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Siglecs in the immune system

PAUL R. CROCKER* & AJIT VARKI† *The Wellcome Trust Biocentre at Dundee, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK, and †Glycobiology Research and Training Center, Department of Medicine and Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

INTRODUCTION

Siglecs[1] (sialic acid binding Ig-like lectins) are I-type (Ig-type) lectins[2] characterized by an N-terminal V-set Ig domain that mediates sialic acid binding,[3] followed by varying numbers of C2-set Ig domains (Fig. 1). The initial discovery of this lectin family came about through independent studies on sialoadhesin (Siglec-1/CD169), a macrophage lectin-like adhesion molecule,[4] and CD22 (Siglec-2), a B-cell restricted member of the Ig superfamily (IgSF)[5] that plays an important role in regulating B-cell activation. Both molecules were found to mediate cell–cell interactions *in vitro* via recognition of sialylated glycoconjugates.[6–10] The cloning of sialoadhesin[11] revealed striking sequence similarities to CD22 and led to the demonstration that two other related IgSF proteins, myelin-associated glycoprotein (MAG/Siglec-4) and CD33 (Siglec-3), which were not previously known to bind sialic acids, were also members of the Siglec family (Table 1).[12,13]

Six additional human Siglecs (Siglecs 5–10) have been identified and characterized over the last 3 years. These previously unknown molecules share a high degree of sequence similarity with CD33 in their extracellular and intracellular regions, and are hence collectively referred to as 'CD33-related Siglecs'. A striking feature of the CD33-related Siglecs is their differential expression pattern amongst the cell lineages of the haemopoietic system (see Table 1). This, together with the presence of two conserved immunoreceptor tyrosine-based inhibition motif (ITIM)-like motifs in their cytoplasmic tails, suggests that, like CD22, CD33-related Siglecs are involved in regulating cellular activation within the immune system. Here we discuss how sialic acid recognition by Siglecs might contribute to the regulation of immune functions.

SIALIC ACID RECOGNITION BY SIGLECS AND *CIS* INTERACTIONS WITH ENDOGENOUS GLYCANS

Sialic acid is a generic term for a large family of 9-carbon sugars that are all derivatives of neuraminic acid (Neu) or keto-deoxynonulosonic acid (KDN). They are typically found at the exposed, non-reducing ends of oligosaccharide chains attached to a wide variety of proteins and lipids. Many different types of sialic acid exist in mammals and each can occur in a variety of glycosidic linkages. Thus, in addition to their well-known roles in preventing cell–cell interactions through charge-repulsion effects and in masking subterminal sugars, sialic acids are also well situated to act as ligands for mediating selective cell–cell interactions (reviewed in 14–16). With the exception of Siglec-6, which shows restricted specificity for the sialyl Tn antigen,[17] all of the known Siglecs recognize forms and linkages of sialic acid that are commonly found at cell surfaces and in the extracellular environment. One consequence is that the Siglec binding site can be masked by *cis* interactions with sialic acids on the same cell surface, thereby preventing them from mediating cell–cell interactions (Fig. 2).

This phenomenon of Siglec masking has been systematically studied with regard to CD22. When human or mouse CD22 was expressed in COS or CHO cells, high levels of sialic acid-dependent binding were observed.[5,12,18–21] However, both these cell types lack ST6Gal-I, the sialyltransferase that creates the sialylated sequence recognized by CD22·[22] When ST6Gal-I was coexpressed with CD22, all CD22 binding activity was lost, but could be restored by sialidase treatment of the transfected cells.[23,24] Since B cells normally express ST6Gal-I and therefore carry CD22 ligands, these observations raised the issue of whether CD22 expressed naturally on B cells can mediate binding to exogenous ligands. Using an α2-6-sialyllactose-based synthetic probe that binds strongly to recombinant CD22, there was indeed no detectable binding to human peripheral blood B lymphocytes[25] and to most mouse B cells[26] due to masking of the sialic acid binding site (see below). However, activation of human B cells or sialidase treatment led to unmasking of CD22 and readily detectable probe binding.[25] Similar findings were obtained using human peripheral blood leucocytes which collectively express all of the CD33-related Siglecs[27] (Table 1). These studies indicate that most Siglecs exist on resting cells in a masked form, but that unmasking may occur during cellular activation. Sialoadhesin appears to be an exception to this general theme since it is able to mediate binding to other cells when expressed naturally on stromal tissue macrophages[4,7] (Fig. 2a).

Cis interactions with sialic acids in the same plasma membrane could potentially regulate Siglec functions in a variety of different ways. For example, they could prevent cell–cell interactions until they are needed or facilitate specific interactions with other proteins on the same cell surface. Conversely, they could be preventing specific protein–protein interactions by non-specifically engaging the Siglecs to other

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Correspondence: Paul R. Crocker, The Wellcome Trust Biocentre at Dundee, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK. E-mail: p.r.crocker@dundee.ac.uk

sialylated molecules. Regardless of which of these mechanisms is operative in a given cell type, the actual 'unmasking' of the Siglec could be mediated by a variety of possible mechanisms, including a cell surface sialidase, via altered glycosylation, or by reorganization of plasma membrane domains (Fig. 2a).

SIALOADHESIN AND CELLULAR INTERACTIONS IN THE IMMUNE SYSTEM

Sialoadhesin differs from other Siglecs expressed in the haemopoietic system in several significant ways. First, it has 17 Ig-like domains (compared with a maximum of seven found in other Siglecs; see Table 1). Secondly, unlike other Siglecs, it is able to mediate sialic acid-dependent binding to other cells when expressed in its native environment (i.e. macrophages).[4,7] Thirdly, it lacks tyrosine-based motifs in the cytoplasmic tail. It would therefore appear that sialoadhesin has been selected during evolution to mediate extracellular rather than intracellular functions. This is supported by the recent cloning of the human sialoadhesin orthologue[28] which also has 17 Ig-like domains, and shares high sequence similarity (72% identity) with its mouse counterpart in the extracellular domains, but only low homology (30% identity) in the cytoplasmic tail. Sialoadhesin is expressed on resident macrophages in many tissues and also by inflammatory macrophages such those found in rheumatoid arthritis.[28] Tumour-infiltrating macrophages in breast cancer also express high levels of sialoadhesin, and these macrophages were shown to make intimate contact with carcinoma cells expressing MUC-1, a membrane mucin which can be bound specifically by sialoadhesin.[29] Sialoadhesin has also been implicated in functionally important interactions with T lymphocytes that lead to generation of cytotoxic T cells in a graft-vs.-leukaemia model.[30] CD43, a cell surface mucin-like molecule on T cells, has been identified as a potentially important counter-receptor for sialoadhesin.[31] The widespread expression of ligands for this Siglec on cell surfaces and the extracellular matrix and its ability to mediate cellular interactions, even in the presence of 100% plasma,[4] suggest that sialoadhesin may be involved in diverse cell-cell and cell–matrix interactions of macrophages.

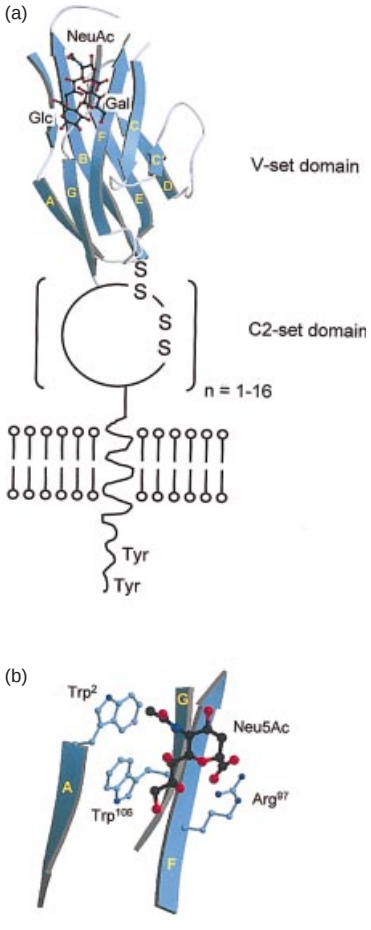


Figure 1. Structural features of Siglecs. (a) Siglecs are type I membrane proteins with an extracellular region containing a sialic acid binding V-set Ig-like domain at the N-terminus and 1–16 C2-set Ig-like domains. The cytoplasmic tails of all Siglecs apart from sialoadhesin have one or more tyrosine residues within potential signalling motifs. A crystal structure of the sialoadhesin N-terminal domain complexed to 3′-sialyllactose[3] revealed some key features of sialic acid recognition by Siglecs, including the presence of an essential arginine (Arg^{97}) on the F strand (conserved in the other Siglecs) which forms a salt bridge with the carboxylate of sialic acid (b). The V-set and adjacent C2-set domains of Siglecs contain an unusual arrangement of conserved cysteines that give rise to an intrasheet disulphide (rather than the more usual intersheet disulphide) within the V-set domain and a disulphide between the domains. The conserved intrasheet disulphide in sialoadhesin results in widening of the Ig β-sandwich and exposure of two tryptophans (Trp2 and Trp106) on the A and G strands that form hydrophobic contacts with the N-acetyl and glycerol side groups of NeuAc, respectively (see 3 for further details).

CD22 AND CELL–CELL INTERACTIONS

Despite the fact that CD22 is generally masked on B cells, recent studies suggest that CD22 plays a role in homing of B cells to the bone marrow through interactions with sialylated ligands expressed on bone marrow sinusoidal endothelium.[26,32,33] CD22-deficient mice were found to lack mature (IgD^{hi}) B cells in the bone marrow but not in the spleen.[32] Adoptive transfer of CD22-deficient B cells into RAG–/– mice resulted in reduced entry into the bone marrow when compared with normal B cells.[32] Staining of mouse bone marrow cryostat sections with recombinant mCD22-Fc chimaeras revealed high levels of CD22 ligands on bone marrow endothelium that were absent from endothelial cells in other tissues.[33] In addition, injected recombinant CD22-Fc was able to bind *in vivo* to marrow endothelium and reduced the number of mature B cells in the bone marrow by around 50%. These findings prompted a careful re-examination of the masking status of CD22 on B cells from bone marrow, spleen and lymph node.[26] Although the majority of B cells in all tissues expressed the typical masked forms of CD22, the number of B cells with unmasked CD22 was enriched 2–5-fold in the bone marrow, when compared with those from the spleen or mesenteric lymph nodes. One interpretation is that the subset of transiently circulating B cells with unmasked CD22 is able to bind bone marrow endothelial cell ligands, leading to transmigration into the marrow parenchyma and local enrichment of these cells. Although the biological significance of CD22-dependent lymphocyte homing is not well understood, the bone marrow is a major site of Ig production.[34] CD22-deficient mice have reduced numbers of IgM-secreting plasma cells in

Table 1. Properties of Siglecs

Siglec Alternative name (Ig domains)	Species	Cell types	Comments	References
Sialoadhesin	Human	Macrophages	Subsets of resident and inflammatory macrophages	11, 28
Siglec-1/CD169	Murine		Does not require unmasking to mediate binding	
(17)			No Tyr-based motifs in cytoplasmic tail	
			Prefers α2-3 linked sialic acid over α2-6 linkage	
CD22	Human	B cells	Negative regulator of B-cell activation	8, 9, 33, 43
Siglec-2	Murine		Recruits SHP-1 to three cytoplasmic ITIMs	
(7)			Potential role in B-cell homing to bone marrow	
			Binding site masked on resting B cells	
			Strong preference for α2-6-linked sialic acids	
CD33	Human	Myeloid progenitors	Commonly used as a marker of early myeloid cells that is absent from haemopoietic stem cells	13, 64, 65
Siglec-3	Murine	Monocytes	Two cytoplasmic ITIM-like motifs	
(2)		Macrophage subsets	Recruits SHP-1 and SHP-2 to ITIM-like motifs	
			Cocross-linking with Fc receptor inhibits Ca flux	
MAG	Human	Oligodendrocytes	Role in myelin maintenance, inhibitor of axonal growth	12, 84
Siglec-4	Murine	Schwann cells	Alt. spliced cytoplasmic tail with Tyr-based motif	
(4)	Rat		Prefers α2-3 linked sialic acid over α2-6 linkage	
Siglec-5	Human	Monocytes	Two ITIM-like motifs	48, 77
CD170	Murine?[1]	Neutrophils	Binds equally to α2-3- and α2-6-linked sialic acids	
(4)		B cells[1]		
Siglec-6	Human	Trophoblasts	Identified as low-affinity leptin receptor	17, 77
(3)	Murine?[1]	B cells	Two ITIM-like motifs	
			Restricted specificity for sialyl Tn structure	
Siglec-7	Human	NK cells	Inhibitory receptor of NK cells	49, 52, 54, 58, 59
p75/AIRM-1	Murine?[2]	Monocytes	Alt. spliced form lacking Ig-like domain 2	
(3)			Two ITIM-like motifs	
			Recruits SHP-1	
			Binds equally to α2-3 and α2-6-linked sialic acids	
Siglec-8	Human	Eosinophils	Alt. spliced forms with two ITIM-like motifs	50, 51, 53, 63
(3)	Murine?[2]	Basophils (lo)	Prefers α2-3 linked sialic acid over α2-6 linkage	
		Mast cells (grown *in vitro*)		
Siglec-9	Human	Monocytes	Two ITIM-like motifs	56, 57
(3)	Murine?[2]	Neutrophils	Binds equally to α2-3 and α2-6-linked sialic acids	
		B cells (lo)	Binds strongly to SLe^x	
		NK cells (lo)		
Siglec-10	Human	NK-like cells	Two ITIM-like motifs, one Grb-2-like motif	53
(5)		B cells	Binds equally to α2-3 and α2-6-linked sialic acids	
		Eosinophils (lo)		
		Monocytes (lo)		

[1]Unpublished observations (P. Crocker).
[2]A mouse CD33-related Siglec designated mSiglec-E/MIS has recently been characterized that shares greatest sequence similarity with hSiglecs 7, 8 and 9.

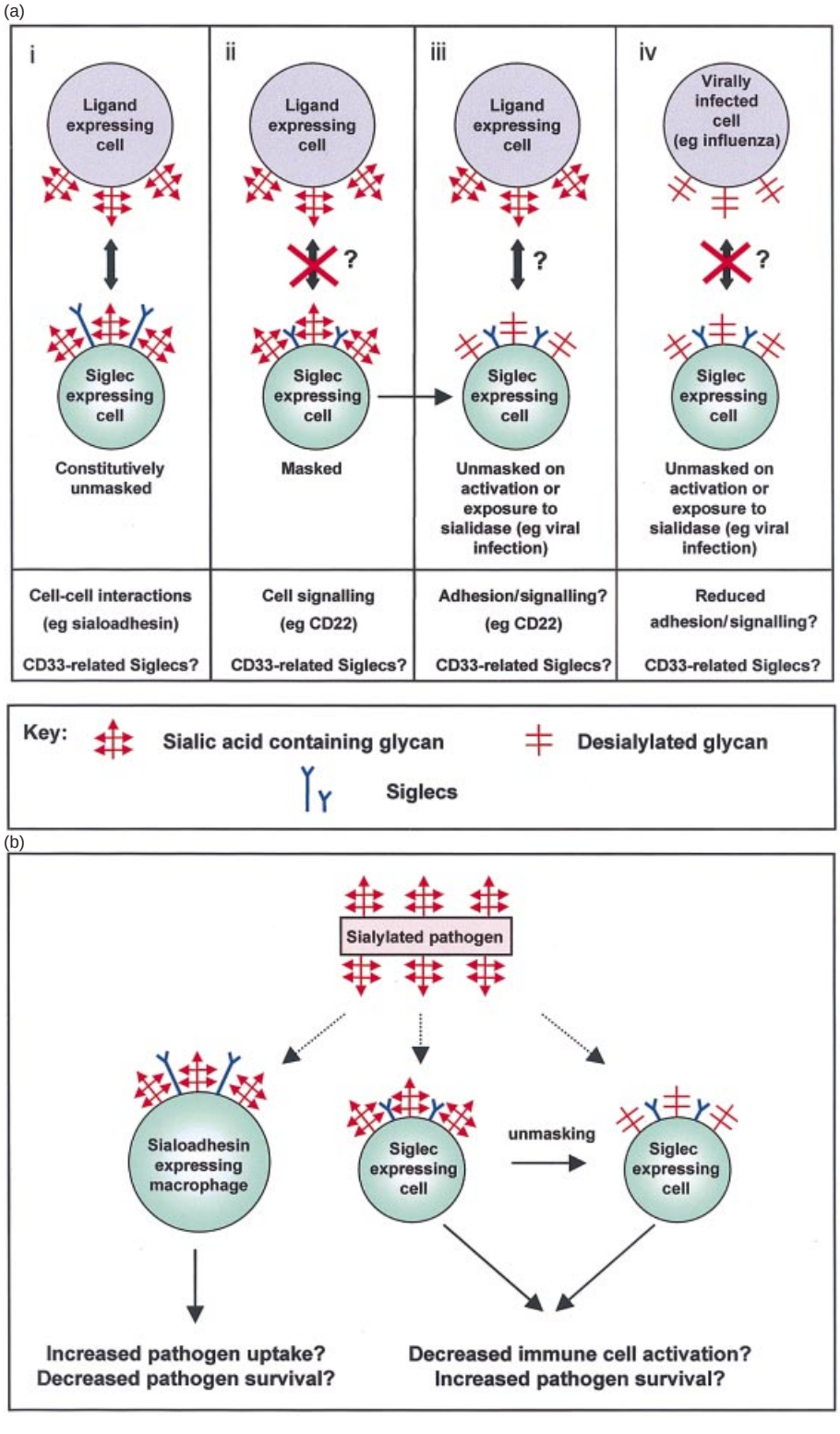
bone marrow, indicating that some plasma cell precursors use a CD22-dependent pathway to enter the bone marrow.[33] Interestingly, TNF-α and IL-1-β were shown to cause an up-regulation of CD22 ligands on endothelial cells, suggesting a possible effect on B-cell trafficking in conditions of inflammation.[20]

CD22 AND B CELL SIGNALLING

The importance of balancing positive and negative signals is an emerging theme in the immune system (see 35 for a recent excellent review). When cellular activation is triggered by receptors with immunoreceptor tyrosine-based activation motifs (ITAMs), counteracting inhibitory signals can be delivered through receptors bearing ITIMs. Following phosphorylation by Src family kinases, ITIMs can recruit phosphatases, either the inositol phosphatase SHIP, or more commonly the protein tyrosine phosphatases SHP-1 and SHP-2. These phosphatases inhibit signalling pathways through distinct mechanisms, resulting in raised activation thresholds.

CD22 is a well-characterized inhibitory receptor for B-cell receptor (BCR) signalling. This has been most clearly demonstrated using B cells from CD22-deficient mice[32,36–38] which show an enhanced and prolonged Ca influx after BCR stimulation in comparison to normal B cells. B cells from CD22-deficient mice also exhibit a mildly activated phenotype, respond more robustly to LPS and have a tendency to generate autoantibodies with increasing age.[39] However, B-cell responses to T-dependent antigens appear not to be grossly affected, as shown by normal germinal centre formation and switched Ig isotypes in CD22-deficient mice.[32,36–38]

CD22 is constitutively associated with the BCR complex.[40,41] Following BCR engagement it becomes tyrosine phosphorylated in its cytoplasmic tail by Lyn.[42] This leads to recruitment and activation of SHP-1 through binding to three ITIMs (reviewed in 43). Phosphorylated CD22 can also bind other signalling molecules such as Syk, PLCγ, PI3 kinase, Grb-2 and Shc, suggesting a potential role in positive as well as negative signalling.[44,45] Sequestration of CD22 away from the BCR using anti-CD22 coated beads lowered the activation threshold by a factor of 100, and resulted in a strong activation



of the MAP kinase pathway in comparison to unligated CD22.[46,47] It was proposed that interactions with other lymphocytes expressing high levels of α2–6 sialylated glycans could result in a similar sequestration, thus providing a molecular switch that may bias signalling through BCR to anatomical sites rich in lymphoid tissues. However, key unresolved issues are whether sequestration can occur physiologically, whether sialylation of the BCR is important for its association with CD22 and whether alterations in sialylation that are known to occur on B-cell activation influence the association of CD22 with the BCR and subsequent activation response to antigens. Interestingly, ST6Gal-I-deficient mice which lack CD22 ligands display a B-cell immunodeficiency phenotype somewhat unlike that of CD22-deficient mice.[22] These findings suggest a more general role of ST6Gal-I in regulating B-cell responsiveness, perhaps also involving interactions with other Siglecs expressed on B cells (see Table 1). In the final analysis, the precise biological role of the sialic acid binding activity of CD22 remains unresolved.

NEW CD33-RELATED SIGLECS IN THE INNATE IMMUNE SYSTEM

Human Siglecs 5, 7, 8 and 10 were identified in expressed sequence tag (EST) databases as being homologous to CD33, and cloning of full-length cDNAs showed that they possess the key features required for sialic acid recognition (Fig. 1).[48–53] Siglec-6 was isolated in a screen for leptin-binding proteins[17] and Siglec-7 was independently identified as a NK cell inhibitory receptor in a redirected killing assay and designated p75/AIRM-1.[54] Siglec-9, a close homologue of Siglec-7, was identified as a Siglec-like gene[55] and also during characterization of Siglec-like cDNAs.[56,57] Most recently, a novel mouse Siglec with three Ig-like domains and two ITIM-like motifs, similar to hSiglecs 7, 8 and 9, was identified by one group as a CD33-like EST sequence (MIS)[58] and by another as a binding partner for SHP-1 in a yeast two-hybrid screen (mSiglec-E).[59]

Human CD33-related Siglecs contain between two and five Ig-like domains and share a high degree of sequence similarity (~50–80%). The genes encoding them are all clustered on chromosome 19q13.3–13.4[60] and seem to have evolved by gene duplication. Until recently, the only published murine CD33-related Siglec was a CD33-like protein with two Ig domains which, however, lacked the cytoplasmic ITIM-like motifs.[61] Phylogeny analyses revealed that this molecule does not form an exclusive clade with hCD33, being instead loosely associated with several human CD33-related Siglecs.[56] The eventual classification of CD33-related Siglec orthologues in mice will require the identification of all candidates from the mouse genome project, and the detailed characterization of each with regard to expression patterns, binding specificities and functions. It remains to be seen if all of the human CD33-like Siglecs share a corresponding mouse orthologue and *vice versa*.

Monoclonal antibodies recognizing each of the new human Siglecs have allowed a detailed analysis of their expression patterns on blood leucocytes (Table 1). Some are expressed quite broadly; e.g. Siglec-9 is found on neutrophils, monocytes and a substantial fraction of NK cells and B cells.[57] Others are much more restricted in their expression, notably Siglec-8 which is found only on circulating eosinophils[50,51] and at very low levels on basophils.[51] On the other hand, several Siglecs can be present on the same cell type (e.g. monocytes express CD33 and Siglecs 5, 7, 9 and 10[48,49,53,56,57,62]), suggesting some degree of functional redundancy at the cellular level. Regardless, each CD33-related Siglec exhibits a unique expression pattern amongst haemopoietic cells, indicative of specific functions. Siglec-6 is also prominently expressed by cyto- and syncytiotrophoblasts of the placenta, in addition to B cells.[17] Limited information is currently available on the expression of the other CD33-related Siglecs on cells outside the haemopoietic system.

CD33-RELATED SIGLECS AS INHIBITORY RECEPTORS

The presence of two conserved ITIM-like motifs in the cytoplasmic regions of CD33-related Siglecs and the differential expression of these proteins on different types of leucocytes suggests a role in regulating cellular activation. Siglec-8, which was originally thought to lack ITIM-like motifs,[50,51] has now been found to exist in alternatively spliced forms containing such sequences.[53,63] While the membrane proximal motif of all the CD33-related Siglecs fits the consensus ITIM sequence (Ile/Val/Leu/Ser)-X-Tyr-X-X-(Leu/Val), the membrane distal motif does not. Following treatment of cells with pervanadate to inhibit tyrosine phosphatases, hCD33, Siglec-7 and MIS/mSiglec-E became tyrosine phosphorylated.[54,58,59,64–66] Under these conditions, Siglec-7 recruited only SHP-1, whereas CD33 and MIS/mSiglec-E bound both SHP-1 and SHP-2. Mutation of the tyrosine in the distal motif did not significantly affect recruitment of SHP-1

Figure 2. Diagram illustrating how the interaction of Siglecs with cells or sialylated pathogens could be affected by *cis* interactions with sialylated glycoconjugates. (a) (i) Some Siglecs such as sialoadhesin appear to be unmasked and able to mediate cell–cell interactions constitutively whereas (ii) the majority of Siglecs appear to be naturally masked due to *cis* interactions with adjacent sialic acids. *Cis* interactions may be important for signalling functions; for example CD22 is closely associated with the B-cell receptor complex and provides inhibitory signals that raise B-cell activation thresholds. (iii) Unmasking of Siglecs can occur in some cases by cellular activation or by exposure to sialidases. The unmasked Siglec may now be capable of stronger interactions with ligands on other cells. This could result in increased cellular interactions and/or signalling. (iv) In another scenario, a virally infected cell expressing a viral sialidase (neuraminidase) on the cell surface would result in loss of Siglec ligands and reduced interactions with unmasked Siglecs on cells of the innate immune system. In this situation, unmasking of Siglecs could also occur by close cell–cell contact with the virally infected cell. (b) Some pathogens can express sialylated glycoconjugates (see text for details) which may interact with Siglecs. In the case of sialoadhesin, this may lead to increased uptake of pathogens by macrophages and therefore be detrimental to the pathogen. In the case of CD22- and CD33-related Siglecs, interactions with sialylated pathogens may lead to subversion of the normal signalling functions of Siglecs (e.g. reduced activation) which could result in increased pathogen survival.

and SHP-2. In contrast, an equivalent mutation in the proximal motif had a major effect.[58,59,64,65] The much weaker binding of the distal motif to SHP-1 and SHP-2 presumably reflects its departure from the consensus ITIM sequence.[59,64] However, the distal motif is highly conserved in all CD33-related Siglecs, suggesting that it may be important for interacting with other regulatory molecules. In this regard, for some CD33-related Siglecs it is similar to a motif [Thr-Ile-Tyr-X-X-(Val/Ile)] found in signalling lymphocyte activation molecule (SLAM) and SLAM-related proteins that bind SLAM associated protein (SAP), a T-cell specific molecule that is known to inhibit SHP-2 recruitment to SLAM.[67,68]

Functional evidence indicating that Siglecs can mediate inhibitory signals has been obtained using antibodies to cocross-link CD33[65,66] or MIS/mSiglec-E[58] with an activating human receptor, *Fc*γRI. This resulted in reduced Ca influx compared with cross-linking *Fc*γRI alone. Likewise, Siglec-7 was identified as an inhibitory NK cell receptor in a redirected killing assay in which anti-Siglec-7 antibodies were used to cluster Siglec-7 at the NK cell–target cell interface.[54] The physiological relevance of these interesting findings remains unclear, since antibodies rather than natural ligands were used to cluster the Siglecs. In other functional studies the addition of intact anti-CD33 or Siglec-7 monoclonal antibodies to haemopoietic cell cultures resulted in reduced cell growth[62] and prevention of dendritic cell development.[69] However, it is not clear if the intact antibodies used in these studies could also have interacted with myeloid cell Fc receptors, giving rise to 'confused signals' not necessarily relevant to Siglec functions. Clearly, additional experimental approaches are needed to elucidate the functions of CD33-related Siglecs.

COULD SIALIC ACID ACT AS A MARKER OF 'SELF' TO REGULATE CELL ACTIVATION?

Many of the recently characterized ITIM-containing receptors are expressed by myeloid cells of the innate immune system (reviewed in 35). An emerging theme is that these inhibitory receptors can interact with broadly expressed 'self' ligands and thus regulate myeloid cell activation. This could be an important mechanism for preventing inappropriate autoreactivity during immune responses. For example, MHC class I molecules are displayed on most nucleated cell types and serve as ligands for both NK cell inhibitory receptors, and also for the Ig-like transcript (ILT)-2 and ILT-4 inhibitory receptors found on myeloid cells.[35]

Recently, an inhibitory receptor of myeloid and neuronal cells called SIRP-α was shown to bind the widely expressed molecule CD47.[70–72] When CD47-deficient erythrocytes were injected into normal mice they were rapidly phagocytosed by splenic red pulp macrophages, unlike normal erythrocytes.[73] Thus, CD47 may act as a marker of 'self' that prevents inappropriate macrophage phagocytosis by interacting with the inhibitory receptor SIRP-α. Another inhibitory receptor of the Ig superfamily is the OX2 receptor (OX2R), expressed specifically by macrophages and granulocytes.[74] OX2R contains three tyrosine-based motifs and interacts with OX2, a structurally similar molecule that lacks signalling motifs but is expressed widely.[74] In OX2-deficient mice, the major phenotype was associated with the myeloid cells that express OX2R, generating microglia (brain macrophages) with an 'activated' phenotype[75] and increased numbers of myeloid cells in lymphoid organs.[76] OX2-deficient mice also exhibited a more rapid onset and greater susceptibility to experimental autoimmune diseases.

In a similar way, we suggest that sialic acids might act as broadly expressed 'self' ligands that interact with CD33-related Siglecs on myeloid cells, thereby preventing inappropriate self-reactivity. Unlike most other commonly found sugars, sialic acids are thought to have appeared relatively late in evolution, and therefore should be absent from the majority of potential pathogens. Indeed, recent genomic database searches of *Caenorhabditis elegans and Drosophila melanogaste*r failed to uncover obvious sequences encoding known enzymes of the sialic acid biosynthesis pathway.[56]

The interaction of Siglecs with host sialic acids is likely to be affected by a number of different factors. On the one hand, a Siglec on an effector cell that becomes unmasked upon activation might become re-engaged if there is an adjacent normal host cell with cell surface sialic acids (Fig. 2a). On the other hand, if the host cell is infected with a sialidase-producing virus (e.g. influenza A or B), or has been extensively exposed to a secreted bacterial sialidase, its cell surface would be devoid of sialic acids, and hence could not engage the unmasked Siglec. In these hypothetical scenarios, the re-engagement of the unmasked Siglec (or the lack thereof) could affect its clustering, and thus the phosphorylation state of the cytoplasmic ITIM motifs, eventually modulating the activation state of the effector cell in the most appropriate direction. All of these possibilities should be testable in *in vitro* systems.

DO MICROORGANISMS EXPRESS SIALIC ACIDS TO SUBVERT SIGLEC FUNCTION?

Despite the rarity of sialic acids in lower organisms, it is striking that many known pathogens have independently evolved the capacity to express sialic acids.[15] For example, pathogenic bacteria such as groups B and C *Neisseria meningitidis*, *Haemophilus influenzae*, *Haemophilus ducreyi*, *Escherichia coli* K1 and group B *Streptococci* synthesize their own sialic acids. In contrast, *Trypanosoma cruzi* and *Corynebacterium diphtheriae* transfer sialic acids from host glycoconjugates using a *trans*-sialidase. Sialic acids can also be captured from host CMP-sialic acid by a cell surface sialyltransferase, e.g. by *Neisseria gonorrhoea*. In some instances, the source of the sialic acids is unknown, e.g. fungal pathogens such as *Cryptococcus neoformans* and *Candida albicans*. With several of these organisms, the expression of sialic acid has been shown to be essential for pathogenicity and survival within the host. Whilst the sialic acids on these pathogens are likely to be important for host mimicry, prevention of complement activation, attenuation of antibody production and non-specific charge-repulsion effects, an additional possibility is that they interact with Siglecs and thus modulate the outcome of the host–pathogen encounter (Fig. 2b). On the one hand, sialylated molecules on pathogens could interact with unmasked Siglecs and reduce activation responses, leading to increased survival within the host. On the other hand, interactions with sialoadhesin expressed on macrophages might lead to increased uptake and therefore be detrimental to the pathogen. Clearly, many unanswered questions remain. For

example, can resting leucocytes with masked Siglecs engage sialic acids on pathogens or are activation and unmasking required first (Fig. 2b)?

ARE SIALIC ACID MODIFICATIONS AND UNDERLYING GLYCAN STRUCTURE RELEVANT TO SIGLEC FUNCTION?

Most Siglecs seem to recognize only a terminal sialic acid, usually in conjunction with its linkage to the next sugar. Thus, the primary regulation of Siglec ligand formation is likely to be dictated by the differential expression of various sialyltransferases in different cell types. However, in some instances, the structure of the underlying sugar chain can also affect binding. For example, the addition of a fucose residue to certain α2–3 sialylated sugar chains can markedly reduce binding by most Siglecs.[77] Interestingly, this fucosylation also generates the structure Sialyl Lewis X (SLe^X), a well-known signature motif for recognition by the selectin family of cell adhesion molecules.[78] Of note, the fucosyltransferases responsible for adding this fucose residue are also highly regulated in their expression amongst various haemopoietic cell types.[78] Further complexity in Siglec biology arises from the fact that certain natural modifications of sialic acids can enhance or abrogate recognition. For example, the addition of a 9-O-acetyl group to α2-6-linked or α2-3-linked sialic acids can block recognition by CD22[8] or sialoadhesin,[79–81] respectively, and such modifications are found in potentially important locations, such as the spleen and bone marrow. In another example, the presence of an N-glycolyl instead of an N-acetyl group at the 5-position of sialic acids can abrogate binding by sialoadhesin[79] and MAG,[81] whilst enhancing recognition by mouse CD22.[79,82] In this regard, a human-specific mutation in the enzyme that generates the N-glycolyl group might explain a markedly different distribution of sialoadhesin-positive macrophages found between humans and chimpanzees.[83]

CONCLUSIONS

Siglecs are expressed abundantly on many cells of the immune system and are therefore likely to be important in both innate and acquired immune responses. Sialoadhesin is a macrophage-restricted molecule that has the potential to interact with a wide variety of ligands on other cells and in the extracellular matrix. CD22 is a well-characterized inhibitory receptor of B cells that may also mediate cell–cell interactions following unmasking of its sialic acid binding site. CD33 is a marker of myeloid progenitors with the potential to function as an inhibitory receptor. The discovery of six new CD33-related haemopoietic Siglecs with features of inhibitory receptors indicates that sialic acid recognition plays a more important role in regulation of the immune system than had been hitherto considered. Although at the present time we can only speculate about how this might occur, the paradigms established with CD22 and other ITIM-containing receptors of the haemopoietic system provide a useful starting point for future investigations. It is hoped that the generation and characterization of genetically manipulated mice lacking Siglecs and Siglec ligands or expressing mutated Siglecs will shed considerable light on the roles of Siglecs and their sialic acid-recognizing properties in the immune system.

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