Cryptic sialic acid binding lectins on human blood leukocytes can be unmasked by sialidase treatment or cellular activation

Nahid Razi and Ajit Varki

Glycobiology Program and Cancer Center, Divisions of Hematology-Oncology, and Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA, USA

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We recently reported that the sialic acid–specific binding sites of CD22 molecules on B cells are masked by endogenous ligands, and can be unmasked by sialidase treatment or cellular activation. Here, we show that many other human blood leukocyte types have endogenous sialic acid binding sites that can be unmasked by sialidase treatment. Truncation of sialic acid side chains on the soluble probes used for detection abolishes all binding, indicating the specificity of the interaction for the details of sialic acid structure. There is limited overlap between α2-6- and α2-3-sialic acid–specific binding sites, which are unmasked on monocytes, natural killer cells, a minority of mature T cells, neutrophils, and some cultured human leukemic cell lines. Activation with phorbol ester and calcium ionophore causes spontaneous exposure of some of the binding sites, occurring over a period of minutes on neutrophils and several hours on monocytes and U937 leukemia cells. Activation is accompanied by some evidence for desialylation of cell surface molecules. Thus, many human blood cells have specific binding sites for sialic acids, masked by endogenous sialylated ligands. Cellular activation can unmask these sites, possibly by the action of an endogenous sialidase. The nearly universal masking of such sites in unactivated blood cells could explain why many of these sialic acid–binding lectins have not been previously discovered. Similar considerations may apply to sialic acid binding lectins of other cell types and tissues.

Key words: human/blood/cell-to-cell interactions/cellular activation/neutrophils

Introduction

Sialic acids are a family of 9-carbon acidic sugars that are ubiquitously expressed on vertebrate cell surfaces (Varki, 1992; Kelm and Schauer, 1997). Given their location, their remarkable structural diversity and the variety of their linkages to underlying sugar chains, it is not surprising that sialic acids are recognized with exquisite specificity by many viral hemagglutinins and bacterial adhesins (Karlsson, 1995; Varki, 1997b). It is reasonable to suppose that this diversity might also be utilized to generate biological ligands for endogenous sialic acid–binding lectins. A decade ago, the only known vertebrate sialic acid binding lectin was the H protein of the alternative complement pathway (Pangburn and Muller-Eberhard, 1978; Kazatchkine et al., 1979; Ram et al., 1998). The discovery and characterization of the selectins (Rosen and Bertozzi, 1994; Lasky, 1995; Nelson et al., 1995; Tedder et al., 1995; Butcher and Picker, 1996; Kansas, 1996; Lowe and Ward, 1997; McEver and Cummings, 1997) added three vertebrate “C-type” lectins that utilized sialic acids as part of their ligands. However, the only requirement for selectin recognition of sialic acids seems to be the negatively charged carboxylate at the 1-position, and the adjacent α2–3 linkage (Rosen and Bertozzi, 1994; Varki, 1994; Lasky, 1995; Nelson et al., 1995; Crocker and Feizi, 1996; Kansas, 1996; Vestweber, 1996; McEver and Cummings, 1997; Varki, 1997a). The discovery of the sialic acid–binding property of human CD22 (Sgroi et al., 1993; Powell et al., 1993) and the purification and cloning of mouse macrophage sialoadhesin (Crocker et al., 1991, 1994) led to the realization that Ig-superfamily members other than immunoglobulins can specifically recognize vertebrate oligosaccharides (Powell and Varki, 1995). Amongst these “I-type” lectins, CD22 and sialoadhesin are part of a distinct subset that specifically bind sialic acids (Kelm et al., 1994a,b; Powell and Varki, 1995; Crocker et al., 1996), and have been recently renamed as the Siglecs (for sialic acid binding immunoglobulin superfamily lectins) (Crocker et al., 1998). Apart from sialoadhesin (siglec-1) and CD22 (siglec-2), the other members are CD33 (siglec-3), myelin associated glycoprotein (siglec-4a), and Schwann cell myelin protein (siglec-4b). (Dulac et al., 1992; Crocker et al., 1994, 1995, 1997; Kelm et al., 1994a,b; Powell and Varki, 1994, 1995; Sjoberg et al., 1994; Tchilian et al., 1994; Hanasaki et al., 1995a,b; Freeman et al., 1995; Powell et al., 1995; Crocker and Feizi, 1996; Kelm et al., 1996; Shi et al., 1996; Yang et al., 1996; Collins et al., 1997a,b). An additional member of the family (siglec-5) has recently been discovered by database homology searching (Cornish et al., 1998). Throughout this family of molecules, the sialic acid binding property appears to be mediated primarily by an amino-terminal V-set Ig domain, with some contribution by the next C2-set domain (Engel et al., 1993, 1995; Kelm et al., 1994b; Law et al., 1995; Nath et al., 1995; Van der Merwe et al., 1996; Vinson et al., 1996). Available data suggest that recognition involves the entire sialic acid molecule, including the carboxylate group at the 1-position, the linkage from the 2-position, the N-acyl group at the 5-position, and the exocyclic hydroxyl groups at the 7-, 8-, and 9-positions.

1To whom correspondence should be addressed

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(Kelm et al., 1994b, 1998; Powell and Varki, 1994; Sjoberg et al., 1994; Hanasaki et al., 1995a,b; Powell et al., 1995; Shi et al., 1996; Yang et al., 1996; Collins et al., 1997a,b; Crocker et al., 1997; Kelm et al., 1998) In the case of sialoadhesin (siglec-1), a crystal structure has been elucidated in the presence of bound ligand, confirming that all of these aspects of the sialic acid molecule are actually in direct contact with the binding site (May et al., 1998).

The question arises as to why so few endogenous vertebrate sialic acid binding lectins have been discovered to date, despite exploration by many investigators over a long period of time. One possibility is that sialic acids are so common on cell surface and secreted glycoconjugates (Kitagawa and Paulson, 1994; Hanasaki et al., 1995a) that they cannot be effectively used as discriminable biological ligands for endogenous functions. Another possibility is that, in order to prevent undesirable interactions with inappropriate targets, such lectins are kept in a masked state until they are ready to be used in specific situations. With regard to the latter possibility, masking by sialylated ligands on the same cell surface has been reported for the lectin activities of several siglecs when they were studied as recombinant forms expressed in cultured cells (Freeman et al., 1995; Hanasaki et al., 1995b; Sgroi et al., 1996; Collins et al., 1997b; Tropak and Roder, 1997). We recently extended this experimental paradigm to a more natural situation, showing that the CD22 lectin on resting B cells from normal human peripheral blood is naturally masked from detection by a soluble sialylated probe (Razi and Varki, 1998). The masking appears to be due to endogenous sialylated ligands that can be removed by treatment of the cell surface with a bacterial sialidase. Interestingly, partial unmasking of the lectin sites could also be achieved by pharmacological or physiological activation of the B cells. This provides a mechanism by which the CD22 lectin can regulate its exposure and function, within a milieu of natural glycoproteins that carry copies of the basic ligand structure, and are therefore low affinity ligands (Hanasaki et al., 1995a). Thus, the receptor could expose its activity only when it is really needed, perhaps only in a protected environment, away from potential inhibitors. Here we show that the situation with CD22 can be extended to sialic acid binding lectins on many blood cell types, several of which were not previously known to express such lectins.

**Results**

**Sialidase treatment unmasks binding sites for sialyl-6' - and 3' -lactose probes on human PBMCs**

Mononuclear cells from the peripheral blood of normal human volunteers were washed, treated with sialidase, washed again, and then probed with biotinylated polyacrylamide molecules substituted with arrays of sialyl-6'- or 3'-lactose (hereafter called 6'PAA-B and 3'PAA-B, respectively). As can be seen from Figure 1, both the 6' and 3' probes detect binding sites on a subpopulation of the PBMCs, but only after sialidase treatment. We have previously shown that the 6'PAA-B can detect CD22 lectin binding sites that are unmasked on normal human peripheral blood B cells by sialidase treatment (Razi and Varki, 1998). The only other known sialic acid binding lectin on human peripheral blood cells is CD33. However, this lectin has a very low affinity, and cannot be detected with soluble probes even when overexpressed in recombinant form on sialidase-treated COS cells (Cornish et al., 1998). Thus, the 3'PAA-B binding must be due to other previously unknown lectins. Also, since B cells represent a minority (typically ~10%) of these mixed populations, at least some sites detected by the 6'PAA-B probe must be due to novel lectins. Likewise, siglec 5 was found on monocytes (Cornish et al., 1998), which also form a minor component of the PBMC mixture.

The sialidase preparation used has no known contamination with proteases or other glycosidases, and similar results were obtained using another sialidase from *Clostridium perfringens* (data not shown). However, the apparent unmasking of sialic

![Fig. 1. Sialidase pretreatment of PBMCs unmasks lectin activity recognizing α2–3 and α2–6-linked sialic acids. Freshly isolated PBMCs were washed, treated with sialidase, washed again, then probed with 3' or 6'-PAA-B followed by streptavidin-PE, and analyzed by single color flow cytometry as described under Materials and methods. Background control incubations were probed with the secondary reagent alone.](image-url)
Unmasking of sialic acid–binding lectins

Acid binding sites on the PBMCs could also be due to a general loss of negative charge repulsion resulting from the desialylation of the cell surface. To rule out this possibility, we pre-treated aliquots of the PBMCs with mild peridote oxidation under conditions that are known to selectively oxidize and cleave the side chain of cell surface sialic acids, without affecting the negative charge of the sialic acids, or any of the underlying sugar chains (Van Lenten and Ashwell, 1971a,b).

Although this chemical approach was not as efficient as sialidase treatment, we found that binding sites were also exposed by this method (data not shown).

Truncation of sialic acid side chains of the sialylated probes by mild periodate oxidation abolishes binding

To confirm that probe binding to sialidase-treated cell surfaces involves more than just the negative charge of the probes, we pretreated the probes themselves with mild periodate under similar conditions as above, causing selective truncation of the sialic acid side chains on the sialyllactose units. After the treatment, the residual periodate was destroyed by an excess of glycerol. Sham treatment involved mixing of the periodate and glycerol prior to addition to the probe. As shown in Figure 2, prior periodate oxidation of either the 6′- or 3′-PAA-B probes abolished the binding to desialylated PBMCs. These data indicate that all of the unmasked binding sites detected by these probes require their sialic acid side chain for the interaction. This finding rules out a non-specific interaction based on negative charge only, and indicates a binding phenotype typical of the siglec family (which require the sialic acid side chain for recognition) (Powell et al., 1993; Kelm et al., 1994a, 1996, 1998; Powell and Varki, 1994, 1995; Sjoberg et al., 1994; Hanasaki et al., 1995a,b; Powell et al., 1995; Crocker and Feizi, 1996; Shi et al., 1996; Yang et al., 1996; Collins et al., 1997a,b), rather than that of the selectins (wherein binding is unaffected, or even enhanced upon oxidation of the side chain) (Varki, 1994).
There is limited overlap between the unmasked binding sites for 6' and 3'-PAA

To date, most reported siglecs and selectins have also shown a preference for the linkage of the cognate sialic acid residue to the underlying sugar chain (Powell et al., 1993, 1995; Kelm et al., 1994a, 1996, 1998; Powell and Varki, 1994, 1995; Sjoberg et al., 1994; Hanasaki et al., 1995a,b; Crocker and Feizi, 1996; Shi et al., 1996; Yang et al., 1996; Collins et al., 1997a,b). Thus, while CD22 strongly prefers an $\alpha_2$–6 linkage, CD33, MAG, and Sn all prefer an $\alpha_2$–3 linkage (with sialoadhesin, an $\alpha_2$–8 linkage also permits some recognition, and siglec 5 may be able to bind both linkages). To examine this issue for the newly exposed binding sites on the sialidase-treated PBMCs, we incubated the desialylated PBMCs with a mixture of 6'-PAA-FITC and 3'-PAA-B probes. As shown in Figure 3, only a limited overlap was seen between the cells binding the two kinds of probes. This indicates that most of the exposed binding sites are highly selective for the sialic acid linkage recognized.

Binding sites are expressed on several leukocyte types not previously known to carry sialic acid binding lectins

To examine which blood cell types express the unmasked sialic binding sites, we carried out two-color flow cytometry analysis using cell surface markers specific for various types of leukocytes, including CD19 (B cell-specific), CD14 (monocyte-specific), CD56 (natural killer cell-specific), and CD3 (T cell-specific). As expected, a subpopulation of the CD19 positive B cells have binding sites for the 6'-PAA-B probe (presumably those cells carrying CD22, data not shown). As seen...
in Figure 4, the 6'-PAA-B and 3'-PAA-B probes also showed binding to subpopulations of CD14+ monocytes and CD56+ NK cells. There was limited binding to a subset of CD3+ positive T cells (data not shown). To further study the subsets of mature T cells, we explored the distribution of binding sites on CD4 and CD8-positive cells. As shown in Figure 5, the unmasked sialic acid binding sites were enriched on the CD4lo population (staining with anti-CD4 in the fluorescence intensity range 10^2–10^3) and only to a limited extent on the mature CD4hi (fluorescence intensity range 10^2–10^3) and CD8+ T cells. In human peripheral blood, these CD4lo cells are known to represent mostly monocytes, with a minor component of dendritic cells and immature T cells. Thus, the data indicate that only a small subpopulation of mature CD4hi and CD8+ T cells carry these masked sialic acid binding sites. However, these binding sites appear to be specific, because these cells were not detected when using control anti-mouse antibodies (data not shown).

Up to this point, all of our studies had excluded one other type of peripheral blood leukocyte, the neutrophil, which is not enriched for in the standard PBMC preparation. We therefore turned to the use of a modified Ficoll gradient that allows the
simultaneous isolation of neutrophils in a separate band. As shown in Figure 6, neutrophils also carry binding sites for both the 6'- and 3' -PAA-B probes, that are unmasked upon sialidase treatment. Among the T cell lines, only CEM cells expressed low levels of unmasked binding sites that were expressed whether or not prior sialidase treatment was done (data not shown). However, this low level of binding to CEM cells was only present when the cells were grown in serum-free media, and the matter was not pursued further.

**Cellular activation causes spontaneous exposure of lectin binding sites**

With the exception of the low levels of exposed binding sites found in CEM cells, all other sites detected in both normal and leukemic cells were masked, and needed to be exposed by exogenous addition of sialidase. We next asked if these binding sites could be spontaneously exposed upon cellular activation. Pharmacological activation of normal PBMCs or U937 cells with PMA/ionomycin exposed some binding sites for both the 6'- and 3' probes (data not shown). The kinetics of this unmasking occurred over a period of hours, with substantial further exposure occurring in the U937 cells after several days (data not shown). Since this unmasking is occurring within a mixture of multiple cell types in the PBMCs and over a long period of time in both cultures, we did not pursue the mechanistic details further at this point. The same treatment gave partial exposure of both 6'- and 3'-PAA-B binding sites on neutrophils over a very short period of time (see Figure 8, maximum exposure in 15 min). These experiments indicate that the phenomenon of unmasking of sialic acid binding sites upon activation that we first observed in B cells (Razi and Varki, 1998) can be extended to some other blood cell types.

**Does an endogenous sialidase activity explain the unmasking of binding sites?**

There are several prior reports indicating the activation of cell surface sialidases and/or the shedding of cell surface sialic acids upon activation of T cells, B cells, and neutrophils (see Discussion). To pursue the role of such sialidases in the unmasking of sialic acid binding sites, we choose to focus on neutrophils, since unmasking is very rapid, and cannot be ascribed to new synthesis of receptor molecules. Activation of neutrophils was associated with a variable but small increase in binding sites for peanut agglutinin (PNA, which binds nonsialylated Galβ1–3GalNAc units) and a small decrease in binding of *Maackia amurensis* lectin II (MAL-II, which binds molecules with terminal α2–3-linked sialic acids). These data (not shown) suggest the rapid action of an endogenous sialidase that appears to be acting at the cell surface. To examine if the unmasking of sialic acid binding sites is mediated by this sialidase, we carried out the activation experiments in the presence of the potent bacterial and mammalian sialidase inhibitor 2,3-dehydro-2,6-anhydro-N-acetyl-neuraminic acid (Neu2en5Ac) (Warner *et al.*, 1991,1993). However, even when adding this inhibitor at 5 mM concentration, there was no effect on the appearance of the PNA-binding sites, nor the loss of the MAL-II binding sites. The inhibitor also did not suppress the
unmasking process in U937 cells or PBMCs (data not shown). Thus, if a sialidase is responsible for the unmasking, it is not susceptible to this inhibitor.

Discussion

We have previously shown that biotinylated α2–6 sialyllactose-substituted polyacrylamide arrays can be used to specifically probe the lectin binding site of cell surface CD22 on normal and leukemic B cells. Detection of this activity required prior unmasking by sialidase treatment or cellular activation (Razi and Varki, 1998). Here we use biotinylated α2–6 and α2–3 sialyllactose-substituted polyacrylamide probes to detect additional masked binding sites on a variety of human blood cell types. In all cases, binding was abolished simply by truncating the side chain of the sialic acid residues.
on the probe, indicating that recognition involves not just the acidic charge of the sialic acid carboxylate, but also the rest of the molecule. This requirement for the exocyclic side chain of sialic acids is typical for most siglec family members described so far, as well as for the Complement H protein. The only two previously reported sialic binding lectins on circulating human blood cells are CD22 and CD33. The latter has a very poor affinity, and cannot be detected using these types of probes, instead requiring the use of intact cell assays (Cornish et al., 1998). Thus, all of the binding sites uncovered in this study on NK cells, neutrophils, monocytes (and possibly a few mature T cells) must represent previously unknown sialic acid binding lectins. In this regard, another group just reported the cloning of a new siglec-5 that is present on some monocytes and on neutrophils (Cornish et al., 1998). This lectin may account for some but not all of the binding sites that we have uncovered. The binding sites on some NK cells could reflect activity of the previously described NK cell lectins (Bezouska, 1996; Lanier, 1998), whose natural ligands are unknown. The small number of mature T cells with specific binding sites also remains unexplained.

Because of the potentially multivalent nature of the interactions on both sides (probe and cell surfaces) we cannot estimate the true affinity of binding. The existence of binding sites for both α2–6 and α2–3 binding sites on such a variety of different blood cell types might also raise some initial concerns about the significance of the result. However, the double label and competition experiments as well as the loss of binding upon mild periodate oxidation of the probes indicate substantial specificity of the interactions. In almost all instances, we also found that the sialic acid binding sites were masked by endogenous ligands and could be unmasked by sialidase treatment. We also show that cellular activation results in spontaneous unmasking of a portion of such binding sites. The kinetics of this unmasking were slow (over a period of hours) for mononuclear cells and PBMCs and very rapid (over a period of minutes) for neutrophils. The question arises as to how the unmasking takes place. New synthesis of sialic acid binding lectin molecules cannot explain the findings in neutrophils, where the response occurs within minutes.

In the case of PBMCs and the monocytic leukemia cell line, maximum exposure occurs over a period of many hours, and therefore could represent new synthesis. However, the starting cells already have a substantial amount of masked sites, making it unnecessary to invoke this mechanism. The most logical possibility is that unmasking is due to the action of an endogenous sialidase. Indeed, prior studies have suggested the existence of lymphocyte sialidases that can affect activation and cell:cell interactions (Guthridge et al., 1994; Chen et al., 1997) and a neutrophil sialidase (Cross and Wright, 1991) that is released upon activation. We were able to show that some cell surface desialylation is indeed taking place rapidly upon activation of neutrophils. However, our attempts to prevent the unmasking of monocytes, neutrophils, and U937 cells by a well-known inhibitor of sialidases were not successful. Based on changes in cell surface lectin binding, it appears that this inhibitor is not very active against this particular sialidase. Better inhibitors and/or sialidase-deficient cells are required to pursue this matter further. There could be of course be additional cooperating mechanisms, such as a conformational change in the lectin molecules resulting in improved binding, an induced clustering of lectin molecules generating enhanced binding of the multivalent probe, the action of a selective protease that cleaves the endogenous ligands, conformational changes of endogenous masking ligands which lower their affinity, or a redistribution of the lectins to a privileged site on the cell surface (e.g., the filopodia).

Regardless of the mechanism involved, we can also ask when (during the life of each cell) this unmasking actually occurs in vivo, and what the biological consequences might be. Answering these questions will require the isolation, cloning and full characterization of each of the proteins with novel sialic acid binding sites that we have detected. Logical approaches include affinity purification and/or expression cloning after sialidase treatment to remove potentially masking sialic acid residues. Of course, some of these molecules may turn out to be previously cloned leukocyte cell surface proteins whose sialic acid binding properties were missed earlier, because of masking by endogenous ligands.

Materials and methods

General chemicals and biologicals

These were mostly from Sigma or Oxford GlycoSystems. The others were phorbol ester (PMA)² from GIBCO/BRL and Ionomycin from Calbiochem.

Antibodies and probes

Tri-color-conjugated anti-human CD22, CD14, CD56, and CD19 mAbs, Caltag Laboratories; CyChrome-tagged anti human CD4 and FITC-tagged antihuman CD8, Pharmingen, and Phycoerythrin-conjugated streptavidin (SA-PE), Boehringer-Mannheim. The FITC or biotin-conjugated polyclamylamid substituted with α2–6 sialyllactose (6’PAA-FITC and 6’PAA-B, respectively), its analog with α2–3 Sia substitution, (3’PAA-B ), a nonsialylated version (Lac-PAA), and non-biotinylated forms of all conjugates were from GlycoTech. The sialidase inhibitor 2,3 dehydro-2,6 anhydro-N-acetyl-neuraminic acid (Neu2en5Ac) was from Calbiochem.

Peripheral blood mononuclear preparations

Fresh peripheral blood samples from normal human volunteers and mononuclear cells were separated by a standard ficoll-hypaque density gradient, diluted 1/1 with serum-free RPMI medium, followed by two washes with the same medium. In other studies, we used Mono-Poly Resolving Medium Ficoll-hypaque (ICN Biomedical, Inc.) according to the manufacturer’s instructions (except that centrifugation speed and time were adjusted for optimum separation), allowing the simultaneous separation of two bands, one containing mononuclear cells, and the other containing neutrophils.

Cell lines

U937 cells were cultured in RPMI 1640 medium with 10% FCS and l-glutamine. T cell leukemia lines CEM, MOLT-4 and Jurkat were grown in serum-free medium supplemented with 1% ITP (insulin, transferrin, and selenium) supplement (Sigma).

Sialidase treatments

Cells were resuspended in serum-free RPMI medium (1–5 ×106 cells/ml) containing 0.05 M HEPES, pH 6.9 and incubated for 15 min at room temperature (RT) with 20 mU of Arthrobacter ureafaciens sialidase (AUS). Excess sialidase
Mild periodate treatment

Cells were washed with PBS and resuspended (1–5 × 10⁶ cells/ml) in phosphate buffer pH 7.4 containing freshly dissolved 2 mM NaIO₄, and incubated for 30 min at 4°C in the dark. Excess periodate was destroyed by adding 10 µl of 20% glycerol followed by immediate washing with staining buffer. Treatment of the probes with mild periodate followed a similar protocol. In each case, the “sham” treatment consisted of premixing the periodate and the glycerol, and then adding them to the probe sample.

Activation of cells

PBMCs, neutrophils, or U937 cells (1 × 10⁶/ml) were resuspended in RPMI supplemented with 1% L-glutamine, 1% PenStrep (complete RPMI), and activated with 10–100 ng/ml PMA and 1 µM ionomycin (for prolonged activation studies, 10% FCS was added). Cells from each well (3 × 10⁵) were removed in different times for the flow cytometry analysis to probe for the presence of sialic acid binding sites and/or other lectin binding sites.

Flow cytometric analysis

Flow cytometry was performed on a Becton Dickinson FACS can machine. The binding activity of cell surface sialic binding lectins was examined after several washings with staining buffer by incubating the cells (intact or with sialidase or periodate treatment) in 100 µl staining buffer containing 1–1.5 µg 6PAA-B probe for 1 h on ice (conditions of time and probe concentration were determined to be optimal). After washing once with 0.5 ml of the staining buffer, cells were incubated with phycoerythrin-conjugated streptavidin for 30 min to detect binding of the biotinylated probe.

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Abbreviations

PMA, phorbol ester; PAA, polyacrylamide; PAA-B, PAA substituted with biotin; 6PAA, PAA, substituted with α2–6 sialyllectose; 3PAA, PAA substituted with α2–3 sialyllactose; Sia, sialic acid; AUS, Arthrobacter ureafaciens sialidase; PBMCs, peripheral blood mononuclear cells; Tc, Tricolor fluorophore; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SA-PE, streptavidin conjugated with PE.

Note Added in Proof

Since the original acceptance of this manuscript, we have discovered, characterized, and reported an additional member of the human Siglec family, called OB-BP-1/Siglec-6 (Patel et al., 1999). Amongst human leukocytes, this Siglec is found only on B cells, and does not appear to bind to the 6’ or 3’ sialyllactose probes used in this study. Thus, Siglec-6 is not responsible for any of the binding phenomena noted in the present study.

References


Unmasking of sialic acid–binding lectins