Lymph node macrophages, but not spleen macrophages, express high levels of unmasked sialoadhesin: implication for the adhesive properties of macrophages in vivo

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Glycoconjugates may also reduce cell–cell interactions nonspecifically through charge-repulsion effects. There is some evidence that Sn expressed by splenic marginal zone macrophages is partially masked because sialidase treatment significantly impaired the Sn-dependent binding of red blood cells to marginal zone macrophages (Barnes et al., 1999). To gain insight into the potential adhesive functions of Sn, it is important to measure the amount of Sn masked by the endogenous ligands as a function of the total amount of Sn present on native macrophages. To detect the binding activity of Sn, we previously developed a streptavidin-based glycoprobe carrying more than 100 oligosaccharide chains from GT1b (NeuAcα2-3Galβ1-3GalNAcβ1-4[NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ1-1Cer] ganglioside, which contains
N-acetylneuraminylα2-3galactose at the nonreducing terminus (Hashimoto et al., 1998). This high avidity complex only detects the unmasked forms of Sn; masked forms are detectable after a sialidase treatment. This novel binding assay provides a method for estimating both the level of masking and the binding activity of Sn at the cell surface.

In the present study we have utilized the GT1b probe to estimate the activity of Sn on native macrophages isolated from rat secondary lymphoid organs. We demonstrate the existence of macrophages expressing unmasked forms of Sn at high levels in lymph node compared to spleen.

Results

The GT1b probe strongly binds Sn on lymph node macrophages

We estimated the binding activity of rat splenic cells and mesenteric lymph node cells for a glycoprobe carrying approximately 140 GT1b oligosaccharides. The binding activities of these cells differed greatly; mesenteric lymph node cells exhibited 10-fold higher activity than splenic cells (Figure 1). To determine which cells carried the binding activity, distinct leukocyte populations were depleted from the mesenteric lymph node cells. The depletion of either T or B lymphocytes did not reduce the binding activity (Figure 2). The depletion of macrophages, however, from 0.61% to 0.08% of the total cells, reduced binding by more than 90%, suggesting that the majority of activity is carried by macrophages. The binding of the GT1b probe was inhibited by various glycosphingolipids; GM3 (NeuAcα2-3Galβ1-4Glcβ1-1Cer) and GD1a (NeuAcα2-3Galβ1-3GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Cer) exhibited an inhibitory potency similar to GT1b (Figure 3), suggesting that the N-acetylneuraminylα2-3galactose at the nonreducing terminus is important for inhibition. No inhibition was detected with asialoGM1 (Gaβ1-3GalNAcβ1-4Gaβ1-4Glcβ1-1Cer) or GM1 (Gaβ1-3GalNAcβ1-4[NeuAcα2-3]Gaβ1-4Glcβ1-1Cer). This binding specificity is similar to that described for Sn in previous studies (Van den Berg et al., 1992; Collins et al., 1997; Hashimoto et al., 1998). Accordingly, the majority of binding was inhibited by the anti-Sn monoclonal antibody (mAb) (ED3); the residual activity was less than 15% of the total (Figure 4), suggesting that the involvement of lectins other than Sn was likely to be minimal. The activity of the splenic cells was also inhibited by ED3 (Figure 4). Thus we concluded that the majority of activity detected was mediated by Sn on macrophages.

We then calculated the activity per cell based on the total number of macrophages. Macrophages were defined as CD11b/c and CD4 double-positive cells; the content of macrophages in the lymph node and spleen were 1.6% and 9% of the total cells, respectively. As shown in Figure 5, the binding activities of lymph node and splenic macrophages were calculated as 72 and 2 fmole/10^6 cells, respectively. Lymph node macrophages exhibited 36-fold higher activity than splenic macrophages. We also analyzed the binding activity of macrophages prepared from additional secondary lymphatic organs (Figure 5). Macrophages from the axillar and inguinal lymph nodes also exhibited the activity similar to those derived from the mesenteric lymph nod...
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Nodes; the activity of these cells was 74 fmole/l × 10⁶ cells. The activity of macrophages from Peyer’s patches, however, was significantly lower (10 fmole/l × 10⁶ cells) than that of lymph node macrophages. Thus macrophages in lymph nodes are distinguishable from macrophages in spleen and Peyer’s patches in terms of the probe binding activity.

This difference was independent of rat strains, because similar results were obtained from Fisher rats in addition to Wistar rats. Germ-free rats also had macrophages with high binding activity, suggesting that this property is determined constitutively, regardless of the presence or absence of bacterial flora (data not shown).

The Bₘₐₓ value of GT1b probe binding to lymph node macrophages is higher than that of splenic macrophages

Saturation isotherm for GT1b probe binding to lymph node macrophages is shown in Figure 6A. Scatchard analysis revealed that the Bₘₐₓ value of lymph node macrophages is 1.4 fmole/l × 10⁴ cells (Figure 6B), whereas that of splenic macrophages is 0.01 fmole/l × 10⁴ cells (Scatchard plot for the latter is not shown). The Kₛₐ value of GT1b probe binding to lymph node macrophages is approximately 1 nM (Figure 6B), which is indistinguishable from the value of splenic macrophage binding (1–2 nM). These data suggests that the high activity of lymph node macrophages depends on high levels of Sn.

Western blot analysis reveals high levels of Sn in lymph node macrophages

Macrophages were enriched from 2% to 47% in the mesenteric lymph node cell fraction and from 9% to 39% in the splenic cell fraction using a MACS positive selection system. The macrophage-enriched fractions were then subjected to western blot analysis. We detected a single band migrating at 185 kDa region in each lane (Figure 7), consistent with the previous reports (Crocker et al., 1991). We quantified the amount of Sn by chemiluminescence detection and compared the intensity per number of macrophages. The lymph node macrophages had 25-fold higher levels of Sn in comparison with the splenic macrophages.

Flow cytometry reveals the high expression of Sn on lymph node macrophages

We estimated the quantities of Sn on the cell surface of macrophages by flow cytometry. Negative selection was employed, because the macrophages eluted from the positive selection column already carried a mouse marker antibody, which prevented further staining with another mouse antibody. Macrophages were enriched to 12.6% in lymph node cell fraction and 21.9% in splenic cell fraction. Cell surface Sn in these fractions was detected by the anti-Sn antibody ED3 (Figure 8). A comparison of the mean value of the two histograms indicates that the levels of Sn on lymph node macrophages are four times greater than that on splenic macrophages. Approximately 20% of lymph node macrophages exhibit a fluorescence intensity greater than 1 × 10², whereas none of the splenic macrophages do. Macrophages expressing Sn...
such high levels of Sn may contribute to the high binding activity of lymph node macrophages.

**Sialidase treatment does not affect the GT1b probe binding to lymph node macrophages**

Barnes et al. (1999) reported that Sn on rat splenic macrophages is masked by endogenous sialic acid. Therefore, we treated macrophages with sialidase to unmask Sn, before performing the binding assay. Sialidase treatment increased the binding activity of splenic macrophages fourfold but did not increase that of the lymph node macrophages (Figure 9). The unmasking of Sn on splenic macrophages was also demonstrated by mild periodate oxidation, which abolished the sialic acid ligand activity by truncation of the exocyclic glycerol side chains. Mild periodate oxidation increased the binding activity of splenic macrophages 2.8 times but did not increase that of lymph node macrophages (data not shown). These results suggest that the Sn present on lymph node macrophages is not masked, whereas the majority of Sn present on splenic macrophages is masked by endogenous sialic acid.

**Sheep erythrocytes bind well to a lymph node section but not to a spleen one**

Differential expression of Sn between lymph node and splenic macrophages was further confirmed by erythrocyte binding assays. Tissue sections of mesenteric lymph nodes or spleen were incubated at 4°C with sheep erythrocytes as authentic cellular ligands for Sn. Erythrocytes bound well to subcapsular sinus and medullary region of a lymph node section (Figure 10A), whereas erythrocytes poorly bound to a spleen section (Figure 10B). The erythrocyte binding to a lymph node section was completely abolished by the addition of anti-Sn antibody ED3 (Figure 10C), suggesting that the binding is mediated by Sn on macrophages. A spleen section was treated with sialidase and then subjected to the erythrocyte binding assay. After the sialidase treatment erythrocytes bound well to splenic marginal zones (Figure 10D), suggesting that Sn on splenic macrophages are largely masked by endogenous sialic acids. The binding to marginal zones after sialidase treatment was blocked by ED3 (data not shown). Thus we confirmed the presence of masked forms of Sn in spleen in addition to high...
Discussion

Sn is expressed on certain subsets of resident macrophages in lymphoid tissues. Dijkstra et al. (1985) prepared an mAb (ED3) to macrophage subsets that was subsequently shown to be specific for rat Sn. In their analysis of the antigen distribution in lymphatic organs, Sn was observed on macrophages of both the splenic marginal zones and the subcapsular sinus and medullary region of peripheral lymph nodes (Dijkstra et al., 1985). A similar distribution of Sn was observed in mouse organs (Van den Berg et al., 1992). Significant binding activity of Sn was detected in tissue slices using red blood cells or T lymphocyte blasts as cellular ligands, localized to the regions of antibody staining (Van den Berg et al., 1992). The binding activity in marginal zones of a spleen slice was detected at 37°C but not at 4°C, which was consistent with the results of our erythrocyte binding assay (our result at 37°C was not shown). Although these binding assays using cellular ligands are sensitive to detect Sn activity, the assays make a quantitative comparison of different organs difficult. The quantitative binding assay utilizing a GT1b probe can clearly distinguish activity differences between lymph node and splenic macrophages. Lymph node macrophages exhibited a 36-fold higher binding activity than the splenic macrophages.

We also detected similar macrophages in mouse lymphatic organs; lymph node macrophages had a higher binding activity than the splenic macrophages. These activities were abolished by the addition of an mAb specific for mouse Sn, 3D6 (data not shown). It should be noted that mouse splenic macrophages comprises of several subsets, and their expression levels of Sn are quite different (Crocker and Gordon, 1989; Crocker et al., 1997). In spleen, monocytes do not express Sn, nor do tingible body macrophages. Red pulp macrophages, which make up the majority of macrophages in spleen, express low levels of Sn. The highest levels are found on most marginal metallophilic and some marginal zone macrophages. These cells may have similarly high binding activity as lymph node macrophages. To investigate this further, we would need a sensitive method for detecting Sn binding activity at the single-cell level.

High expression of Sn on rat lymph node macrophages was substantiated by western blot analysis and flow cytometry. The total amount of Sn in lymph node macrophage lysates is 25-fold higher than that in splenic macrophages by western blot analysis. In addition, the cell surface expression of Sn on the former is higher than that of the latter, as assessed by flow cytometry. The Sn present on the lymph node cells is not masked by endogenous ligands, in contrast to splenic macrophages, as evidenced by binding assays with or without sialidase treatment. Thus we have identified macrophages expressing high levels of unmasked Sn in rat lymph nodes.

Sn (Siglec-1), a member of Siglec family of proteins, exhibits lectin activity toward sialoglycoconjugates. The lectin activities observed for this family have often been demonstrated in overexpression systems, in which the Siglec cDNA was transfected into COS or Chinese hamster ovary cells. Siglecs expressed on the cell surface under these conditions exhibit no or poor lectin activity without sialidase treatment; that is, endogenous sialoglycoconjugates mask the binding sites of Siglecs through cis-type interactions. This masking is also observed on native cells, which physiologically express Siglec. For example, CD22 (Siglec-2) expressed on resting peripheral blood B cells is naturally masked by endogenous sialoglycoconjugates; the binding of glycoprobes to CD22 was observed after the removal of the cis-competing sialic acid by sialidase treatment (Razi and Varki, 1998; Floyd et al., 2000).
Unmasking of CD22 was also seen to occur following activation of B cells with anti-IgM and anti-CD40 antibodies (Razi and Varki, 1998). Human blood leukocytes express a wide range of Siglecs that also appear to be masked by cis-interactions with sialic acids (Razi and Varki, 1999). These observations suggest that Siglec masking is a commonly used in vivo mechanism controlling lectin activity and cell–cell binding properties. For instance, Floyd et al. (2000) recently reported that a minor subset of murine B cells, enriched in the bone marrow, expresses unmasked forms of CD22. Together with their previous observations that the bone marrow sinusoidal endothelium expresses CD22 ligands, the unmasked forms of CD22 are implicated as receptors that control homing of B cells to the bone marrow (Nitschke et al., 1999). Thus unmasked forms of Siglecs may mediate the physiological tissue distribution of lymphohematopoietic cells through interactions with oligosaccharide ligands.

Barnes et al. (1999) demonstrated that the activity of Sn is partially masked on splenic macrophages, and they hypothesized that this masking on splenic macrophages results from the modification with sialic acid on the Sn molecule. The molecular mechanisms governing the unmasking of Sn on lymph node macrophages remain unknown, although the possibilities include the absence of endogenous ligands, poor accessibility of the ligands, or the presence of a large excess of Sn over endogenous ligands. These unmasked forms are available for Sn-dependent adhesive processes, recognizing ligands on neighboring cells or the substratum. The heavily sialylated mucin MUC1 on breast cancer cells was recently shown to be a potential counterreceptor for Sn (Nath et al., 1999). Similarly, CD43, an extended molecule carrying multiple O-linked glycans, was shown to function as a counterreceptor for Sn on T lymphocytes. The interaction of CD43 with Sn may promote the initial physical contacts between T cells and macrophages and play a role in the antigen-presentation functions of these cells (Van den Berg et al., 2001).

In conclusion, we have identified the macrophage populations expressing large quantities of unmasked Sn. These macrophages may interact with other hematopoietic cells, including T cells, which express CD43. Like the unmasked CD22, unmasked forms of Sn may contribute to both the physiological and pathological tissue distribution of macrophages and other cell types through the interactions with their oligosaccharide ligands localized on both interstitial cells and the substratum in lymph nodes. Future studies will seek to clarify the function of this interesting populations of macrophages.

Materials and methods

Materials

GT1b, GD1a, and GM1 ganglioside were prepared from bovine brain. GM3 ganglioside was purified from dog erythrocytes. Additional glycosphingolipids were purchased from Wako Pure Chemical Industries (Tokyo). An anti-Sn polyclonal antibody was prepared by immunization of a rabbit with purified mouse Sn. This polyclonal antibody reacted with both rat and mouse Sn, as previously reported (Van den Berg et al., 1992). Anti-rat Sn mAb ED3 (mouse IgG2a) was purchased from Serotec (Raleigh, NC). The irrelevant mouse IgG2a, used as a control, was obtained from Sigma-Aldrich (St. Louis, MO). The peroxidase-conjugated anti-rabbit IgG [Fab′], utilized in western blotting, was purchased from Amersham (Arlington Heights, IL).

Animals

Eight- to 14-week-old Male Wistar rats, maintained in specific pathogen-free or germ-free conditions, were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). Fischer strain germ-free rats were the generous gift of Dr. Yoshinori Umezaki (Yakuruto Institute, Kunitachi, Japan).

Cell preparation

Following sacrifice of rats by deep ether anesthesia, the spleen, mesenteric lymph nodes, axillary lymph nodes, inguinal lymph nodes, and Peyer’s patches were removed. A single cell suspension was prepared from these organs in phosphate buffered saline (PBS) supplemented with 2% fetal calf serum (FCS) and 0.05% sodium azide. To remove contaminating erythrocytes if present, the cell suspensions were subjected to osmotic lysis using a hypotonic ammonium chloride solution. In the preparation of splenic macrophages, spleens were treated with or without collagenase (0.5% solution of type III collagenase in Hanks balanced salt solution) in an attempt to recover macrophages that were deeply embedded in the stroma. In a comparison of the macrophage recoveries under these conditions, collagenase treatment increased the percent of isolated macrophages from 6% to 7–9% but did not affect the binding activity. Therefore the collagenase digestion was not employed for the standard protocol.

Either T cells, B cells, or macrophages were depleted from the total lymph node cells by magnetic cell sorting using a MACS system, according to the manufacturer’s protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). Briefly, 5–7 × 10⁷ lymph node cells suspended in 2% FCS-PBS containing 0.05% sodium azide were incubated for 30 min at 4°C with either a biotinylated anti-rat T cell receptor mAb (0.5 µg/10⁶ cells; Pharmingen, San Diego, CA), a biotinylated anti-rat panB mAb (1 µg/10⁶ cells; Seikagaku kogyo, Tokyo) or a biotinylated anti-rat CD11b/c mAb (0.5 µg/10⁶ cells, Pharmingen). Following two subsequent washes in 2% FCS-PBS, the cells were resuspended in 90 µl (per 10⁷ cells) staining buffer (PBS containing 2 mM ethylenediamine tetra-acetic acid (EDTA) and 0.01% sodium azide) with 10 µl (per 10⁷ cells) additional streptavidin-MicroBeads (Miltenyi Biotec). Following a 15-min incubation at 4°C, the cells were washed in staining buffer and suspended in 0.5% bovine serum albumin (BSA)-PBS containing 2 mM EDTA and 0.01% sodium azide. To remove T cells, B cells, or macrophages, the cells were applied to a MACS LS⁺ column. The pass-through fraction was then subjected to the GT1b probe binding assay.

Preparation of GT1b probe

The GT1b probe was prepared as previously described (Hashimoto et al., 1998). Briefly, GT1b oligosaccharides were coupled to streptavidin by reductive amination. The resulting oligosaccharyl streptavidin (GT1b-streptavidin) was mixed with biotinylated BSA, radiodinated prior to mixing. The components formed a complex based on the streptavidin–biotin interaction, composed of 1 molecule of [¹²⁵I]biotinylated-BSA and 11 molecules of GT1b-streptavidin. The complex carried approximately 140 GT1b oligosaccharides. After the...
purification by Sephacryl S-200 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) column chromatography, the complex was utilized as a probe (GT1b probe) in the binding assays.

**GT1b probe binding assay**

The binding of the standard assay method for cytokine receptor activity. Briefly, 2–3 × 10^6 cells, prepared from various lymphoid tissues, were mixed with [125I]GT1b probe (2.5 nM) in 30 µl of 1% FCS-PBS containing 0.05% sodium azide (incubation buffer). After a 90-min incubation at room temperature, the mixture was overlaid in a plastic tube (3 mm ID × 45 mm) onto 200 µl of an oil mixture (dibutyl phthalate/bis[2-ethylhexyl] phthalate) (3:2, v/v) and centrifuged for 2 min at 7000 rpm. The plastic tube was cut at the center of oil layer to isolate the lower part of the tube containing pelleted cells. The radioactivity associated with the pelleted cells was measured with a gamma counter. The specific binding activity was defined as the total binding, performed in the absence of GT1b ganglioside, minus the nonspecific binding in the presence of 330 µM of GT1b ganglioside.

**Western blot analysis**

Macrophage-enriched fractions were subjected to western blot analysis. We utilized the MACS positive selection to enrich the percentage of macrophages from either total splenocytes or lymph node cells. Briefly, either 4 × 10^6 splenocytes or 5 × 10^6 lymph node cells were incubated with physocytirine (PE)-conjugated -anti-rat CD11b/c mAb (0.5 µg/10^6 cells, Pharmingen), on ice for 30 min. After washing with 1% FCS-PBS containing 0.05% sodium azide, cells were stained at 4°C for 15 min with anti-PE mAb-MicroBeads (10 µl/10^6 cells, Miltenyi Biotech) and a biotinylated streptavidin-MicroBeads (Miltenyi Biotech) in 90 µl of per 10^7 cells) staining buffer (PBS containing 2 mM EDTA and 0.01% sodium azide). The labeled cells were enriched using a MACS LS+ positive selection column. After washing, the cells retained in the column were eluted in staining buffer by removing the column from the magnetic field. Cells (about 5 × 10^6 cells) were then washed with PBS and lysed in 600 µl of buffer containing 1% octyl glucoside, 30 mM Tris–HCl (pH 7.3), 5 mM EDTA, and 120 mM NaCl with a protease inhibitor cocktail (Roche, Mannheim, Germany). Lysates, isolated by centrifugation for 10 min at 10,000 × g, were stored at −20°C until use. An aliquot of the lysate was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 5–20% gel, followed by electrophoretic transfer onto a nitrocellulose membrane (BioRad, Melviller, NY). The membrane was blocked by an incubation with 0.1% Tween–PBS containing 0.3% milk for 1 h. The membrane was further incubated with a rabbit anti-Sn polyclonal antibody at 1 µg/ml in 0.05% Tween–PBS containing 0.3% milk for 1.5 h and followed by incubation with a peroxidase-conjugated anti-rabbit IgG [F(ab)′2] as a second antibody (1:1500; Amersham). Between each incubation, the membrane was washed several times with 0.1% Tween–PBS. Sn was visualized by an enhanced chemiluminescence using SuperSignal (Pierce, Rockford, IL). The levels of Sn were estimated using CoolSaver (ATTO, Tokyo).

**Flow cytometry**

Positive selection could not be employed for flow cytometry, because the macrophages eluted from the positive selection column had already been labeled with a mouse antibody, which prevented further staining with another mouse antibody. Negative selection was therefore employed for flow cytometry. Briefly, 5 × 10^7 cells in 2 ml of 2% FCS-PBS containing 0.05% sodium azide were incubated for 30 min with both a bioinylated anti-rat panB mAb (100 µl, Seikagaku kogyo) and a bioinylated anti-rat CD3 mAb (30 µl, Seikagaku kogyo) at 4°C. The cells were then washed twice with 2% FCS-PBS and resuspended in 450 µl of staining buffer (PBS containing 2 mM EDTA and 0.01% sodium azide). Fifty microliters streptavidin-MicroBeads (Miltenyi Biotech) were then added for a 15-min incubation at 4°C. After washing in staining buffer, the cells were resuspended in 700 µl of 0.5% BSA-PBS, containing 2 mM EDTA and 0.01% sodium azide, and applied to a MACS VX negative selection column for magnetic separation. T and B lymphocytes remained trapped in the column; the macrophages were recovered in the flow-through fractions. Macrophages were enriched to 12.6% in the mesenteric lymph node fraction and to 21.9% in the splenic fraction. A single-cell suspension of the macrophage-enriched fraction (5 × 10^5 cells) was incubated with either 15 µg/ml of anti-Sn mAb, ED3, or an irrelevant mouse IgG2a (Sigma) in 2% FCS-PBS containing 0.05% sodium azide. Fluorescein isothiocyanate (FITC)-anti-mouse IgG mAb (5 µg/ml, Serotec) was used as second antibody. PE-anti-rat CD11b/c mAb (5 µg/ml, Serotec) were also utilized to stain the cells. The cells were analyzed on a FACCaliber flow cytometer using Cellquest software (Becton Dickinson, Franklin Lakes, NJ). Macrophages were tentatively defined as double positive cells for the FITC-anti-rat CD11b/c mAb (1 µg/10^6 cells, Pharmingen) and the PE-anti-rat CD4 mAb (1 µg/10^6 cells, Pharmingen) (Jefferies et al., 1985). These double-positive cells carried almost all the sugar-binding activity on lymph node cells. Macrophages are not defined as CD11b/c single-positive cells because in the spleen this population contains significant numbers of granulocytes.

**Sialidase treatment**

Sialidase treatment of spleen and lymph node cells was performed by the method described by Barnes et al. (1999) with the following modifications. Briefly, 6 × 10^7 cells were incubated at 37°C for 30 min with 0.6 U Vibrio cholerae sialidase (EC 3.2.1.18, Calbiochem, Darmstadt, Germany) in 2% BSA-PBS. Cells were then washed with 2% FCS-PBS and subjected to the GT1b probe binding assay.

**Adhesion assay of sheep erythrocytes**

Frozen cryostat sections (7 µm) of the mesenteric lymph nodes and spleen were placed on glass slides and air-dried for 60 min. These sections were washed three times with PBS and then cold PBS containing 2 mM EDTA. Sheep erythrocytes suspended in EDTA-PBS were placed on the sections. After the incubation at 4°C for 30 min, the sections were washed three times with cold EDTA-PBS to remove unbound erythrocytes. The binding was immediately examined by light microscopy. Sialidase treatment was performed as follows: a cryostat section washed with PBS was incubated at 37°C for 60 min with 0.02 U V. cholerae sialidase (Calbiochem) in 200 µl of PBS. Antibody blocking studies were performed as follows: a lymph node section or a sialidase-treated spleen section was preincubated with 100 µg/ml of anti-Sn mAb (ED3) in PBS at 4°C.
room temperature for 30 min and then incubated with sheep erythrocytes at 4°C in the presence of 30 μg/ml ED3.

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Abbreviations
BSA, bovine serum albumin; EDTA, ethylenediamine tetra-acetic acid; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GA1, Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer; GD1a, NeuAcα2-3Galβ1-3GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Cer; GM1, Galβ1-3GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Cer; GM3, NeuAcα2-3Galβ1-4Glcβ1-1Cer; GT1b, NeuAcα2-3Galβ1-3GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Cer; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PE, phycoerythrin; Sn, sialoadhesin.

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