Semin Immunopathol (2012) 34:353–364
DOI 10.1007/s00281-012-0310-3

REVIEW

Sialoadhesin in recognition of self and non-self

Mariliis Klaas · Paul R. Crocker

Received: 3 November 2011 / Accepted: 5 March 2012 / Published online: 27 March 2012

Abstract The immune system is tightly regulated to maintain an appropriate balance between immune activation and tolerance. Macrophages play a key role in this process since they express many pathogen recognition molecules as well as receptors for 'self'. Sialoadhesin is a major macrophage receptor that specifically recognizes sialic acid, an abundant component of host glycoconjugates but which can also be found on several human pathogens. In recent years, several studies have demonstrated that sialoadhesin can contribute to the uptake and processing of sialylated pathogens as well as playing an important role in regulating inflammatory and autoimmune responses via recognition of self.

Keywords Sialoadhesin · Sialic acid · Innate immunity · Pathogens

Introduction

A key role of the immune system is to discriminate between 'self' (the host) and 'non-self' (e.g. pathogens) and make appropriate responses to maintain tissue homeostasis. Macrophages (Mϕs) express several proteins on their surface that distinguish 'self' from 'non-self', and therefore these cells are central to immune regulation via sensing and responding to pathogens [47]. Carbohydrates on the surfaces of both host cells and pathogens are critically involved in this discrimination process. Here we focus on the role of the Mϕ sialic acid receptor sialoadhesin (Sn) in the 'self' versus 'non-self' recognition and phagocytic functions of Mϕs during immune responses.

This article is published as part of the Special Issue on Glycosylation and Immunity [34:3].

M. Klaas · P. R. Crocker (✉)
Division of Cell Signalling and Immunology,
College of Life Sciences, University of Dundee,
Dow Street,
Dundee, UK
e-mail: p.r.crocker@dundee.ac.uk

Discovery and characterisation of Sn

Sn was first described in 1985 as a non-phagocytic sheep erythrocyte receptor (SER) on resident bone marrow Mϕs (RBBMs) which naturally cluster with developing haemopoietic cells [12, 13]. SER activity was unaffected in the temperature range 0–37 °C, divalent cation independent and destroyed by trypsin. Interestingly, treatment of the erythrocytes with *Vibrio cholerae* sialidase abolished binding by up to 90%. The lectin-like properties of SER for binding sialic acid were further demonstrated by the ability of trisaccharide 3′-sialyllactose and ganglioside GD1a, but not lactose or GM1, to inhibit erythrocyte rosette formation [13].

SER activity was present on tissue 'stromal' Mϕs isolated by collagenase digestion from the bone marrow, lymph nodes and spleen, but was absent from 'free' Mϕs isolated from the peritoneal and pleural cavities. However, in vitro upregulation of SER on these Mϕs could be mediated by a ~70 kDa species-restricted component in mouse plasma and serum [14]. This finding led to speculation that tissue Mϕ expression of SER could be regulated by access to or local production of this component.

The isolation of a blocking monoclonal antibody called SER-4 from rats immunized with mouse serum-induced peritoneal Mϕs was a key step in the characterisation [15], purification [17] and molecular cloning [18] of this receptor, which was subsequently renamed sialoadhesin (Sn). Sn is also sometimes referred to as CD169 and/or siglec-1. SER-4

antibody was shown to immunoprecipitate a protein with a molecular weight of 185 kDa under non-reducing or 170 kDa under reducing conditions. By immunohistochemistry, SER-4 antigen was shown to be Mϕ-restricted, with high expression on F4/80+ Mϕs in the bone marrow, subcapsular sinus and the medullary cords of the lymph nodes, marginal metallophils in the inner marginal zone of the spleen and weak expression on red pulp Mϕs in the spleen and Kupffer cells in the liver [15]. In the nervous system, Sn is not expressed by the resident Mϕs, microglia, but it is present on some Mϕs that reside outside of the blood–brain barrier, such as in the choroid plexus and leptomeninges [56]. Sn was found to be induced on inflammatory Mϕs in the central nervous system after sterile injury, expression correlating with leakiness of the blood–brain barrier and therefore providing additional evidence for a macromolecular factor in plasma that regulates Sn expression [56].

Definitive evidence that Sn is a lectin-like receptor came from studies of the purified protein following its isolation by antibody affinity chromatography from murine spleen [17]. Visualization of the isolated receptor following low angle shadowing and electron microscopy showed that it consisted of a globular head, measuring 9±2 nm in diameter and tail, measuring 35±4 nm in length. Sn binding properties were characterised using desialylated human erythrocytes reconstituted enzymatically with sialic acid in α2 → 3 or α2 → 6 linkages. This showed that Sn preferentially recognizes Neu5Acα2 → 3Galβ1 → 3GalNAc- over Neu5Acα2 → 6Galβ1 → 4GlcNAc-derivatized erythrocytes. The preference of Sn for α2 → 3 over α2 → 6 linked sialic acid in both O- and N-linked glycans was also demonstrated in inhibition experiments performed with sialylated derivatives of antifreeze glycoprotein and glycophorin. Similar results were obtained when gangliosides were used. GD1a and GM3, which express terminal α2 → 3 linked sialic acid, were recognized by Sn, whereas binding was absent or weak to the gangliosides GM1 and GM2 which only express an 'internal' sialic acid residue and to GD3 and GQlb which contain terminal α2 → 8 linked sialic acid. This suggested that specific sialoglycoconjugates containing terminal α2 → 3 linked sialic acid are preferred targets of Sn.

Sn protein primary structure

In 1994, the molecular cloning of murine Sn was reported based on screening a lambda phage cDNA library with degenerate probes derived from C-terminal peptide sequence of purified protein [18]. Unexpectedly, Sn was shown to be a member of the immunoglobulin (Ig) superfamily with 17 Ig-like domains (Fig. 1). Murine Sn was predicted to be a type I transmembrane glycoprotein consisting of 1,694 amino acids, with a short intracellular region of 35 amino acids, a 21-amino acid transmembrane segment and a long extracellular region of 1,619 amino acids containing up to 15 N-linked glycans. The short cytoplasmic tail of Sn did not show any similarities with other proteins, but 5 potential phosphorylation sites were identified. The first domain of the extracellular N terminus of the molecule was shown to be a V-set domain containing an unusual arrangement of predicted disulphide bonds, including an intra-sheet disulphide bond between β-strands B and E (as opposed to the normal inter-sheet bond between β-strands B and F) and an interdomain disulphide linking the V-set domain to the adjacent Ig domain. The other 16 extracellular domains were shown to be C2-like, with similarities in length and sequence between domains 4, 6, 8, 10, 12, 14 and 16 and between domains 5, 7, 9, 11, 13, 15 and 17, whereas domain 3 was shown to be similar to even-numbered domains. Domains 1 and 2 at the N terminus were distinct. Therefore the 'stem region' of domains 4–17 within Sn appears to have evolved by repeated duplication of a two exon unit.

The first two Ig domains of Sn were seen to share both sequence similarity and structural features with the previously described Ig superfamily proteins CD22, myelin-associated glycoprotein and CD33. This raised the intriguing possibility that these proteins had all evolved to mediate sialic acid recognition, a prediction that was subsequently borne out using recombinant proteins and resialylated human red blood cells in rosetting assays [40]. Although initially described as the 'sialoadhesin family', these proteins were subsequently renamed the 'sialic acid binding Ig-like lectins', or 'siglecs' by consensus [19]. Following the discovery and characterisation of the species-variable CD33-related siglec subgroup, this family is now known to be made up of at least 14 members in humans, most of which are expressed in cells of the immune system (reviewed in [21]).

Molecular basis for Sn recognition of carbohydrates

The location of the sialic acid binding region of Sn was determined by generating a series of extracellular domain deletion constructs fused to the Fc portion of human IgG1 [51] which revealed that the N-terminal V-set domain is both necessary and sufficient. The molecular basis for carbohydrate binding by Sn was investigated by site-directed mutagenesis [70], X-ray crystallography [45] and nuclear magnetic resonance analysis [20]. The Sn V-set domain was crystallized as a complex with its ligand 3′-sialyllactose [45]. It was shown that arginine-97, which is conserved in Sn from all species (Table 1), forms a bidentate salt bridge with the carboxylate group of *N*-acetylneuraminic acid and two conserved tryptophans at positions 2 and 106 to make hydrophobic interactions with the *N*-acetyl and glycerol

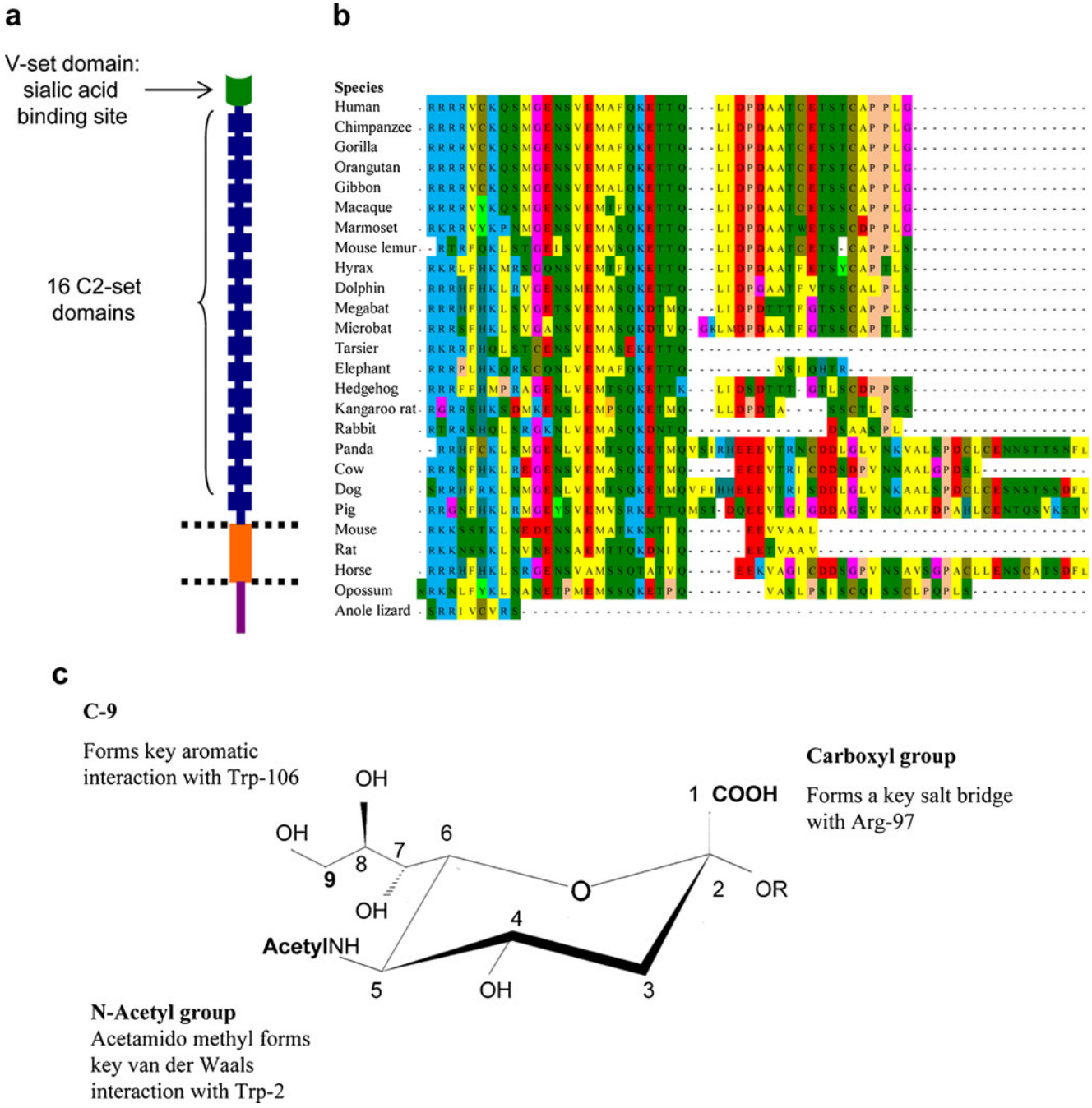

Fig. 1 The features of Sn. **a** Schematic diagram showing the domain organization of Sn protein. **b** Alignment of cytoplasmic tails of mammalian and reptilian Sn. **c** *N*-acetylneuraminic acid showing the functional groups that make key contacts with the binding site of Sn. The Sn residues indicated are conserved in all Sn orthologues

moieties respectively (Fig. 1c, Table 1). Arginine-97 was shown to be crucially important for binding to sialic acid as it was demonstrated that even a conservative substitution of arginine with lysine results in about 10-fold loss in binding affinity of Sn [20]. The functional groups of sialic acids involved in binding to Sn were investigated using synthetic *N*-acetylneuraminic acid analogues either on resialylated human erythrocytes or as free α-glycosides in hapten inhibition [41]. This study and others [39] have confirmed that Sn is specific for αNeu5Ac, the most abundant of the mammalian sialic acids, and does not recognize two other important sialic acids, Neu5Gc or Neu5Ac9Ac.

In common with many other lectins, the glycan binding site of Sn exhibits low millimolar binding affinities for naturally sialylated ligands [20]. Therefore, cell adhesion depends on clustering of both the receptor and the ligand

Table 1 Comparison of mammalian and a reptilian Sn orthologues

				Cytoplasmic tail	
Species	Ig domains	Sia binding[a]	% Seq Id[b]	Length	% Seq Id
Human	17	WRW	100	44	100
Chimpanzee	17	WRW	99	44	100
Gorilla	17	WRW	99	44	100
Orangutan	17	WRW	95	44	100
Macaque	17	WRW	95	44	93
Marmoset	17	WRW	89	44	81
Gibbon	17	WRW	89	44	95
Mouse Lemur	17	WRW	83	42	78
Dolphin	17	WRW	82	44	68
Cow	17	WRW	79	49	38
Horse	17	WRW	79	60	31
Microbat	17	W?W[c]	79	46	65
Megabat	17	WRW	79	44	65
Rabbit	17	WRW	78	33	43
Pig	17	WRW	77	64	34
Dog	17	WRW	77	65	34
Panda	17	WRW	77	64	38
Elephant	17	WRW	77	32	53
Mouse	17	WRW	73	32	34
Hedgehog	17	WRW	73	43	53
Rat[d]	17	WRW	72	32	32
Hyrax	17	WRW	71	44	70
Kangaroo Rat	17	WRW	67	40	50
Tarsier	17	WRW	57	25	64
Opossum	17	WRW	52	45	31
Anole Lizard	17	WRW	39	9	55

[a] Designates the three amino acids in murine Sn required for interaction with sialic acid: W2 with the glycerol, R97 with the carboxylate and W106 with the *N*-acetyl moieties referred to in Fig. 1c and demonstrated in the crystal structure of Sn complexed with 3′-sialyllactose [45]

[b] Sequence identity of full-length transcript compared to human Sn

[c] The microbat sequence does not align over the 'R' residue

[d] Domain 6 not fully sequenced in rat genome sequence GenBank: EDL80222.1

to mediate high avidity. Experimental evidence for the importance of ligand clustering was obtained using transfected CHO cells presented with up to 140 GT1b oligosaccharides attached to streptavidin and complexed with biotin-BSA [32]. In general, a linear increase in the number of ligands per molecule leads to a logarithmic increase in the binding affinity. Likewise, increasing the number of Sn molecules in the plasma membrane is important for achieving a critical density required for stable binding to appropriate targets. Following induction on Mϕs with mouse serum, it was shown using ^{125}I labelled SER-4 Fab fragment that up to 10^6 molecules of Sn can be expressed per cell, resulting in high avidity binding to red blood cells [15]. By altering the expression of Sn over a fairly narrow range, fine control over cellular interactions can be achieved via this avidity effect.

Expression of Sn on Mϕ subsets and its induction by type I IFN

In all species examined so far, including human, pig, mouse and rat, Sn has been shown to be highly restricted in expression to cells of the mononuclear phagocyte system with highest expression on Mϕ subsets in secondary lymphoid organs, such as lymph nodes and spleen (Fig. 2). In human samples, immunohistochemistry and FACS studies showed that Sn is not normally expressed on blood monocytes but is present on a wide variety of tissue Mϕs in the spleen, lymph node, bone marrow, liver, colon and lungs [31]. Sn was also found to be highly expressed on inflammatory Mϕs in rheumatoid synovial membrane in tissue samples from rheumatoid arthritis patients [31]. In humans, Sn+ Mϕs are not found in the marginal zone of the spleen in contrast to rodents [63]. Human spleens contain an extra compartment, the perifollicular zone, which is located between the marginal zone and the red pulp (Fig. 2). It is a dynamic region which appears to be similar to the red pulp containing erythrocyte-filled spaces and strongly Sn+ Mϕs that form sheaths around capillaries in the perifollicular zone. Weakly Sn-expressing Mϕs could also be found in the perifollicular zone and the red pulp. In contrast to rodents, the marginal metallophils are absent in humans and the human marginal zone does not contain any Sn+ Mϕs.

A key factor that has been shown to upregulate Sn in all species examined is interferon-α (IFN-α). Molecules that trigger type I IFN responses can also upregulate Sn expression in Mϕs, as demonstrated for LPS and poly I:C [72] that drive TRIF-dependent pathway of type I interferon induction via TLR-4 and TLR-3 respectively. Kirchberger and colleagues also demonstrated that Sn can be induced on monocyte-derived dendritic cell (DC) populations in vitro after treatment with inactivated human rhinovirus, probably via intracellular RNA sensors such as MDA-5 and RIG-I that drive type I IFN production [42]. Despite these in vitro effects on monocyte-derived DCs, no expression of Sn has been shown on bona fide DCs in vivo so the role of Sn in modulation of DC functions such as antigen presentation to naive T cells is unknown. In addition, Sn expression has been reported in monocytes after HIV-1 infection [58] and in patients suffering from systemic lupus erythematosus and systemic sclerosis [72]. These observations are consistent with the upregulation of type I interferon levels in the serum of patients with viral infections and autoimmune diseases [7, 55].

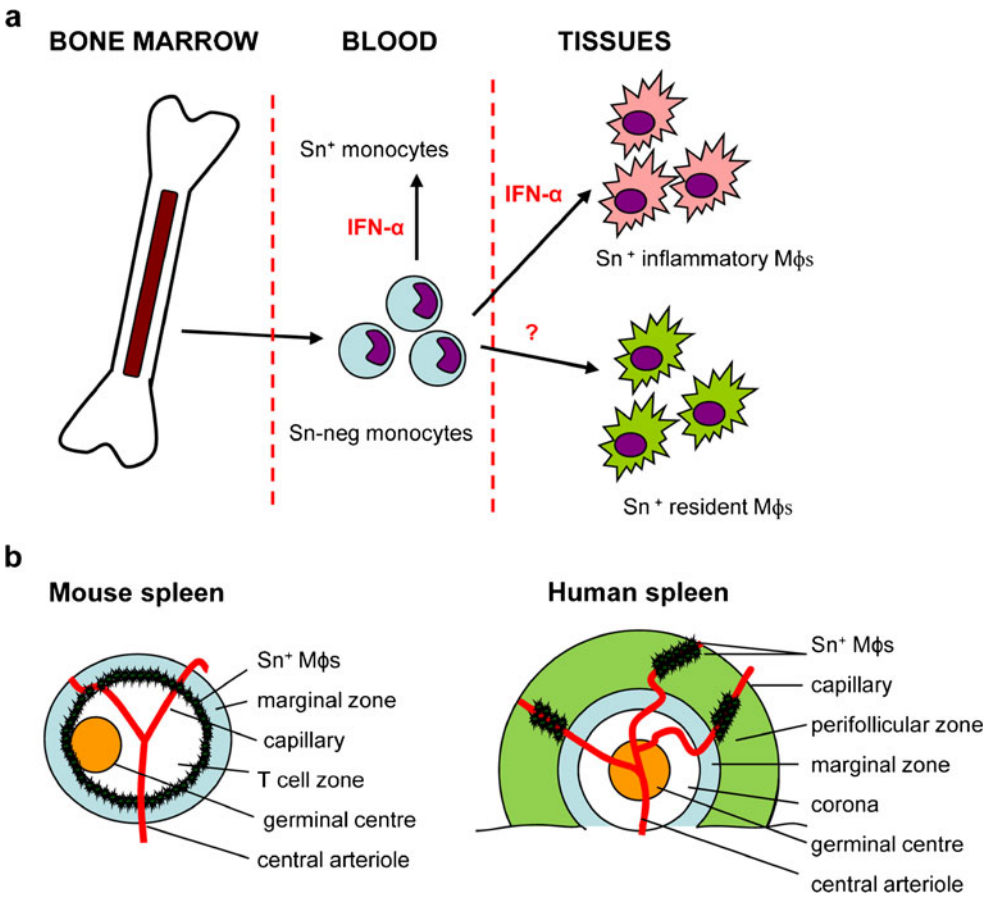

Fig. 2 The development and regulation of Sn expression on myelomonocytic cells. **a** Blood monocytes are derived from the haematopoietic precursor cells in the bone marrow where they move to tissues, either to become resident tissue Mϕs that constitutively express Sn via unknown mechanisms or under the influence of IFN-α to become infiltrating Sn+ Mϕs in inflammatory tissues. Virus-infected monocytes, e.g. in HIV-1 infection, can also upregulate Sn expression in the presence of IFN-α. **b** The anatomy and localization of Sn+ Mϕs in mouse and human spleen

Conserved biological functions of Sn

A phylogenetic analysis of Sn using currently available genomic sequences shows that a Sn orthologue with 17 Ig domains and a highly conserved sialic acid binding site is present in all mammals (Table 1). Interestingly, a clear orthologue with 17 Ig domains and a sialic acid binding site is also present in the anolis lizard genome [1] showing that the Sn gene must have evolved prior to the split of the amniote lineage into the ancestral lineages of mammals and reptiles ~320 million years ago.

It has been proposed that Sn has evolved the unusually large number of 17 Ig domains to extend the sialic acid binding site above the dense glycocalyx to avoid *cis*-inhibition by the abundant sialic acids at the cell surface and thereby permit *trans* interaction with ligands on other cells [18, 50]. This feature of Sn is in striking contrast to other siglecs that usually contain between 2 and 7 Ig domains and are typically masked at the cell surface by *cis* interactions with sialic acids [21].

In comparison to the extracellular region, the cytoplasmic tail of Sn is very poorly conserved in both sequence and length varying from only 9 residues in the anolis lizard to 65 in the dog (Fig. 1b, Table 1). Taken together, these observations would indicate that the main conserved intrinsic role of Sn is in mediating cellular interactions rather than cell signalling. However, via collaboration with other receptors, Sn may be important for triggering key signalling functions in Mϕs that influence host responses to the extracellular environment (Fig. 3). In addition, the extended cytoplasmic tail seen in some species (Table 1) may provide novel species-specific signalling functions, as discussed below for porcine Sn.

Sn-deficient ($Sn^{-/-}$) mice were generated to study the biological functions of Sn in detail [53]. $Sn^{-/-}$ mice are viable and fertile with no developmental abnormalities. Analysis of different tissues revealed that $Sn^{-/-}$ mice show a small increase in CD8+ T cells and a small decrease in B220+ B cells in spleens and lymph nodes with slightly less follicular B cells and an increase in numbers of marginal zone B cells in spleen. The reduction of B cell number correlated with reduced immunoglobulin M (IgM) levels in $Sn^{-/-}$ mice but similar titers of IgG subclasses were found. These observations suggested that the main role for Sn may be in regulating the immune system and not in directly regulating haematopoiesis as had previously been considered [16]. This contention is also consistent with the results of several studies investigating the role of Sn in autoimmune diseases. These include experimental autoimmune uveoretinitis, two models of inherited demyelinating neuropathies of the central and nervous systems [35, 43],

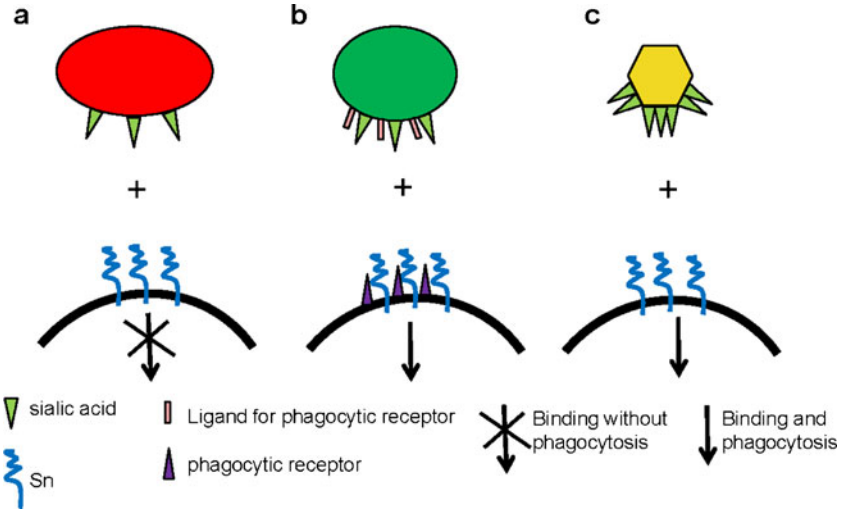

Fig. 3 Models of Sn-mediated uptake of sialylated particles. **a** In the case of cellular targets that have relatively low sialic acid density, such as erythrocytes, the particle binds to Sn+ Mϕ, but the avidity is not high enough to trigger phagocytosis. **b** If the sialylated particle displays additional ligands for a phagocytic receptor on Sn+ Mϕ, Sn and the phagocytic receptor may synergize to mediate phagocytosis. **c** If the sialic acid density on the sialylated particle is high enough to create high avidity binding to Sn, as with certain pathogens, the particle may be directly phagocytosed by Sn+ Mϕs

and experimental autoimmune encephalomyelitis (EAE) as a model for human multiple sclerosis [71]. In all cases, $Sn^{-/-}$ mice developed less severe inflammatory responses, with reduced clinical signs of disease and lowered numbers of inflammatory Mϕs and T effector cells. In the most recent study of EAE, it was shown that the proinflammatory role of Sn correlated with reduced numbers of anti-inflammatory CD4+ T regulatory cells (T-regs) which expressed high levels of Sn ligand [71]. It was demonstrated that co-culture of these T-regs with Sn+ Mϕs led to reduced proliferation in vitro indicating that Sn-dependent Mϕ/T-reg interactions could lead to stronger adaptive immune responses. In the case of pathogens, this could be important in setting an appropriate balance of T-reg versus T effector populations during host adaptive immune responses. Thus, pathogen-driven upregulation of Sn expression on Mϕs would be expected to suppress local T-reg expansion, resulting in stronger T effector activity, both for CD4+ and CD8+ T cell populations.

Sn as a putative endocytic and/or phagocytic receptor

Binding and uptake by either phagocytosis or endocytosis of self-constituents has to be highly regulated to lower the risk of autoimmune responses. Given that Mϕs are the major phagocytic cells in the body with the potential to trigger autoimmune responses, regulation of uptake via receptors for self, such as Sn, is of considerable importance. The presence of Sn in endocytic vesicles of the subcapsular sinus Mϕs (SSMs) in the lymph node [61] indicates that Sn is constitutively involved in endocytic functions of these cells.

Initial studies with Sn showed that it was a non-phagocytic receptor for red blood cells, but subsequent studies with *Neisseria meningitidis* challenged this view, as these bacteria were shown to be taken up by Mϕs in a Sn-dependent manner [37]. Furthermore, and as discussed further below, Sn has been shown to mediate endocytosis of sialylated enveloped viruses and porcine Sn can endocytose the mouse anti-Sn monoclonal antibody 41D3 in a clathrin-dependent manner [27, 60, 68]. Endocytosis was claimed to require a porcine-specific motif F^{1671}-YKL in the cytoplasmic tail of Sn that is recognized by the clathrin-associated endocytic adapter complex AP-2 as a first step in the formation of clathrin-coated vesicles [22].

A recent study showed that liposomes displaying a high affinity sialic acid-based ligand for Sn, but no other ligands, were rapidly cleared in mouse liver in a Sn-dependent manner [11]. Therefore, under certain circumstances such as when particles contain a high density of sialylated ligands, Sn may be capable of directly mediating uptake into Mϕs. This could be of considerable importance in the host–pathogen interaction as discussed below as well as in some autoimmune diseases. However, under steady-state conditions, Sn-dependent uptake of material into Mϕs is likely to involve synergy with additional 'professional' phagocytic receptors (Fig. 3).

Resident Sn+ Mϕ populations and antigen presentation

In the absence of infection, Sn is abundantly expressed on Mϕs in the subcapsular sinus and medullary cords of the

lymph node, as well as on marginal metallophilic Mφs in rodent spleens or perifollicular zone Mφs in human spleen (Fig. 2b) [63]. Due to the strategic localization of splenic and lymph node Sn+ Mϕs at portals of entry for blood and lymph respectively, it is likely that Sn plays a role in regulating the immune responses to pathogens and self-antigens that they encounter in blood or lymph.

Spleen marginal zone Mϕs have been shown to collaborate with CD8+ DCs in generation of cytotoxic T cells [6]. Den Haan and colleagues showed that injection of ovalbumin-coupled anti-Sn monoclonal antibodies targeted to marginal metallophilic Mϕs in the spleen results in strong activation of antigen-specific CD8+ T cell responses. These Sn+ Mϕs were shown to transfer antigens exclusively to CD8+ splenic DCs, which led to efficient cross-presentation and activation of cytotoxic T lymphocytes. This leads to speculation whether sialylated pathogens could follow the same route of uptake by marginal metallophilic Mϕs, transfer to CD8+ DCs and eventually activation of cytotoxic T cells and whether Sn has a role in the interactions between marginal metallophilic Mϕs and DCs.

SSMs in the lymph node have been shown to act directly as antigen presenting cells as they capture antigens from the blood and translocate them across the sinus to the follicular space where they present the antigen to B cells [10, 38, 54, 57]. The B cells then transport antigens to germinal centers and initiate immune responses. The SSMs are highly positive for Sn but it is yet to be investigated whether Sn has a role in antigen presentation or phagocytosis/endocytosis of antigens that SSMs capture from the afferent lymph. In addition to being able to present antigens to B cells, recently it was shown that these cells can also capture and present lipid antigens to *i*NKT cells and therefore mediate early *i*NKT cell activation in lymph nodes [8].

In view of the role of SSMs as gatekeepers of adaptive immunity, the regulation of phagocytosis in this population of Mϕs is likely to be very important in determining which antigens are available to B cells as opposed to those that are taken up by the SSMs and degraded and rendering them cryptic to the immune system. Dysregulation of phagocytosis, for example the failure to efficiently clear damaged or apoptotic host cells via SSMs, could therefore contribute to breakage of tolerance and development of autoimmune responses such as those seen in systemic lupus erythematosus. Conversely, exploitation of this tolerance pathway by pathogens might reduce their antigenicity and favor their survival in the host.

Sn+ Mϕs have also been reported to have a crucial role in antitumour immunity by cross-presenting antigens from dead tumour cells to CD8+ T cells [4]. A recent publication showed that subcutaneously injected dead cells are transported to lymph nodes and SSMs phagocytose and directly cross-present dead cell-associated antigens to CD8+ T cells. The depletion of Sn+ Mϕs resulted in loss of tumour-specific CD8+ T cell activation and proliferation, indicating a critical role for these Mϕs in antigen presentation. Subsequent tumour destruction was also shown to be controlled by Sn + SSMs because mice lacking Sn+ Mϕs at the time of vaccination were shown to be unable to reject viable tumour cells. This Sn-mediated cross-presentation of tumour cell associated antigens was shown to be independent of migratory DCs and lymph node-resident conventional DCs.

Investigation of porcine Sn has revealed that Sn could directly participate in antigen uptake and/or presentation by antigen presenting cells (APCs) [60]. In order to investigate whether Sn can be targeted for delivery of antigens to APC for T cell stimulation, T lymphocytes from pigs immunized with mouse immunoglobulins were incubated with IFN-α treated monocytes or monocyte-derived DCs. Monoclonal antibody to Sn was shown to induce T cell proliferation in vitro at concentrations 100-fold lower than control antibody. These data indicate ligands that bind Sn gain access efficiently to the major histocompatibility complex processing and presentation pathways. However, a major caveat of experiments, such as these using intact IgG to target Mϕ receptors like Sn, is that interactions with FcR cannot be ruled out. FcR are well-known signalling molecules that trigger multiple effects in Mϕs including delivery of antigens to MHC class I and class II molecules for antigen presentation (reviewed in [52]). Nevertheless, the potential role of Sn in endocytosis and antigen presentation is an important area for future study as it could be exploited in vaccine or drug development by specifically targeting antigens, toxins or other compounds to immunomodulatory Sn+ Mϕs.

While there is no clear evidence that DCs can express Sn in vivo, treatment of human monocyte-derived DCs with HRV14, a member of the major group of human rhinovirus family has been reported to induce Sn expression and inhibit the accessory function of DCs [42]. Despite the high levels of MHC and costimulatory molecules on infected DCs, the induction of Sn was shown to diminish DC-T cell stimulatory capacity and cause an anergic state in cocultured T cells. This inhibitory phenotype was shown to be reverted by a monoclonal antibody to Sn, suggesting that induction of Sn in infected DCs can lead to reduction in adaptive response during viral infection. A subsequent study showed that human DCs that are activated by rhinoviruses induce the production of IL-35 secreting T-regs, a pathway that can be blocked by antibodies to Sn and B7-H1 [62].

Sn and the regulation of tolerance for self-antigens

Sn+ Mϕs in the marginal zone have been shown to have a critical role in regulating suppression of immune responses

to apoptotic cell associated antigens [48]. Miyake and colleagues showed that intravenously injected apoptotic cells that express a fragment of myelin oligodendrocyte glycoprotein (MOG) reduced MOG-specific T cell response and prevented the development of EAE. These apoptotic cells were shown to be selectively captured by CD8α+ DCs which are responsible for suppression of immune responses to cell-associated antigens. By using transgenic mice in which Sn+ Mϕs could be deleted by injection of diphtheria toxin, the apoptotic cells were made available to CD8α– CD11b + DCs which resulted in breach of tolerance, T cell activation and accelerated development of EAE.

The role of Sn+ marginal zone Mϕs in regulation of immune tolerance was also supported by studies performed by McGaha and colleagues who also showed altered localization pattern and increased immune responses in mice after depletion of Sn+ marginal zone Mϕs [46]. In the absence of Sn+ cells, apoptotic cells were found to be associated with T cell areas of the lymphoid follicles but also with red pulp Mϕs which led to increased production of proinflammatory cytokines and enhanced lymphocyte responsiveness to apoptotic cell antigens. The breakage of tolerance to self-antigens in Sn+ cell depleted mice was also shown to promote SLE.

Although the mechanism of the phagocytosis of apoptotic cells is not completely understood, it has been proposed to be a two-step process. First, an interaction between putative ligands and receptors occurs and then the apoptotic cell is engulfed through recognition of PS exposed on the apoptotic cell surface [34]. Sn could fit into the proposed model by binding sialylated ligands on apoptotic cells and therefore increasing the contact area between the Mϕ and the sialylated target to promote uptake in synergy with phagocytic receptors such as the TIMs that recognize phosphatidylserine on apoptotic cells [29].

Sn as a potential receptor for sialylated pathogens

Although sialic acids are abundant in all higher organisms, they are less common amongst microorganisms and potential pathogens (reviewed in [3, 69]). It is therefore of great interest that several medically important pathogens have evolved various mechanisms to synthesize or capture sialic acids and display them on their surfaces. Understanding the role of Sn in the immune response to these bacteria is particularly relevant because they include important human pathogens, such as *N. meningitidis*, *Haemophilus influenzae*, group B *Streptococcus*, *Campylobacter jejuni* and several pathogenic strains of *Escherichia coli*. In addition, enveloped viruses that use the host cell's glycosylation machinery to display glycans at the cell surface can present sialic acids recognized by Sn.

Due to strategic positioning in the spleen and lymph nodes of Sn+ Mϕs on the border of lymphocyte rich regions and the entry of blood or lymph (Fig. 2), it has been speculated that Sn performs a role in the uptake and processing of sialylated pathogens. This view is also supported by observations that Sn can mediate recognition and promote uptake of various sialylated pathogens in vitro, such as PRRSV [2, 23–26, 64, 68], HIV-1 [58, 59, 65, 73], *N. meningitidis* [37], *C. jejuni* [33] and *Trypanosoma cruzi* [49] (Table 2). Each will be discussed in turn.

Porcine reproductive and respiratory syndrome virus

Porcine reproductive and respiratory syndrome virus (PRRSV) induces respiratory tract illness and reproductive failure in pigs which severely affects the swine industry worldwide. It belongs to the *Arteriviridae* family of single-stranded RNA viruses and infects cells of the monocyte/Mϕ lineage via receptor-mediated endocytosis. In 1998, it was reported that a monoclonal antibody named 41D3 could block the infection of PRRSV of porcine alveolar Mϕs [28]. It was later demonstrated that this antibody recognises porcine Sn [68]. When porcine Sn was cloned and transfected into a cell line resistant to virus entry, the cells became permissive. Sialidase treatment of the virus was shown to prevent the attachment and infection of porcine alveolar Mϕs with PRRSV, in addition, enzymatic removal of N-linked glycans reduced PRRSV infection. Another receptor that has been associated with PRRSV infection is heparin-like material which recognizes a viral matrix protein

Table 2 Human pathogens that can present sialic acid on the cell surface leading to recognition by Sn

Pathogen	Disease	Sialic acid ligand	References
PRRSV	Reproductive failure in breeding stock and respiratory tract illness in young pigs	M/GP5 glycoprotein complex in viral envelope	[64, 68]
HIV-1	AIDS	gp120 in viral envelope	[59, 73]
Trypanosoma cruzi	American trypanosomiasis (Chagas disease)	Neu5Acα2 → 3Gal in mucins	[49]
Neisseria meningitidis	Meningitis	Neu5Acα2 → 3Gal in LOS	[37]
Campylobacter jejuni	Gastroenteritis, Guillain–Barré syndrome	Neu5Acα2 → 3Gal in LOS, GD1a, GM1b, GM3 mimic	[33]

[23]. Analysis of the kinetics of PRRSV entry revealed that heparin first mediates attachment of the virus but not the entry and it is gradually followed by Sn mediated internalization [25]. Using transfected CHO cells, it was shown that heparin enhances Sn mediated internalization but Sn-dependent binding and internalization of PRRSV occurs independently.

Using site-directed mutagenesis, it was shown that similar to mouse and human Sn, the V-set domain of porcine Sn functions as the sialic acid binding domain [26]. When R^{116} to E mutation was introduced in the V-set domain of porcine Sn, the PRRSV could no longer attach or enter the transfected CHO cells, suggesting that sialic acid binding capacity of Sn is required for the entry of the virus. This was confirmed by studies from a different research group, who showed using a series of truncated porcine Sn mutants that the first 150 amino acids that comprise the entire first N-terminal V-set domain is necessary and sufficient for PRRSV binding to cells [2].

To identify the sialic acid components of PRRSV, a soluble form of Sn was used [64]. The soluble Sn-Fc protein was shown to bind the virus in a sialic acid dependent manner and was able to block virus entry into porcine alveolar Mϕs confirming the essential role for PRRSV infection. Sn was shown to recognize only the M/GP_5 glycoprotein complex of PRRSV in a sialic acid dependent manner, although in addition to GP_5, sialic acids are present on the GP_3 and GP_4 envelope glycoproteins of the virus as well. This insight into Sn-PRRSV interactions could be useful for vaccine development.

In addition to Sn, CD163, a group B scavenger receptor has also been described as a receptor for PRRSV [9]. Studies using transfected PK-15 cell line showed that when cells were transfected only with Sn, the virus was internalized but not uncoated; however, when Sn and CD163 were co-expressed, the virus was uncoated upon internalization and successfully replicated [66]. CD163 expression alone was shown not to be sufficient for virus entry, although cells became productively infected; but compared to Sn and CD163, the transfected cells expressing only CD163 produced less virus. These data indicate that Sn is required for the internalization of the virus and CD163 for virus uncoating. The same group also showed using dominant-negative Rab5 and Rab7 mutants, that PRRSV needs CD163+ early endosomes but does not continue through the endocytic pathway to late endosomes for productive infection [67]. The virus was shown to colocalize with Sn on the cell surface and beneath the plasma membrane, while CD163 colocalizes with the virus only in early endosomes in porcine alveolar Mϕs.

HIV-1

The role of Sn in inflammatory and infectious diseases gained increased interest after the demonstration that Sn is upregulated in monocytes from HIV-1 infected patients and that expression increases with disease progression [58, 59, 65]. Analysis of HIV patients has revealed that Sn mRNA is significantly elevated early after HIV-1 infection and a further increase is seen in AIDS patients [65]. This was confirmed by upregulation of Sn protein by FACS that showed that in infected patients up to 90% of CD14+ monocytes express Sn. This was followed by studies that showed that Sn expression directly correlates with viral load [59]. Monocytes were shown to bind to HIV-1 via Sn, as Sn + monocytes bound to approximately 3-fold more virus particles compared to normal control monocytes. Sn was shown to bind the virus through recognizing the sialic acid residues on the viral envelope glycoprotein gp120. Furthermore, Sn + monocytes enhanced HIV-1 infectivity by absorbing the cell-free virus from the culture and trans infecting permissive reporter cells [59].

A recent study showed that Sn and siglec-9 directly recognize viral gp120 proteins in HIV-1 envelope, which promotes the adhesion of viral particles to Mϕs [73]. Compared to Sn and siglec-9, which showed affinities of recombinant dimeric proteins varying between 0.01–1 mM to different gp120 envelope proteins, siglecs-3, -5 and -7 displayed low affinity binding to gp120 using similar receptor densities. However, increasing the receptor immobilization density by 3- to 5-fold improved binding affinities for siglec-3, -5 and -7 between 100- to 1,000-fold. This indicates an important avidity contribution to the siglec-gp120 recognition by siglecs. In addition, the sialic acid on gp120 binding to Sn was shown to have a crucial role in infection of Mϕs. This suggests that viruses can target Sn-mediated endocytosis to increase infectivity.

N. meningitidis

In parallel to the role of Sn in PRRSV infection, the role of mouse Sn in binding and uptake of sialylated *N. meningitidis* emerged [37]. Although Sn was not thought to function directly as a phagocytic receptor, the experimental data indicated that it could enhance Mϕ phagocytosis of bacteria by binding to sialylated lipooligosaccharides (LOS) on the surface of *N. meningitidis* and therefore have an important role in the uptake of intact bacteria by Mϕs [37]. Using sialylated and non-sialylated LPS derivatives of two serogroups of *N. meningitidis*, it was shown that the sialylated bacteria are specifically recognised by Sn in a sialic acid dependent manner. In addition to Sn, soluble recombinant siglec-5 was shown to bind sialylated *N. meningitidis* in vitro and Sn or siglec-5 transfected CHO cells showed increased binding to sialylated variants of *N. meningitidis*. The role of Sn in uptake of the sialylated bacteria was confirmed by analysis of phagocytosis by Mϕs from wild-type and $Sn^{-/-}$ mice which showed that Sn bound and phagocytosed sialylated bacteria in a sialic acid-dependent

manner. These results indicated that Sn can have a role in host defence against sialylated bacteria.

C. jejuni

C. jejuni is a common human pathogen that causes gastroenteritis and has been linked with an autoimmune disease called the Guillain–Barré syndrome [36]. Some clinical isolates have been shown to expresses sialylated LOS that display ganglioside-like terminal structures on the core oligosaccharides. Previous studies have demonstrated that sialylation of *C. jejuni* can enhance pathogenity by increasing invasiveness in epithelial cells [44] and act as an immunosuppressor by blocking complement activation [30]. The initial report that siglecs might have a role in binding to sialylated *C. jejuni* showed that out of 10 siglecs screened, siglec-7 was found to bind purified LOS HS:19(GM1(+) GT1a(+)) from a sialylated isoform of *C. jejuni* in solid-phase binding assays and to intact bacteria [5]. A later study showed that Sn can also bind sialylated *C. jejuni* strains that carry LOS-ganglioside mimics with terminal $\alpha 2 \rightarrow 3$-linked sialic acids, including GD1a, GM1b and GM3 [33]. Sn was shown to enhance the binding of sialylated *C. jejuni* to transfected CHO cells, which indicates that Sn can have an important role in immune responses to *C. jejuni*.

T. cruzi

In addition to sialylated viruses and bacteria, Sn has been shown to recognize a sialylated parasite, *T. cruzi* that naturally infects Mϕs [49]. The proliferative forms of *T. cruzi*, epimastigotes, and the infective adhering forms of *T. cruzi*, trypomastigotes, were shown to associate with mouse peritoneal Mϕs, in which Sn expression was induced with homologous mouse serum and this association could be blocked by monoclonal antibodies to mouse Sn. Desialylation of parasites was shown to decrease the Mϕ association, indicating a sialic acid dependent binding to Sn. Scanning electron microscopy confirmed the association of trypomastigotes to Sn-expressing Mϕs and showed plasma membrane projections from the Mϕs. Cytochalasin D, which blocks phagocytosis, abolished internalization of trypomastigotes. These data indicate that Sn may have an important role in the initial *T. cruzi* internalization and thus in the establishment of Chagas disease.

Conclusions

As a major Mϕ sialic acid receptor conserved in all mammals, Sn has evolved to mediate 'self' cell–cell interactions. Recent studies indicate that cross-talk between Sn and its ligands on lymphocytes play an important role in fine-tuning T and B cell responses to antigens. A secondary consequence of this role is that Sn can also recognize a wide range of sialylated pathogens. Recent studies have shown that this Sn-dependent recognition can promote pathogen uptake in Mϕs which could be important in antigen presentation and immune activation. A key unresolved question is whether this latter function is of benefit to the host in protective immunity or whether certain pathogens exploit this pathway to their advantage. In the case of porcine arterivirus, sialylated glycoproteins in the envelope provide Sn ligands that play an important role in binding to alveolar Mϕs leading to infection of pigs. Whether a similar paradigm applies to other sialylated pathogens requires further study.

References

1. Alfoldi J, Di Palma F, Grabherr M, Williams C, Kong L, Mauceli E, Russell P, Lowe CB, Glor RE, Jaffe JD, Ray DA, Boissinot S, Shedlock AM, Botka C, Castoe TA, Colbourne JK, Fujita MK, Moreno RG, ten Hallers BF, Haussler D, Heger A, Heiman D, Janes DE, Johnson J, de Jong PJ, Koriabine MY, Lara M, Novick PA, Organ CL, Peach SE, Poe S, Pollock DD, de Queiroz K, Sanger T, Searle S, Smith JD, Smith Z, Swofford R, Turner-Maier J, Wade J, Young S, Zadissa A, Edwards SV, Glenn TC, Schneider CJ, Losos JB, Lander ES, Breen M, Ponting CP, Lindblad-Toh K (2011) The genome of the green anole lizard and a comparative analysis with birds and mammals. Nature 477:587–591
2. An TQ, Tian ZJ, He YX, Xiao Y, Jiang YF, Peng JM, Zhou YJ, Liu D, Tong GZ (2010) Porcine reproductive and respiratory syndrome virus attachment is mediated by the N-terminal domain of the sialoadhesin receptor. Vet Microbiol 143:371–378
3. Angata T, Varki A (2002) Chemical diversity in the sialic acids and related alpha-keto acids: an evolutionary perspective. Chem Rev 102:439–469
4. Asano K, Nabeyama A, Miyake Y, Qiu CH, Kurita A, Tomura M, Kanagawa O, Fujii S, Tanaka M (2011) CD169-positive Mϕs dominate antitumor immunity by crosspresenting dead cell-associated antigens. Immunity 34:85–95
5. Avril T, Wagner ER, Willison HJ, Crocker PR (2006) Sialic acid-binding immunoglobulin-like lectin 7 mediates selective recognition of sialylated glycans expressed on Campylobacter jejuni lipooligosaccharides. Infect Immun 74:4133–4141
6. Backer R, Schwandt T, Greuter M, Oosting M, Jungerkes F, Tuting T, Boon L, O'Toole T, Kraal G, Limmer A, den Haan JM (2010) Effective collaboration between marginal metallophilic Mϕs and CD8+ dendritic cells in the generation of cytotoxic T cells. Proc Natl Acad Sci USA 107:216–221
7. Banchereau J, Pascual V (2006) Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunity 25:383–392
8. Barral P, Polzella P, Bruckbauer A, van Rooijen N, Besra GS, Cerundolo V, Batista FD (2010) CD169(+) Mϕs present lipid antigens to mediate early activation of iNKT cells in lymph nodes. Nat Immunol 11:303–312
9. Calvert JG, Slade DE, Shields SL, Jolie R, Mannan RM, Ankenbauer RG, Welch SK (2007) CD163 expression confers susceptibility to porcine reproductive and respiratory syndrome viruses. J Virol 81:7371–7379
10. Carrasco YR, Batista FD (2007) B cells acquire particulate antigen in a Mϕ-rich area at the boundary between the follicle and the subcapsular sinus of the lymph node. Immunity 27:160–171

11. Chen WC, Completo GC, Sigal DS, Crocker PR, Saven A, Paulson JC (2010) In vivo targeting of B-cell lymphoma with glycan ligands of CD22. Blood 115:4778–4786
12. Crocker PR, Gordon S (1985) Isolation and characterization of resident stromal Mϕs and hematopoietic cell clusters from mouse bone marrow. J Exp Med 162:993–1014
13. Crocker PR, Gordon S (1986) Properties and distribution of a lectin-like hemagglutinin differentially expressed by murine stromal tissue Mϕs. J Exp Med 164:1862–1875
14. Crocker PR, Hill M, Gordon S (1988) Regulation of a murine Mϕ haemagglutinin (sheep erythrocyte receptor) by a species-restricted serum factor. Immunology 65:515–522
15. Crocker PR, Gordon S (1989) Mouse Mϕ hemagglutinin (sheep erythrocyte receptor) with specificity for sialylated glycoconjugates characterized by a monoclonal antibody. J Exp Med 169:1333–1346
16. Crocker PR, Werb Z, Gordon S, Bainton DF (1990) Ultrastructural localization of a Mϕ-restricted sialic acid binding hemagglutinin, SER, in Mϕ-hematopoietic cell clusters. Blood 76:1131–1138
17. Crocker PR, Kelm S, Dubois C, Martin B, McWilliam AS, Shotton DM, Paulson JC, Gordon S (1991) Purification and properties of sialoadhesin, a sialic acid-binding receptor of murine tissue Mϕs. EMBO J 10:1661–1669
18. Crocker PR, Mucklow S, Bouckson V, McWilliam A, Willis AC, Gordon S, Milon G, Kelm S, Bradfield P (1994) Sialoadhesin, a Mϕ sialic acid binding receptor for haemopoietic cells with 17 immunoglobulin-like domains. EMBO J 13:4490–4503
19. Crocker PR, Clark EA, Filbin M, Gordon S, Jones Y, Kehrl JH, Kelm S, Le Douarin N, Powell L, Roder J, Schnaar RL, Sgroi DC, Stamenkovic K, Schauer R, Schachner M, van den Berg TK, van der Merwe PA, Watt SM, Varki A (1998) Siglecs: a family of sialic-acid binding lectins. Glycobiology 8:v
20. Crocker PR, Vinson M, Kelm S, Drickamer K (1999) Molecular analysis of sialoside binding to sialoadhesin by NMR and site-directed mutagenesis. Biochem J 341:355–361
21. Crocker PR, Paulson JC, Varki A (2007) Siglecs and their roles in the immune system. Nat Rev Immunol 7:255–266
22. Dancourt J, Barlowe C (2010) Protein sorting receptors in the early secretory pathway. Ann Rev Biochem 79:777–802
23. Delputte PL, Vanderheijden N, Nauwynck HJ, Pensaert MB (2002) Involvement of the matrix protein in attachment of porcine reproductive and respiratory syndrome virus to a heparinlike receptor on porcine alveolar Mϕs. J Virol 76:4312–4320
24. Delputte PL, Nauwynck HJ (2004) Porcine arterivirus infection of alveolar Mϕs is mediated by sialic acid on the virus. J Virol 78:8094–8101
25. Delputte PL, Costers S, Nauwynck HJ (2005) Analysis of porcine reproductive and respiratory syndrome virus attachment and internalization: distinctive roles for heparan sulphate and sialoadhesin. J Gen Virol 86:1441–1445
26. Delputte PL, Van Breedam W, Delrue I, Oetke C, Crocker PR, Nauwynck HJ (2007) Porcine arterivirus attachment to the Mϕ-specific receptor sialoadhesin is dependent on the sialic acid-binding activity of the N-terminal immunoglobulin domain of sialoadhesin. J Virol 81:9546–9550
27. Delputte PL, Van Gorp H, Favoreel HW, Hoebeke I, Delrue I, Dewerchin H, Verdonck F, Verhasselt B, Cox E, Nauwynck HJ (2011) Porcine sialoadhesin (CD169/Siglec-1) is an endocytic receptor that allows targeted delivery of toxins and antigens to Mϕs. PLoS One 6:e16827
28. Duan X, Nauwynck HJ, Favoreel HW, Pensaert MB (1998) Identification of a putative receptor for porcine reproductive and respiratory syndrome virus on porcine alveolar Mϕs. J Virol 72:4520–4523
29. Freeman GJ, Casasnovas JM, Umetsu DT, DeKruyff RH (2010) TIM genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. Immunol Rev 235:172–189
30. Guerry P, Ewing CP, Hickey TE, Prendergast MM, Moran AP (2000) Sialylation of lipooligosaccharide cores affects immunogenicity and serum resistance of Campylobacter jejuni. Infect Immun 68:6656–6662
31. Hartnell A, Steel J, Turley H, Jones M, Jackson DG, Crocker PR (2001) Characterization of human sialoadhesin, a sialic acid binding receptor expressed by resident and inflammatory Mϕ populations. Blood 97:288–296
32. Hashimoto Y, Suzuki M, Crocker PR, Suzuki A (1998) A streptavidin-based neoglycoprotein carrying more than 140 GT1b oligosaccharides: quantitative estimation of the binding specificity of murine sialoadhesin expressed on CHO cells. J Biochem 123:468–478
33. Heikema AP, Bergman MP, Richards H, Crocker PR, Gilbert M, Samsom JN, van Wamel WJ, Endtz HP, van Belkum A (2010) Characterization of the specific interaction between sialoadhesin and sialylated Campylobacter jejuni lipooligosaccharides. Infect Immun 78:3237–3246
34. Hoffmann PR, deCathelineau AM, Ogden CA, Leverrier Y, Bratton DL, Daleke DL, Ridley AJ, Fadok VA, Henson PM (2001) Phosphatidylserine (PS) induces PS receptor-mediated macropinocytosis and promotes clearance of apoptotic cells. J Cell Biol 155:649–659
35. Ip CW, Kroner A, Crocker PR, Nave KA, Martini R (2007) Sialoadhesin deficiency ameliorates myelin degeneration and axonopathic changes in the CNS of PLP overexpressing mice. Neurobiol Dis 25:105–111
36. Jacobs BC, van Doorn PA, Schmitz PI, Tio-Gillen AP, Herbrink P, Visser LH, Hooijkass H, van der Meche FG (1996) Campylobacter jejuni infections and anti-GM1 antibodies in Guillain–Barre syndrome. Ann Neurol 40:181–187
37. Jones C, Virji M, Crocker PR (2003) Recognition of sialylated meningococcal lipopolysaccharide by siglecs expressed on myeloid cells leads to enhanced bacterial uptake. Mol Micro 49:1213–1225
38. Junt T, Moseman EA, Iannacone M, Massberg S, Lang PA, Boes M, Fink K, Henrickson SE, Shayakhmetov DM, Di Paolo NC, van Rooijen N, Mempel TR, Whelan SP, von Andrian UH (2007) Subcapsular sinus Mϕs in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. Nature 450:110–114
39. Kelm S, Schauer R, Manuguerra JC, Gross HJ, Crocker PR (1994) Modifications of cell surface sialic acids modulate cell adhesion mediated by sialoadhesin and CD22. Glycoconj J 11:576–585
40. Kelm S, Schauer R, Crocker PR (1996) The Sialoadhesins–a family of sialic acid-dependent cellular recognition molecules within the immunoglobulin superfamily. Glycoconj J 13:913–926
41. Kelm S, Brossmer R, Isecke R, Gross HJ, Strenge K, Schauer R (1998) Functional groups of sialic acids involved in binding to siglecs (sialoadhesins) deduced from interactions with synthetic analogues. Eur J Biochem 225:663–672
42. Kirchberger S, Majdic O, Steinberger P, Bluml S, Pfistershammer K, Zlabinger G, Deszcz L, Kuechler E, Knapp W, Stockl J (2005) Human rhinoviruses inhibit the accessory function of dendritic cells by inducing sialoadhesin and B7-H1 expression. J Immunol 175:1145–1152
43. Kobsar I, Oetke C, Kroner A, Wessig C, Crocker P, Martini R (2006) Attenuated demyelination in the absence of the Mϕ-restricted adhesion molecule sialoadhesin (Siglec-1) in mice heterozygously deficient in P0. Mol Cell Neurosci 31:685–691
44. Louwen R, Heikema A, van Belkum A, Ott A, Gilbert M, Ang W, Endtz HP, Bergman MP, Nieuwenhuis EE (2008) The sialylated lipooligosaccharide outer core in Campylobacter jejuni is an important determinant for epithelial cell invasion. Infect Immun 76:4431–4438
45. May AP, Robinson RC, Vinson M, Crocker PR, Jones EY (1998) Crystal structure of the N-terminal domain of sialoadhesin in complex with 3′ sialyllactose at 1.85 A resolution. Mol Cell 1:719–728

46. McGaha TL, Chen Y, Ravishankar B, van Rooijen N, Karlsson MC (2011) Marginal zone Mϕs suppress innate and adaptive immunity to apoptotic cells in the spleen. Blood 117:5403–5412
47. Medzhitov R, Janeway CA Jr (2002) Decoding the patterns of self and nonself by the innate immune system. Science 296:298–300
48. Miyake Y, Asano K, Kaise H, Uemura M, Nakayama M, Tanaka M (2007) Critical role of Mϕs in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. J Clin Invest 117:2268–2278
49. Monteiro VG, Lobato CS, Silva AR, Medina DV, de Oliveira MA, Seabra SH, de Souza W, DaMatta RA (2005) Increased association of Trypanosoma cruzi with sialoadhesin positive mice Mϕs. Parasitol Res 97:380–385
50. Munday J, Floyd H, Crocker PR (1999) Sialic acid binding receptors (siglecs) expressed by Mϕs. J Leukoc Biol 66:705–711
51. Nath D, van der Merwe PA, Kelm S, Bradfield P, Crocker PR (1995) The amino-terminal immunoglobulin-like domain of sialoadhesin contains the sialic acid binding site. Comparison with CD22. J Biol Chem 270:26184–26191
52. Nimmerjahn F, Ravetch JV (2011) FcgammaRs in health and disease. Curr Top Microbiol Immunol 350:105–125
53. Oetke C, Vinson MC, Jones C, Crocker PR (2006) Sialoadhesin-deficient mice exhibit subtle changes in B- and T-cell populations and reduced immunoglobulin M levels. Mol Cell Biol 26:1549–1557
54. Pape KA, Catron DM, Itano AA, Jenkins MK (2007) The humoral immune response is initiated in lymph nodes by B cells that acquire soluble antigen directly in the follicles. Immunity 26:491–502
55. Pascual V, Farkas L, Banchereau J (2006) Systemic lupus erythematosus: all roads lead to type I interferons. Curr Opin Immunol 18:676–682
56. Perry VH, Crocker PR, Gordon S (1992) The blood–brain barrier regulates the expression of a Mϕ sialic acid-binding receptor on microglia. J Cell Sci 101:201–207
57. Phan TG, Grigorova I, Okada T, Cyster JG (2007) Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. Nat Immunol 8:992–1000
58. Pulliam L, Sun B, Rempel H (2004) Invasive chronic inflammatory monocyte phenotype in subjects with high HIV-1 viral load. J Neuroimmunol 157:93–98
59. Rempel H, Calosing C, Sun B, Pulliam L (2008) Sialoadhesin expressed on IFN-induced monocytes binds HIV-1 and enhances infectivity. PLoS One 3:e1967
60. Revilla C, Poderoso T, Martinez P, Alvarez B, Lopez-Fuertes L, Alonso F, Ezquerra A, Dominguez J (2009) Targeting to porcine sialoadhesin receptor improves antigen presentation to T cells. Vet Res 40:14
61. Schadee-Eestermans IL, Hoefsmit EC, van de Ende M, Crocker PR, van den Berg TK, Dijkstra CD (2000) Ultrastructural localisation of sialoadhesin (siglec-1) on Mϕs in rodent lymphoid tissues. Immunobiology 202:309–325
62. Seyerl M, Kirchberger S, Majdic O, Seipelt J, Jindra C, Schrauf C, Stockl J (2010) Human rhinoviruses induce IL-35-producing Treg via induction of B7-H1 (CD274) and sialoadhesin (CD169) on DC. Eur J Immunol 40:321–329
63. Steiniger B, Barth P, Herbst B, Hartnell A, Crocker PR (1997) The species-specific structure of microanatomical compartments in the human spleen: strongly sialoadhesin-positive Mϕs occur in the perifollicular zone, but not in the marginal zone. Immunology 92:307–316
64. Van Breedam W, Van Gorp H, Zhang JQ, Crocker PR, Delputte PL, Nauwynck HJ (2010) The M/GP(5) glycoprotein complex of porcine reproductive and respiratory syndrome virus binds the sialoadhesin receptor in a sialic acid-dependent manner. PLoS Pathog 6:e1000730
65. van der Kuyl AC, van den Burg R, Zorgdrager F, Groot F, Berkhout B, Cornelissen M (2007) Sialoadhesin (CD169) expression in CD14 + cells is upregulated early after HIV-1 infection and increases during disease progression. PLoS One 2:e257
66. Van Gorp H, Van Breedam W, Delputte PL, Nauwynck HJ (2008) Sialoadhesin and CD163 join forces during entry of the porcine reproductive and respiratory syndrome virus. J Gen Virol 89:2943–2953
67. Van Gorp H, Van Breedam W, Delputte PL, Nauwynck HJ (2009) The porcine reproductive and respiratory syndrome virus requires trafficking through CD163-positive early endosomes, but not late endosomes, for productive infection. Arch Virol 154:1939–1943
68. Vanderheijden N, Delputte PL, Favoreel HW, Vandekerckhove J, Van Damme J, van Woensel PA, Nauwynck HJ (2003) Involvement of sialoadhesin in entry of porcine reproductive and respiratory syndrome virus into porcine alveolar Mϕs. J Virol 77:8207–8215
69. Varki A (2008) Sialic acids in human health and disease. Trends Mol Med 14:351–360
70. Vinson M, van der Merwe PA, Kelm S, May A, Jones EY, Crocker PR (1996) Characterization of the sialic acid-binding site in sialoadhesin by site-directed mutagenesis. J Biol Chem 271:9267–9272
71. Wu C, Rauch U, Korpos E, Song J, Loser K, Crocker PR, Sorokin LM (2009) Sialoadhesin-positive Mϕs bind regulatory T cells, negatively controlling their expansion and autoimmune disease progression. J Immunol 182:6508–6516
72. York MR, Nagai T, Mangini AJ, Lemaire R, van Seventer JM, Lafyatis R (2007) A Mϕ marker, Siglec-1, is increased on circulating monocytes in patients with systemic sclerosis and induced by type I interferons and toll-like receptor agonists. Arthritis Rheum 56:1010–1020
73. Zou Z, Chastain A, Moir S, Ford J, Trandem K, Martinelli E, Cicala C, Crocker P, Arthos J, Sun PD (2011) Siglecs Facilitate HIV-1 Infection of Mϕs through Adhesion with Viral Sialic Acids. PLoS One 6:e24559