozaki, T., Nakagawara, A., Kadomatsu, K., and Muramatsu, T. (1998) J. Biochem. (Tokyo) 124, 670–678
43. Uchimura, K., El Fasakhany, F. M., Hori, M., Hemmerich, S., Blink, S. E., Kansas, G. S., Kanamori, A., Kumamoto, K., Kannagi, R., and Muramatsu, T. (2002) J. Biol. Chem. 277, 3979–3984
44. Uchimura, K., Gauguet, J. M., Singer, M. S., Tsay, D., Kannagi, R., Muramatsu, T., Von Andrian, U. H., and Rosen, S. D. (2005) Nat. Immunol. 6, 1105–1113