

# Chapter 8

## Siglecs at the Host–Pathogen Interface



Yung-Chi Chang and Victor Nizet

**Abstract** Siglecs are sialic acid (Sia) recognizing immunoglobulin-like receptors expressed on the surface of all the major leukocyte lineages in mammals. Siglecs recognize ubiquitous Sia epitopes on various glycoconjugates in the cell glycocalyx and transduce signals to regulate immunological and inflammatory activities of these cells. The subset known as CD33-related Siglecs is principally inhibitory receptors that suppress leukocyte activation, and recent research has shown that a number of bacterial pathogens use Sia mimicry to engage these Siglecs as an immune evasion strategy. Conversely, Siglec-1 is a macrophage phagocytic receptor that engages GBS and other sialylated bacteria to promote effective phagocytosis and antigen presentation for the adaptive immune response, whereas certain viruses and parasites use Siglec-1 to gain entry to immune cells as a proximal step in the infectious process. Siglecs are positioned in crosstalk with other host innate immune sensing pathways to modulate the immune response to infection in complex ways. This chapter summarizes the current understanding of Siglecs at the host–pathogen interface, a field of study expanding in breadth and medical importance, and which provides potential targets for immune-based anti-infective strategies.

**Keywords** Sialic acid · Streptococcus · Pattern-recognition receptor · Trans-infection

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Y.-C. Chang (✉)

Graduate Institute of Microbiology, College of Medicine, National Taiwan University, No. 1, Sec. 1, Jen-Ai Rd., Taipei 10051, Taiwan

e-mail: [yungchiychang@ntu.edu.tw](mailto:yungchiychang@ntu.edu.tw)

V. Nizet

Division of Host-Microbe Systems and Therapeutics, Department of Pediatrics, and Skaggs School of Pharmacy and Pharmaceutical Sciences, UC San Diego, 9500 Gilman Drive Mail Code 0760, La Jolla, CA 92093, USA

e-mail: [vnizet@ucsd.edu](mailto:vnizet@ucsd.edu)

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## 8.1 Introduction

**Sialic acid-binding immunoglobulin-type lectins** (Siglecs) are cell surface receptors belonging to the immunoglobulin (Ig) superfamily. The extracellular domains of these receptors comprise one ligand-binding V-set domain and a variable number of C2-set domains, with high-sequence similarities to the variable and constant region of antibodies, respectively. Siglecs are mostly located on the cell surface of hematopoietic cells, with the exception of Siglec-4 (Schwann cells) and Siglec-6 (epithelial cells). Until now, 14 human Siglecs and 9 murine Siglecs have been identified with different preferences for binding to terminal sialic acids (Sia) on glycan structures in a linkage-sensitive fashion. The arginine residue in the Siglec V-set domain is critical for contacting the carboxyl group of the Sia to form a salt bridge that stabilizes the binding interaction.

Phylogenetically, Siglec family members can be subdivided into two groups: First, there are Siglecs that are conserved among different species, but showing low-sequence identity to one another, including Siglec-1, Siglec-2, Siglec-4, and Siglec-15. The other group comprises the so-called CD33 (Siglec-3)-related Siglecs (CD33rSiglecs), which show low gene conservation but possess higher degrees of sequence identity among the subfamily members. Both conserved Siglecs and CD33rSiglecs include activating and inhibitory receptors. The inhibitory Siglecs contain an immunoreceptor-based inhibition motif (ITIM) in their intracellular domain, conferring the ability to antagonize immune signaling pathways through the recruitment of the SHP phosphatases. Conversely, activating Siglecs have an aspartic acid residue in their trans-membrane domain that associates with immunoreceptor-based activation motif (ITAM)-containing adaptor DAP12 (DNAX-activation protein of 12 kDa) to promote signaling and immune responses. Lastly, Siglec-1 does not possess a functional intracellular domain and is not known to signal directly, but rather plays a role in cell–cell and cell–microbe interactions.

## 8.2 Siglecs in Bacterial Infection

Glycans are ubiquitous on eukaryotic cell surfaces via their incorporation in glycoproteins, glycosphingolipids, and glycerophosphatides. In mammalian cells, Sia is usually the outmost sugar residue on the oligosaccharide chains of cell surface or serum glycoconjugates, where it functions in recognition and anti-recognition phenomena ranging from the regulation of complement activation to the control of cell–cell apposition (Varki 1993). Like their host cells, bacteria have also evolved complex biosynthetic pathways to produce a diverse array of carbohydrates that form the building blocks of capsular polysaccharides (CPS), lipopolysaccharides (LPS), lipooligosaccharides (LOS), and peptidoglycans. These specialized bacterial glycans play pivotal roles in a number of biological processes, particularly mediating microbe–host interactions during the onset and development of infectious disease.

It has been long known that the bacterial CPS represents a key virulence factor for most encapsulated bacterial pathogens by protecting them from immune clearance within the host. Some bacterial capsules interfere with the binding and activation of complement factors on the bacterial surface by inhibiting the C3b convertase or recruiting inhibitory complement factor H (Cross and Kelly 1990). Bacterial capsules exerting this immune resistance mechanism usually contain sialic or polysialic acids or hyaluronic acid (HA), structurally identical or similar to the polysaccharides found in mammalian tissues, as exemplified by *Escherichia coli* K1, *Neisseria meningitidis* types B and C, or group A or group B *Streptococcus* (Stevens et al. 1978; Wessels et al. 1989; Foley and Wood 1959; Dale et al. 1996). Envelopment in these host-like capsular structures confers resistance to complement-mediated killing and phagocytic uptake by neutrophils and macrophages, increasing the chance of bacterial survival and dissemination into host tissues. In addition to these passive mechanisms of protection, an emerging hypothesis has been raised based upon the discovery of inhibitory members of the Siglec protein family on immune cells. Researchers have explored whether these host-mimicking pathogens can blunt activation of antimicrobial responses via engagement of inhibitory Siglecs using the Sia in their CPS. In parallel, studies have asked how hosts have evolved to better recognize and destroy such camouflaged pathogens. The interplay between a particular sialylated human pathogen, group B *Streptococcus* (GBS), and host immune responses serves as a good first example to illustrate the role of Siglecs and bacterial expression of Sia in the pathogenesis of infection.

GBS is a leading cause of neonatal pneumonia, sepsis, and meningitis and is increasingly recognized as a pathogen in elderly and immunocompromised adult populations (Heath and Schuchat 2007; Thigpen et al. 2011; Skoff et al. 2009). GBS can be classified into ten serotypes varying in structural and antigenic features, but which share in common the presence of a terminal Sia residue that closely resembles the one presented throughout the abundant surface glycocalyx of all human cells (Cieslewicz et al. 2005). Sialylated CPS from different GBS serotypes interacts with several human Siglecs in a Sia- and serotype-specific manner (Carlin et al. 2007). Through this Sia molecular mimicry, GBS engages inhibitory human Siglec-9 receptors on neutrophils, resulting in reduced production of reactive oxidative species (ROS) and neutrophil extracellular traps (NETs), which together impair bactericidal activity (Carlin et al. 2009b). Recently, GBS Sia engagement of Siglec-9 on human platelets to suppress their activation and release of antimicrobial peptides (AMPs) was shown to contribute to GBS resistance to platelet killing (Uchiyama et al. 2019). Corroborating these findings, transgenic mice expressing a soluble Siglec-9 receptor, which acts as a decoy to prevent neutrophil suppression, were more resistant to GBS infection in vivo (Saito et al. 2016). Linkages to the underlying sugar chain as well as the substitution at certain carbon positions of Sias are critical in determining their binding specificity for Siglecs. One study used isogenic mutants with different *O*-acetylation phenotypes to show that Sia *O*-acetylation protects GBS CPS from enzymatic removal by microbial sialidases produced by commensal microbes occupying in the same mucosal niche as GBS (Weiman et al. 2009). However, the same modification markedly reduced GBS binding to human Siglec-9, such that

highly *O*-acetylated GBS mutants were less able to accomplish Sia-mediated neutrophil suppression and showed reduced virulence in vivo (Weiman et al. 2009, 2010). These observations suggest that GBS must balance competing evolutionary selective pressures to fine tune the *O*-acetylation level of CPS Sias to maximize its survival advantage in the host.

The role of GBS CPS Sia engagement of inhibitory Siglecs in the context of in vivo infection was first addressed in Siglec-E deficient mice due to the similarity of function and cellular distribution between human Siglec-9 and murine Siglec-E. Upon lower dose GBS challenge intranasally or intravenously, Siglec-E deficient mice showed increased production of several inflammatory cytokines and had reduced dissemination of the pathogen to the brain (GBS meningitis). However, exaggerated inflammatory mediators and reduced anti-inflammatory cytokine IL-10 production were observed in the Siglec-E deficient mice during a high-dose lethal challenge with GBS (Chang et al. 2014a). Thus, the sum consequence of the GBS molecular mimicry and inhibitory Siglec engagement is likely to vary based upon the site, stage, and magnitude of infection. Importantly, in addition to Sia-dependent Siglec engagement, certain GBS strains can use the surface-anchored  $\beta$  protein to bind human Siglec-5, another inhibitory Siglec preferentially expressed on macrophages and neutrophils. This protein-mediated engagement increased bacterial attachment to the macrophage surface, but simultaneously paralyzed macrophage killing functions, leading to a net reduction of phagocytosis, ROS production, NET formation, and bactericidal activity (Carlin et al. 2009a).

To counteract inhibitory Siglec hijacking strategies of bacterial pathogens, host Siglec-1 (sialoadhesin, CD169) plays a crucial role in limiting bacterial dissemination. This receptor, uniquely expressed in the marginal metallophilic and subcapsular sinus macrophages, recognizing the same key Sia epitope on the GBS surface, however lacks an intracellular ITM motif (Crocker and Gordon 1989; Crocker et al. 1994). Siglec-1 binding to GBS CPS Sia promoted phagocytic and bactericidal activity of macrophages in vitro and restricts GBS dissemination in vivo (Chang et al. 2014b). Loss of Siglec-1 expression not only affected the macrophage sampling and trapping capabilities but also the production of anti-GBS antibodies, suggesting a key role in optimization of antigen presentation and subsequent adaptive immune response against sialylated pathogens (Chang et al. 2014b). Another evolutionary adaption of the host to defeat pathogen Siglec hijacking is the emergence of activating Siglecs with the potential to counteract inhibitory Siglec-mediated immune suppression. In 2006, Angata et al. discovered Siglec-14, which possesses nearly identical Sia-binding domain as inhibitory Siglec-5 through gene conversion, but is coupled with DAP12, an ITAM motif bearing adaptor (Angata et al. 2006). Thus Siglec-5 and -14 represent paired receptors with opposite signaling effects. For example, on neutrophils and amniotic epithelium,  $\beta$  protein-expressing GBS can bind to Siglec-5 and Siglec-14, with the latter engagement stimulating p38 MAP kinase and AKT signaling to promote more efficient bacterial clearance. Notably, a *SIGLEC14*-null polymorphism is present in some humans, caused by fusion between *SIGLEC14* and *SIGLEC5* genes, resulting in functional deletion of Siglec-14 expression (Yamanaka

et al. 2009). A genetic survey of the *SIGLEC14*-null polymorphism and GBS colonization and premature delivery found that *SIGLEC14*-null allele is associated with higher GBS colonization in mothers and more frequent premature birth of infants from GBS-positive pregnancies (Ali et al. 2014).

In addition to GBS, the pathogens *Escherichia coli* K1 and *Neisseria meningitidis* serotype B, important agents of bacterial meningitis in infants and children, possess sialylated capsules. The CPS produced by these two bacteria resembles the same poly- $\alpha$ 2,8-Sia (PSA) structure abundantly expressed on neurons and the glia cells of the developing central nervous systems (CNS) is critical for neuron development and function (Finne 1982; Devi et al. 1991; Rutishauser 2008). The human-specific Siglec-11 shows a unique expression pattern in human microglia and selective binding preference to  $\alpha$ 2,8-linked Sias (Angata et al. 2002; Hayakawa et al. 2005). Engagement of Siglec-11 by endogenous host PSA exerts protective effects against inflammation-mediated neurotoxicity (Wang and Neumann 2010). The mimicry of mammalian PSA by *E. coli* K1 and *N. meningitidis* type B CPS may, therefore, target Siglec-11 expression on microglia to blunt immune responsiveness and facilitate neuroinvasion, consistent with the high rates of mortality and serious neurological sequelae seen with these pathogens (Robbins et al. 1974; Kaper et al. 2004). Interestingly, the activating Siglec-16, which shares over 99% of sequence identity to the first two Ig-like domain of Siglec-11 but is coupled with ITAM-containing DAP12, was later discovered to be a paired receptor of Siglec-11 allowing fine-tuning of immune responses (Cao et al. 2008). Pathogenic *E. coli* K1 engages inhibitory Siglec-11 through their PSA capsule to inhibit macrophage anti-bacterial functions. In contrast, activating Siglec-16 expressing macrophages recognizes the same epitope to promote elimination of pathogen (Schwarz et al. 2017). In sum, macrophage engagement of GBS  $\beta$  protein or *E. coli* K1 PSA capsule illustrates an evolutionary dynamic in which the activating member of the Siglec receptor pair may override the immune suppressive responses generated by pathogen mimicry of the inhibitory Siglec member.

In addition to the CPS, sialylation of LPS and LOS is also a prominent binding target of Siglecs. For example, Siglec-1 and Siglec-5 bind to the sialylated LPS of *N. meningitidis*, and cells expressing either Siglec internalized meningococci in a Siglec- and Sia-dependent manner (Jones et al. 2003). Strains of *Campylobacter jejuni* express various monosialylated and disialylated LOS with  $\alpha$ 2,3- or  $\alpha$ 2,3/2,8-linked Sia residues, respectively, which perfectly mimic host neural gangliosides GM1, GD1a, GD3, or GT1a. Colonization with *C. jejuni* strains possessing sialylated LOS is epidemiologically associated with higher risk of developing a postinfectious autoimmune neuropathy termed Guillain-Barré syndrome (Jacobs et al. 1996; Willison et al. 2016; Willison and Yuki 2002). Several human Siglecs recognize these particular sialylated LOS structures, although the functional consequences of this interaction require further investigation. Siglec-7, which is expressed on natural killer (NK) cells, monocytes, and dendritic cells (DCs), can mediate specific Sia-dependent interaction with *C. jejuni* LOS (Avril et al. 2006). *C. jejuni* strains recognized by Siglec-7 express terminal disialylated residues mimicking host GQ1b-like epitopes, including GD1c and GD3. The Siglec-7 binding signal of *C. jejuni*

correlates with its ability to elicit anti-GQ1b antibodies, and strains recognized by Siglec-7 were particularly associated with oculomotor weakness in Guillain-Barré syndrome and its so-called Miller-Fisher variant (Heikema et al. 2013). On the other hand, *C. jejuni* with  $\alpha$ 2,3-linked Sia on the LOS chain showed strong interaction with Siglec-1 (Heikema et al. 2010), which facilitated bacterial uptake and induce higher macrophage production of the pro-inflammatory cytokine IL-6 (Heikema et al. 2013). A key role of Siglec-1 in recognition of sialylated *C. jejuni* LOS was confirmed in Siglec-1 deficient animals. Bone-marrow-derived macrophages from mice lacking Siglec-1 showed greatly reduced phagocytosis of sialylated *C. jejuni*, coupled with reduced production of proinflammatory cytokines and type I interferon responses (Klaas and Crocker 2012).

Sialylated LOS is important in DC and macrophage activation as well as subsequent T cell polarization and B cell activation. Removal of Sia from the *C. jejuni* LOS causes reduced myeloid cell activation and subsequent B cell responses (Kuijf et al. 2010; Huizinga et al. 2012, 2013). Varied sialylated LOS structures differentially modulate DC-mediated T cell polarization in a Siglec-dependent manner, wherein the GD1a/GM1a mimic induced a more pronounced Th2 skewing, while the GD1c mimic preferentially stimulated Th1 responses (Bax et al. 2011). These observations suggest that targeting distinct DC-expressed Siglecs may represent a potential strategy for manipulating Th cell differentiation programs and forestalling autoimmune disease post *C. jejuni* infection. In addition to modulating host DC functions via its sialylated LOS, *C. jejuni* triggers IL-10 production of DCs via engaging Siglec-10 through the pseudaminic acid residues on its flagella (Stephenson et al. 2014). These abundant Sia and Sia-like *C. jejuni* surface structures (e.g. pseudaminic acid) help mediate a complicated interaction with host immune cells that may impact *C. jejuni* human disease associations, ranging from autoimmune neuropathies to asymptomatic colonization in individuals with repeated exposure to *Campylobacter* spp.

In addition to the well-studied microorganisms mentioned above, a growing list of bacterial species have been discovered to display sialoglycoconjugates on their surfaces, such as *Pseudomonas aeruginosa* (Khatua et al. 2010, 2012), *Klebsiella pneumoniae* (Lee et al. 2014), and nontypeable *Haemophilus influenzae* (NTHi) (Kalograiaki et al. 2016). *P. aeruginosa* recruits host sialoglycoproteins and displays them on the bacterial surface, and these absorbed Sias can enhance bacterial survival by reducing complement deposition and engaging inhibitory Siglec-9 to suppress neutrophil bactericidal machinery (Khatua et al. 2010, 2012). *K. pneumoniae* exhibiting a hypermucoviscosity phenotype possess abundant Sia CPS; blocking of the pathogen's ability to engage inhibitory Siglec-9 enhances neutrophil bactericidal activity (Lee et al. 2014). NTHi LOS also contains a terminal Sia residue that interacts with Siglec-14, which enhances inflammatory cytokine production in Siglec-14-expressing macrophages correlating to COPD exacerbation in human patients (Angata et al. 2013). All these observations suggest that Sia molecular mimicry by many medical important bacteria can interplay with various Siglecs to affect infectious risk and clinical disease manifestations.

Glycosaminoglycans (GAGs) are another family of complex carbohydrates ubiquitously present on mammalian host cell surfaces and in the extracellular matrix, regulating a wide range of biological functions from cell adhesion and cell migration to tissue repair and immune responses (Linhardt and Toida 2004). In another instance of molecular mimicry, GAG structures have been discovered in several gram-positive and gram-negative bacterial capsules (Wessels et al. 1994; Jann and Jann 1992; DeAngelis 2002). The GAG HA is structurally identical in animals and in the capsule of group A *Streptococcus* (GAS) where it is serving as a molecular camouflage to evade host immune responses (Wessels et al. 1994; Dale et al. 1996). Recently, GAS was shown to use its HA capsule to target Siglec-9 on neutrophils, thereby blocking NET formation and oxidative burst to inhibit bactericidal function (Secundino et al. 2016). It is an interesting example of convergent evolution that two structurally unrelated carbohydrates, Sia of GBS and HA of GAS, each target distinct epitopes in the V-set domain of inhibitory Siglec-9 to dampen immune responses and promote pathogen survival in the human host (Secundino et al. 2016).

### 8.3 Siglecs in Viral Infection

Although fundamentally distinct from the Sia molecular mimicry strategies employed by bacterial pathogens to engage inhibitory Siglecs for immune evasion, several lines of evidence indicate viruses can themselves take advantage of Sia-Siglec interactions for cell targeting, spreading, and trans-infection.

Human immunodeficiency virus-1 (HIV-1) exploits a Sia-Siglec axis to facilitate its entry into myeloid cells and *trans*-infection into CD4<sup>+</sup> T cells. Notably, Siglec-1 possesses a unique extended 17 Ig-like extracellular domain structure extending out from the surface glycocalyx in an un-masked state which makes it an ideal surface entry target. Moreover, in contrast to infected CD4<sup>+</sup> T cells, myeloid cells are relatively resistant to HIV-1-induced cytopathic effects and there is no obvious depletion of myeloid cells in HIV-1-infected patients. To escape from host immune surveillance, HIV-1 may have evolved to hijack this Siglec-1-mediated cellular recognition pathway for its own benefit, to hide within infected macrophages and *trans*-infect CD4<sup>+</sup> T cells for efficient viral spread.

In the manner of many enveloped retroviruses, the HIV-1 envelope glycoprotein gp120 is heavily glycosylated, and gp120 mutations that remove N-linked glycan sites on HIV (and simian immunodeficiency virus SIV) impair virus attachment and entry (Auwerx et al. 2008). A direct interaction of HIV-1 and Siglec was first reported by Rempel et al. in 2008, where expression levels of Siglec-1 were correlated to HIV-1 viral load, and Siglec-1 was proven to bind HIV-1 in a Sia-dependent manner and facilitate infection to a permissive reporter cell line (Rempel et al. 2008). Later gp120 was proven to serve as viral ligand for Siglec-1 and for several CD33rSiglecs, including Siglec-3, -5, -7, and -9, with varying avidity and HIV-1 strain dependency. Moreover, this Siglec-gp120 interaction facilitates virus infectivity to macrophages and T cells (Zou et al. 2011; Varchetta et al. 2013). Mature DCs (mDCs) capture HIV-1 and

viral membrane gangliosides, then transfer the virus to bystander CD4<sup>+</sup> T cells via the established immunological synapses between these two immune cell types. Host cell-derived  $\alpha$ 2,3 sialylated glycosphingolipid (GSL) on the HIV-1 particle membrane is the ligand for Siglec-1 on mDC required for triggering mDC-mediated *trans*-infection of CD4<sup>+</sup> T cells in lymphoid organs (Yu et al. 2014; Izquierdo-Useros et al. 2012; Puryear et al. 2013). A population of cervical DCs at the lamina propria of the ectocervix and the endocervix that express Siglec-1 may be particularly important, and *ex vivo* studies suggest that CD4<sup>+</sup> T cell *trans*-infection from these cells can be blocked by addition of anti-Siglec-1 antibodies (Perez-Zsolt et al. 2019a).

A loss-of-function variant (Glu88Ter) of *SIGLEC1* gene was recently identified from the Exome Aggregation Consortium genetic database to be present in 1% of the human population. Monocytes isolated from individuals with this specific variant completely lack Siglec-1 expression and have reduced HIV capture and *trans*-infection phenotypes *ex vivo*. However, individuals carrying this truncated Siglec-1 protein do not show marked difference in HIV-1 acquisition and AIDS outcomes *in vivo*, which suggests an indispensable (and perhaps dominant) role of the classical HIV-1 infectious routes in the HIV-1 dissemination within the infected individuals (Martinez-Picado et al. 2016).

The importance of Siglec-1-mediated viral spreading has also been confirmed *in vivo* in murine retroviral infection models. Murine leukemia virus (MLV) is recognized by murine Siglec-1 through its sialylated gangliosides (Erikson et al. 2015; Sewald et al. 2015). MLV captured by Siglec-1-expressing sinus-lining macrophages and subsequent *trans*-infection into B-1 cells through their synaptic contacts was directly demonstrated by time-lapse intravital 2-photon laser scanning microscopy (Sewald et al. 2015). Virus capture and efficient MLV infection at the lymph node and spleen were significantly reduced by Siglec-1-targeting antibodies as well as in *Siglec1*<sup>-/-</sup> mice (Sewald et al. 2015). These data confirm a pivotal role of Siglec-1 in initial retroviral capture and suggest that Siglec-1 could represent a therapeutic target to reduce viral laden macrophage reservoirs and to prevent *trans*-infection. However, in a murine model of the splenomegaly-inducing retrovirus Friend virus complex (FVC) infection, Siglec-1-expressing macrophages capture of incoming blood-borne retroviruses limited their spread to erythroblasts in the red pulp where FVC manifests its pathogenesis (Uchil et al. 2019). In this case, Siglec-1-mediated FVC capture was beneficial, and further activated DCs and promoted cytotoxic CD8<sup>+</sup> T cell responses, promoting efficient clearing of FVC-infected cells (Uchil et al. 2019).

Another well-documented example of Siglec exploitation for viral entry and immune modulation is the example of porcine reproductive and respiratory syndrome virus (PRRSV). PRRSV infection is one of the most economically devastating diseases in the global pork industry. PRRSV has a narrow cell tropism, primarily targeting cells of the monocyte/macrophage lineage (Duan et al. 1997). Two cellular proteins, CD163 and Siglec-1 have been identified as the primary targets for PRRSV binding and internalization (Duan et al. 1998; Vanderheijden et al. 2003).  $\alpha$ 2-3 and, to a lesser extent,  $\alpha$ 2-6-linked Sias on the PRRSV virion mediate viral

attachment and infection alveolar macrophages, with Siglec-1 serving as the primary entry receptor responsible for Sia-dependent binding through its N-terminal V-set domain (Delputte and Nauwynck 2004; Delputte et al. 2007). Siglec-1 neutralization blocks PRRSV infection in a dose-dependent manner, and overexpression of Siglec-1 in non-permissive cells enhances PRRSV cell attachment and internalization (Duan et al. 1998; Vanderheijden et al. 2003). In subsequent studies, Sias on the viral envelope structural protein M/GP5 heterodimer were identified as the binding target of Siglec-1 (Van Breedam et al. 2010), and the interaction of PRRSV with Siglec-1 interferes with macrophage phagocytic activity, which may increase the incidence and severity of secondary bacterial infections complicating primary PRRSV disease (De Baere et al. 2012).

Since Siglec-1 and CD163 are the two key receptors for PRRSV entry and internalization into porcine alveolar macrophages, neutralization of these two viral receptors has been explored as a potential target to control PRRSV infection in pigs. Two recent reports support this therapeutic concept. Neutralization of PRRSV infectivity for porcine alveolar macrophages was achieved by addition of soluble Siglec-1 and CD163 Fc fusion proteins, or by recombinant adenovirus- or exosome-delivered microRNA that specifically targeted CD163 and Siglec-1 (Chen et al. 2014b; Zhu et al. 2014). In addition, pigs that received recombinant adenovirus-delivered soluble CD163 plus Siglec-1 had reduced PRRSV viral loads and fecal viral emission, concurrent with improved clinical scores and higher survival rates from the contagious infection (Xia et al. 2018). It is notable that an *in vivo* study conducted in *SIGLEC1* knockout pigs found that the absence of Siglec-1 expression does not alter to the clinical course and histopathology of PRRSV infection (Prather et al. 2013), implying a potential redundant function of CD163 and Siglec-1 in PRRSV infection and the importance of dual targeting for potential pharmacological interventions.

Although Siglec-1 and CD163 are general recognized as the key entry mediators for PRRSV infection, a recent report demonstrated that certain PRRSV strains can infect Siglec-1-deficient cells by exploiting Siglec-10 in the presence of CD163 (Frydas and Nauwynck 2016; Xie et al. 2017). Siglec-10 showed a higher affinity to type 2 PRRSV vs. type 1 PRRSV and mediates attachment and endocytosis of PRRSV in a Sia-dependent manner, while transfection of Siglec-10 into non-permissive cells also restored the PRRSV infectivity. These findings indicate that PRRSV can use several Siglecs to enter macrophages and may influence strain differences in pathogenesis (Xie et al. 2017). Of note, PRRSV lung infection induces minimal production of type I interferon and inflammatory cytokines compared to infections caused by swine influenza virus and porcine respiratory coronavirus (Van Reeth et al. 1999). Like other inhibitory Siglecs, porcine Siglec-10 contains one ITIM and one ITIM-like motif possibly, likely counteracting key immune activation pathways induced by viral infection (Crocker et al. 2007).

Budding from the GSL-enriched domain is a conserved feature of other enveloped viruses. Incorporation of host GSL into the viral envelope is found in Hendra and Nipah viruses, two enveloped RNA viruses of the family *Paramyxoviridae*, and these viruses can utilize the Sia-Siglec-1 axis to potentiate mDC-dependent capture and

*trans*-infection of T cells (Akiyama et al. 2014). Ebola viruses cause lethal hemorrhagic fever in humans, with many recent outbreaks on the African continent. Recently, Ebola virus entry into activated DCs was shown to involve Siglec-1 recognition of sialylated gangliosides anchored to Ebola virus membranes (Perez-Zsolt et al. 2019b). Blockade of Siglec-1 by specific monoclonal antibodies interrupted Ebola viral uptake and cytoplasmic entry, providing cross-protection against other GSL-containing viruses including HIV-1 (Perez-Zsolt et al. 2019b). This finding suggests that incorporation of GSLs in virus particle membranes facilitates Siglec-1 binding to mDC and may be a conserved mechanism for enveloped RNA viruses to exploit mDC for systemic virus dissemination.

Finally, Siglec-1 modulates T cell-mediated anti-viral responses during human respiratory syncytial virus (RSV) infection, with key differences between newborns and adults. Upon RSV infection, expression of Siglec-1 is upregulated on monocytes from both newborns and adults, whereas expression of Siglec-1 ligand, CD43, is only highly upregulated on adult CD4<sup>+</sup> T cells (van den Berg et al. 2001; Jans et al. 2018). This finding is consistent with observations that Siglec-1 inhibits IFN- $\gamma$  production by adult CD4<sup>+</sup> T cells but not newborn CD4<sup>+</sup> T cells, although the detailed mechanism remains unknown (Jans et al. 2018).

## 8.4 Siglecs in Parasitic and Fungal Infections

Unlike many bacterial and viral infections, parasitic diseases caused by protozoa and helminths are often chronic, lasting months to years or even lifetimes. Animal parasites have developed remarkable strategies to interfere with or avoid immune clearance mechanisms to establish a chronic infection in their vertebrate hosts, including several examples of Sia-Siglec-mediated interactions that help achieve this immune evasion phenotype.

The protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, illustrates well how Sia can be exploited by animal parasites to escape host immune surveillance, from the very first encounter with innate immune cells to the late-mounted adaptive immune responses. Although *T. cruzi* cannot synthesize Sias de novo, it uses its *trans*-sialidase (TcTS) enzyme to transfer Sias from host sialyl-glycoconjugates to its own surface mucins (Pereira 1983; Pereira et al. 1980). These highly sialylated mucin-like coats generated on the *T. cruzi* surface confer resistance to complement-mediated killing by impeding C3 convertase assembly, and block potential lytic effects of host anti- $\alpha$ -Gal antibodies that would recognize the otherwise exposed  $\alpha$ -galactosyl epitopes on the mucin-like glycoproteins (Pereira-Chioccola et al. 2000; Tomlinson et al. 1994). Moreover, *T. cruzi* sialyl-glycoconjugates facilitate its binding to Siglec-1 on host macrophages, and this interaction may be involved in the initiation of trypomastigote infection (Monteiro et al. 2005). *T. cruzi* can also use its surface sialyl-glycoconjugates to engage Siglec-E on DCs and actively suppress their production of proinflammatory cytokine IL-12, impairing generation of protective Th1 responses (Erdmann et al. 2009).

Presence of  $\alpha$ 2-3 and  $\alpha$ 2-6 linked sialoglycans has also been discovered on another flagellated protozoa, *Leishmania donovani*, the causative organism of Indian visceral leishmaniasis (Chatterjee et al. 2003), such that high Sia-containing virulent strains bind both Siglec-1 and Siglec-5. The Sia-Siglec-1 interaction promotes macrophage uptake of the parasite promoting dissemination to other sites within the body, whereas the Sia-Siglec-5 binding suppresses ROS, NO, and Th2-dominant cytokine responses in infected macrophages by counteracting MAPK and PI3K/Akt signaling pathways (Roy and Mandal 2016).

Lastly, vaginitis caused by the fungal pathogen *Candida* is common and frequently recurrent condition in women's health. Recently, a combined global genetic and immune profiling study of clinical populations identified polymorphisms in *SIGLEC15* as candidate genetic predispositions involved in *Candida* vaginitis susceptibility (Jaeger et al. 2019). A particular *SIGLEC15* polymorphism was associated with great inflammasome signaling and IL-1 $\beta$  production in response to *Candida* in vitro, and in vivo silencing of Siglec15 in a murine vaginitis model was associated with increased fungal burden and neutrophilic inflammation (Jaeger et al. 2019).

## 8.5 Crosstalk Between Siglec and Pattern-Recognition Receptors in Host Defense Mechanism

Recent evidence indicates that Siglecs functionally intersect with other host innate immune sensing pathways to modulate the response to viral and bacterial infection in important ways. For example, the RNA virus vesicular stomatitis virus (VSV) upregulates Siglec-G expression in macrophages through intracellular nucleic acid sensor RIG-I- or NF- $\kappa$ B-dependent mechanisms (Chen et al. 2013). Subsequent recruitment of SHP-2 phosphatase to the Siglec-G intracellular domain initiates a pathway for RIG-I degradation, thus suppressing anti-viral immunity. In corroboration, inactivation of Siglec-G protects mice against lethal VSV infection (Chen et al. 2013). VSV infection also results in upregulation of Siglec-1 in macrophages, which triggers a negative regulation pathway for TBK1 degradation via the ubiquitin ligase TRIM27, suppressing type I interferon production and allowing the virus to escape immune elimination (Zheng et al. 2015). Siglec-G expression is low in CD8 $\alpha^+$  DCs, and Siglec-G deficient mice generate more antigen-specific cytotoxic T cell responses to inhibit intracellular bacterial infection (Ding et al. 2016).

One line of evidence developed in recent years suggests a potential for direct interactions between Siglec receptors, e.g. human Siglec-5 or -9 and murine Siglec-E and -F, and various toll-like receptors (TLRs) to downregulate the inflammatory pathways downstream of their pattern recognition (Chen et al. 2014a). Consequently, Siglec-E deletion was seen to boost DC responses to a broad range of microbial TLR ligands. In this model, activation of the mammalian sialidase Neu1 to the cell surface disrupts Siglec-E-TLR4 interaction, suggesting it can serve to derepress and allow positive feedback of TLR activation during infection (Chen et al. 2014a).

Caution is warranted, however, by another study applied quantitative proteomics to three different strains of Siglec-E-deficient mice. Quantitative proteomics found no consistent differences in TLR4 signaling or TLR4 endocytosis in response to LPS, nor did macrophages from the Siglec-E-deficient mice exhibit significant differences in uptake or killing of *Salmonella enterica* in vitro (Nagala et al. 2017).

## 8.6 Future Perspectives

Siglecs are important and broadly distributed lectin receptors of leukocytes uniquely poised to detect Sias and its perturbation during homeostasis and in disease states. The importance of Siglecs in immunopathology was proposed as early as the identification of Siglec-3 (CD33) and Siglec-2 (CD22) as biological markers of myeloid leukemias and B cell lymphomas, respectively. Mounting evidence supports the concept that Sia molecular mimicry serves as a virulence mechanism to subvert host innate immunity or to infect permissive target cells through an interplay with various Siglecs. As our understanding of Siglec influences on glycans-mediated host–pathogen interactions is now rapidly expanding and deepening, there is considerable interest in exploiting Siglecs for immunotherapy and disease prevention. Design of glycan-based therapeutics and their requirements for potency and specificity will likely provide a new biotechnological approach to effectively intervene in immunological processes to reduce the incidence and severity infectious diseases.

## References

- Akiyama H, Miller C, Patel HV, Hatch SC, Archer J, Ramirez NG, Gummuru S (2014) Virus particle release from glycosphingolipid-enriched microdomains is essential for dendritic cell-mediated capture and transfer of HIV-1 and henipavirus. *J Virol* 88(16):8813–8825. <https://doi.org/10.1128/JVI.00992-14>
- Ali SR, Fong JJ, Carlin AF, Busch TD, Linden R, Angata T, Areschoug T, Parast M, Varki N, Murray J, Nizet V, Varki A (2014) Siglec-5 and Siglec-14 are polymorphic paired receptors that modulate neutrophil and amnion signaling responses to group B Streptococcus. *J Exp Med*. <https://doi.org/10.1084/jem.20131853>
- Angata T, Kerr SC, Greaves DR, Varki NM, Crocker PR, Varki A (2002) Cloning and characterization of human Siglec-11. A recently evolved signaling molecule that can interact with SHP-1 and SHP-2 and is expressed by tissue macrophages, including brain microglia. *J Biol Chem* 277(27):24466–24474
- Angata T, Hayakawa T, Yamanaka M, Varki A, Nakamura M (2006) Discovery of Siglec-14, a novel sialic acid receptor undergoing concerted evolution with Siglec-5 in primates. *Faseb J* 20(12):1964–1973
- Angata T, Ishii T, Motegi T, Oka R, Taylor RE, Soto PC, Chang YC, Secundino I, Gao CX, Ohtsubo K, Kitazume S, Nizet V, Varki A, Gemma A, Kida K, Taniguchi N (2013) Loss of Siglec-14 reduces the risk of chronic obstructive pulmonary disease exacerbation. *Cell Mol Life Sci* 70(17):3199–3210

- Auwerx J, Francois KO, Covens K, Van Laethem K, Balzarini J (2008) Glycan deletions in the HIV-1 gp120 V1/V2 domain compromise viral infectivity, sensitize the mutant virus strains to carbohydrate-binding agents and represent a specific target for therapeutic intervention. *Virology* 382(1):10–19
- 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(7):4133–4141
- Bax M, Kuijf ML, Heikema AP, van Rijs W, Bruijns SC, Garcia-Vallejo JJ, Crocker PR, Jacobs BC, van Vliet SJ, van Kooyk Y (2011) *Campylobacter jejuni* lipooligosaccharides modulate dendritic cell-mediated T cell polarization in a sialic acid linkage-dependent manner. *Infect Immun* 79(7):2681–2689
- Cao H, Lakner U, de Bono B, Traherne JA, Trowsdale J, Barrow AD (2008) SIGLEC16 encodes a DAP12-associated receptor expressed in macrophages that evolved from its inhibitory counterpart SIGLEC11 and has functional and non-functional alleles in humans. *Eur J Immunol* 38(8):2303–2315
- Carlin AF, Lewis AL, Varki A, Nizet V (2007) Group B streptococcal capsular sialic acids interact with siglecs (immunoglobulin-like lectins) on human leukocytes. *J Bacteriol* 189(4):1231–1237
- Carlin AF, Chang YC, Areschoug T, Lindahl G, Hurtado-Ziola N, King CC, Varki A, Nizet V (2009a) Group B *Streptococcus* suppression of phagocyte functions by protein-mediated engagement of human Siglec-5. *J Exp Med* 206(8):1691–1699
- Carlin AF, Uchiyama S, Chang YC, Lewis AL, Nizet V, Varki A (2009b) Molecular mimicry of host sialylated glycans allows a bacterial pathogen to engage neutrophil Siglec-9 and dampen the innate immune response. *Blood* 113(14):3333–3336
- Chang YC, Olson J, Beasley FC, Tung C, Zhang J, Crocker PR, Varki A, Nizet V (2014a) Group B *Streptococcus* engages an inhibitory Siglec through sialic acid mimicry to blunt innate immune and inflammatory responses in vivo. *PLoS Pathog* 10(1):e1003846
- Chang YC, Olson J, Louie A, Crocker PR, Varki A, Nizet V (2014b) Role of macrophage sialoadhesin in host defense against the sialylated pathogen group B *Streptococcus*. *J Mol Med (Berl)*
- Chatterjee M, Chava AK, Kohla G, Pal S, Merling A, Hinderlich S, Unger U, Strasser P, Gerwig GJ, Kamerling JP, Vlasak R, Crocker PR, Schauer R, Schwartz-Albiez R, Mandal C (2003) Identification and characterization of adsorbed serum sialoglycans on *Leishmania donovani* promastigotes. *Glycobiology* 13(5):351–361
- Chen W, Han C, Xie B, Hu X, Yu Q, Shi L, Wang Q, Li D, Wang J, Zheng P, Liu Y, Cao X (2013) Induction of Siglec-G by RNA viruses inhibits the innate immune response by promoting RIG-I degradation. *Cell* 152(3):467–478
- Chen GY, Brown NK, Wu W, Khedri Z, Yu H, Chen X, van de Vlekkert D, D’Azzo A, Zheng P, Liu Y (2014a) Broad and direct interaction between TLR and Siglec families of pattern recognition receptors and its regulation by Neu1. *Elife* 3:e04066
- Chen Y, Guo R, He S, Zhang X, Xia X, Sun H (2014b) Additive inhibition of porcine reproductive and respiratory syndrome virus infection with the soluble sialoadhesin and CD163 receptors. *Virus Res* 179:85–92
- Cieslewicz MJ, Chaffin D, Glusman G, Kasper D, Madan A, Rodrigues S, Fahey J, Wessels MR, Rubens CE (2005) Structural and genetic diversity of group B streptococcus capsular polysaccharides. *Infect Immun* 73(5):3096–3103
- Crocker PR, Gordon S (1989) Mouse macrophage hemagglutinin (sheep erythrocyte receptor) with specificity for sialylated glycoconjugates characterized by a monoclonal antibody. *J Exp Med* 169(4):1333–1346
- Crocker PR, Mucklow S, Bouckson V, McWilliam A, Willis AC, Gordon S, Milon G, Kelm S, Bradfield P (1994) Sialoadhesin, a macrophage sialic acid binding receptor for haemopoietic cells with 17 immunoglobulin-like domains. *EMBO J* 13(19):4490–4503
- Crocker PR, Paulson JC, Varki A (2007) Siglecs and their roles in the immune system. *Nat Rev Immunol* 7(4):255–266

- Cross AS, Kelly NM (1990) Bacteria-phagocyte interactions: emerging tactics in an ancient rivalry. *FEMS Microbiol Immunol* 2(5–6):245–258
- Dale JB, Washburn RG, Marques MB, Wessels MR (1996) Hyaluronate capsule and surface M protein in resistance to opsonization of group A streptococci. *Infect Immun* 64(5):1495–1501
- De Baere MI, Van Gorp H, Delputte PL, Nauwynck HJ (2012) Interaction of the European genotype porcine reproductive and respiratory syndrome virus (PRRSV) with sialoadhesin (CD169/Siglec-1) inhibits alveolar macrophage phagocytosis. *Vet Res* 43(1):47
- DeAngelis PL (2002) Microbial glycosaminoglycan glycosyltransferases. *Glycobiology* 12(1):9R–16R
- Delputte PL, Nauwynck HJ (2004) Porcine arterivirus infection of alveolar macrophages is mediated by sialic acid on the virus. *J Virol* 78(15):8094–8101
- Delputte PL, Van Breedam W, Delrue I, Oetke C, Crocker PR, Nauwynck HJ (2007) Porcine arterivirus attachment to the macrophage-specific receptor sialoadhesin is dependent on the sialic acid-binding activity of the N-terminal immunoglobulin domain of sialoadhesin. *J Virol* 81(17):9546–9550
- Devi SJ, Robbins JB, Schneerson R (1991) Antibodies to poly[(2-8)-alpha-*N*-acetylneuraminic acid] and poly[(2-9)-alpha-*N*-acetylneuraminic acid] are elicited by immunization of mice with *Escherichia coli* K92 conjugates: potential vaccines for groups B and C meningococci and *E. coli* K1. *Proc Natl Acad Sci U S A* 88(16):7175–7179
- Ding Y, Guo Z, Liu Y, Li X, Zhang Q, Xu X, Gu Y, Zhang Y, Zhao D, Cao X (2016) The lectin Siglec-G inhibits dendritic cell cross-presentation by impairing MHC class I-peptide complex formation. *Nat Immunol* 17(10):1167–1175
- Duan X, Nauwynck HJ, Pensaert MB (1997) Effects of origin and state of differentiation and activation of monocytes/macrophages on their susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV). *Arch Virol* 142(12):2483–2497
- Duan X, Nauwynck HJ, Favoreel HW, Pensaert MB (1998) Identification of a putative receptor for porcine reproductive and respiratory syndrome virus on porcine alveolar macrophages. *J Virol* 72(5):4520–4523
- Erdmann H, Steeg C, Koch-Nolte F, Fleischer B, Jacobs T (2009) Sialylated ligands on pathogenic *Trypanosoma cruzi* interact with Siglec-E (sialic acid-binding Ig-like lectin-E). *Cell Microbiol* 11(11):1600–1611
- Erikson E, Wratil PR, Frank M, Ambiel I, Pahnke K, Pino M, Azadi P, Izquierdo-Useros N, Martinez-Picado J, Meier C, Schnaar RL, Crocker PR, Reutter W, Keppler OT (2015) Mouse Siglec-1 mediates trans-infection of surface-bound murine leukemia virus in a sialic acid N-Acyl side chain-dependent manner. *J Biol Chem* 290(45):27345–27359
- Finne J (1982) Occurrence of unique polysialosyl carbohydrate units in glycoproteins of developing brain. *J Biol Chem* 257(20):11966–11970
- Foley MJ, Wood WB Jr (1959) Studies on the pathogenicity of group A streptococci. II. The antiphagocytic effects of the M protein and the capsular gel. *J Exp Med* 110:617–628. <https://doi.org/10.1084/jem.110.4.617>
- Frydas IS, Nauwynck HJ (2016) Replication characteristics of eight virulent and two attenuated genotype 1 and 2 porcine reproductive and respiratory syndrome virus (PRRSV) strains in nasal mucosa explants. *Vet Microbiol* 182:156–162
- Hayakawa T, Angata T, Lewis AL, Mikkelsen TS, Varki NM, Varki A (2005) A human-specific gene in microglia. *Science* 309(5741):1693. <https://doi.org/10.1126/science.1114321>
- Heath PT, Schuchat A (2007) Perinatal group B streptococcal disease. *Best Pract Res Clin Obstet Gynaecol* 21(3):411–424
- 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(7):3237–3246

- Heikema AP, Jacobs BC, Horst-Kreft D, Huizinga R, Kuijf ML, Endtz HP, Samsom JN, van Wamel WJ (2013) Siglec-7 specifically recognizes *Campylobacter jejuni* strains associated with oculomotor weakness in Guillain-Barre syndrome and Miller Fisher syndrome. *Clin Microbiol Infect* 19(2):E106–E112
- Huizinga R, Easton AS, Donachie AM, Guthrie J, van Rijs W, Heikema A, Boon L, Samsom JN, Jacobs BC, Willison HJ, Goodyear CS (2012) Sialylation of *Campylobacter jejuni* lipo-oligosaccharides: impact on phagocytosis and cytokine production in mice. *PLoS ONE* 7(3):e34416
- Huizinga R, van Rijs W, Bajramovic JJ, Kuijf ML, Laman JD, Samsom JN, Jacobs BC (2013) Sialylation of *Campylobacter jejuni* endotoxin promotes dendritic cell-mediated B cell responses through CD14-dependent production of IFN $\beta$  and TNF $\alpha$ . *J Immunol* 191(11):5636–5645. <https://doi.org/10.4049/jimmunol.1301536>
- Izquierdo-Useros N, Lorizate M, Puertas MC, Rodriguez-Plata MT, Zangger N, Erikson E, Pino M, Erkizia I, Glass B, Clotet B, Keppler OT, Telenti A, Krausslich HG, Martinez-Picado J (2012) Siglec-1 is a novel dendritic cell receptor that mediates HIV-1 trans-infection through recognition of viral membrane gangliosides. *PLoS Biol* 10(12):e1001448
- Jacobs BC, van Doorn PA, Schmitz PI, Tio-Gillen AP, Herbrink P, Visser LH, Hooijckas H, van der Meche FG (1996) *Campylobacter jejuni* infections and anti-GM1 antibodies in Guillain-Barre syndrome. *Ann Neurol* 40(2):181–187
- Jaeger M, Pinelli M, Borghi M, Constantini C, Dindo M, van Emst L, Puccetti M, Pariano M, Ricano-Ponce I, Bull C, Gresnigt MS, Wang X, Gutierrez Achury J, Jacobs CWM, Xu N, Oosting M, Arts P, Joosten LAB, van de Veerdonk FL, Veltman JA, Ten Oever J, Kullberg BJ, Feng M, Adema GJ, Wijmenga C, Kumar V, Sobel J, Gilissen C, Romani L, Netea MG (2019) A systems genomics approach identifies SIGLEC15 as a susceptibility factor in recurrent vulvovaginal candidiasis. *Sci Transl Med* 11:496
- Jann K, Jann B (1992) Capsules of *Escherichia coli*, expression and biological significance. *Can J Microbiol* 38(7):705–710
- Jans J, Unger WWJ, Vissers M, Ahout IML, Schreurs I, Wickenhagen A, de Groot R, de Jonge MI, Ferwerda G (2018) Siglec-1 inhibits RSV-induced interferon gamma production by adult T cells in contrast to newborn T cells. *Eur J Immunol* 48(4):621–631
- Jones C, Virji M, Crocker PR (2003) Recognition of sialylated meningococcal lipopolysaccharide by siglecs expressed on myeloid cells leads to enhanced bacterial uptake. *Mol Microbiol* 49(5):1213–1225
- Kalograiki I, Euba B, Proverbio D, Campanero-Rhodes MA, Aastrup T, Garmendia J, Solis D (2016) Combined bacteria microarray and quartz crystal microbalance approach for exploring glycosignatures of nontypeable *Haemophilus influenzae* and recognition by host lectins. *Anal Chem* 88(11):5950–5957
- Kaper JB, Nataro JP, Mobley HL (2004) Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2(2):123–140
- Khatua B, Ghoshal A, Bhattacharya K, Mandal C, Saha B, Crocker PR, Mandal C (2010) Sialic acids acquired by *Pseudomonas aeruginosa* are involved in reduced complement deposition and siglec mediated host-cell recognition. *FEBS Lett* 584(3):555–561
- Khatua B, Bhattacharya K, Mandal C (2012) Sialoglycoproteins adsorbed by *Pseudomonas aeruginosa* facilitate their survival by impeding neutrophil extracellular trap through siglec-9. *J Leukoc Biol* 91(4):641–655
- Klaas M, Crocker PR (2012) Sialoadhesin in recognition of self and non-self. *Semin Immunopathol* 34(3):353–364
- Kuijf ML, Samsom JN, van Rijs W, Bax M, Huizinga R, Heikema AP, van Doorn PA, van Belkum A, van Kooyk Y, Burgers PC, Luidert TM, Endtz HP, Nieuwenhuis EE, Jacobs BC (2010) TLR4-mediated sensing of *Campylobacter jejuni* by dendritic cells is determined by sialylation. *J Immunol* 185(1):748–755

- Lee CH, Chang CC, Liu JW, Chen RF, Yang KD (2014) Sialic acid involved in hypermucoviscosity phenotype of *Klebsiella pneumoniae* and associated with resistance to neutrophil phagocytosis. *Virulence* 5(6):673–679
- Linhardt RJ, Toida T (2004) Role of glycosaminoglycans in cellular communication. *Acc Chem Res* 37(7):431–438
- Martinez-Picado J, McLaren PJ, Erkizia I, Martin MP, Benet S, Rotger M, Dalmau J, Ouchi D, Wolinsky SM, Penugonda S, Gunthard HF, Fellay J, Carrington M, Izquierdo-Useros N, Telenti A (2016) Identification of Siglec-1 null individuals infected with HIV-1. *Nat Commun* 7:12412
- 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 macrophages. *Parasitol Res* 97(5):380–385
- Nagala M, McKenzie E, Richards H, Sharma R, Thomson S, Mastroeni P, Crocker PR (2017) Expression of Siglec-E alters the proteome of lipopolysaccharide (LPS)-activated macrophages but does not affect LPS-driven cytokine production or toll-like receptor 4 endocytosis. *Front Immunol* 8:1926
- Pereira ME (1983) A developmentally regulated neuraminidase activity in *Trypanosoma cruzi*. *Science* 219(4591):1444–1446
- Pereira ME, Loures MA, Villalta F, Andrade AF (1980) Lectin receptors as markers for *Trypanosoma cruzi*. Developmental stages and a study of the interaction of wheat germ agglutinin with sialic acid residues on epimastigote cells. *J Exp Med* 152(5):1375–1392
- Pereira-Chioccola VL, Acosta-Serrano A, Correia de Almeida I, Ferguson MA, Souto-Padron T, Rodrigues MM, Travassos LR, Schenkman S (2000) Mucin-like molecules form a negatively charged coat that protects *Trypanosoma cruzi* trypomastigotes from killing by human anti-alpha-galactosyl antibodies. *J Cell Sci* 113(Pt 7):1299–1307
- Perez-Zsolt D, Cantero-Perez J, Erkizia I, Benet S, Pino M, Serra-Peinado C, Hernandez-Gallego A, Castellvi J, Tapia G, Arnau-Saz V, Garrido J, Tarrats A, Buzon MJ, Martinez-Picado J, Izquierdo-Useros N, Genesa M (2019a) Dendritic cells from the cervical mucosa capture and transfer HIV-1 via Siglec-1. *Front Immunol* 10:825
- Perez-Zsolt D, Erkizia I, Pino M, Garcia-Gallo M, Martin MT, Benet S, Chojnacki J, Fernandez-Figueras MT, Guerrero D, Urrea V, Muniz-Trabudua X, Kremer L, Martinez-Picado J, Izquierdo-Useros N (2019b) Anti-Siglec-1 antibodies block Ebola viral uptake and decrease cytoplasmic viral entry. *Nat Microbiol*. <https://doi.org/10.1038/s41564-019-0453-2> (Epub ahead of print)
- Prather RS, Rowland RR, Ewen C, Triple B, Kerrigan M, Bawa B, Teson JM, Mao J, Lee K, Samuel MS, Whitworth KM, Murphy CN, Egen T, Green JA (2013) An intact sialoadhesin (Sn/SIGLEC1/CD169) is not required for attachment/internalization of the porcine reproductive and respiratory syndrome virus. *J Virol* 87(17):9538–9546
- Puryear WB, Akiyama H, Geer SD, Ramirez NP, Yu X, Reinhard BM, Gummuluru S (2013) Interferon-inducible mechanism of dendritic cell-mediated HIV-1 dissemination is dependent on Siglec-1/CD169. *PLoS Pathog* 9(4):e1003291
- Rempel H, Calosing C, Sun B, Pulliam L (2008) Sialoadhesin expressed on IFN-induced monocytes binds HIV-1 and enhances infectivity. *PLoS ONE* 3(4):e1967
- Robbins JB, McCracken GH Jr, Gotschlich EC, Orskov F, Orskov I, Hanson LA (1974) *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. *N Engl J Med* 290(22):1216–1220
- Roy S, Mandal C (2016) *Leishmania donovani* utilize sialic acids for binding and phagocytosis in the macrophages through selective utilization of siglecs and impair the innate immune arm. *PLoS Negl Trop Dis* 10(8):e0004904. <https://doi.org/10.1371/journal.pntd.0004904>
- Rutishauser U (2008) Polysialic acid in the plasticity of the developing and adult vertebrate nervous system. *Nat Rev Neurosci* 9(1):26–35
- Saito M, Yamamoto S, Ozaki K, Tomioka Y, Suyama H, Morimatsu M, Nishijima KI, Yoshida SI, Ono E (2016) A soluble form of Siglec-9 provides a resistance against group B *Streptococcus* (GBS) infection in transgenic mice. *Microb Pathog* 99:106–110

- Schwarz F, Landig CS, Siddiqui S, Secundino I, Olson J, Varki N, Nizet V, Varki A (2017) Paired Siglec receptors generate opposite inflammatory responses to a human-specific pathogen. *EMBO J* 36(6):751–760
- Secundino I, Lizcano A, Roupe KM, Wang X, Cole JN, Olson J, Ali SR, Daheh S, Amayreh LK, Henningham A, Varki A, Nizet V (2016) Host and pathogen hyaluronan signal through human siglec-9 to suppress neutrophil activation. *J Mol Med (Berl)* 94(2):219–233
- Sewald X, Ladinsky MS, Uchil PD, Beloor J, Pi R, Herrmann C, Motamedi N, Murooka TT, Brehm MA, Greiner DL, Shultz LD, Mempel TR, Bjorkman PJ, Kumar P, Mothes W (2015) Retroviruses use CD169-mediated trans-infection of permissive lymphocytes to establish infection. *Science* 350(6260):563–567
- Skoff TH, Farley MM, Petit S, Craig AS, Schaffner W, Gershman K, Harrison LH, Lynfield R, Mohle-Boetani J, Zansky S, Albanese BA, Stefonek K, Zell ER, Jackson D, Thompson T, Schrag SJ (2009) Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990–2007. *Clin Infect Dis* 49(1):85–92
- Stephenson HN, Mills DC, Jones H, Miliaris E, Copland A, Dorrell N, Wren BW, Crocker PR, Escors D, Bajaj-Elliott M (2014) Pseudaminic acid on *Campylobacter jejuni* flagella modulates dendritic cell IL-10 expression via Siglec-10 receptor: a novel flagellin-host interaction. *J Infect Dis* 210(9):1487–1498
- Stevens P, Huang SN, Welch WD, Young LS (1978) Restricted complement activation by *Escherichia coli* with the K-1 capsular serotype: a possible role in pathogenicity. *J Immunol* 121(6):2174–2180
- Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL, Harrison LH, Farley MM, Reingold A, Bennett NM, Craig AS, Schaffner W, Thomas A, Lewis MM, Scallan E, Schuchat A (2011) Bacterial meningitis in the United States, 1998–2007. *N Engl J Med* 364(21):2016–2025
- Tomlinson S, Pontes de Carvalho LC, Vandekerckhove F, Nussenzweig V (1994) Role of sialic acid in the resistance of *Trypanosoma cruzi* trypomastigotes to complement. *J Immunol* 153(7):3141–3147
- Uchil PD, Pi R, Haugh KA, Ladinsky MS, Ventura JD, Barrett BS, Santiago ML, Bjorkman PJ, Kassiotis G, Sewald X, Mothes W (2019) A protective role for the lectin CD169/Siglec-1 against a pathogenic murine retrovirus. *Cell Host Microbe* 25(1):87–100 (e110)
- Uchiyama S, Sun J, Fukahori K, Ando N, Wu M, Schwarz F, Siddiqui SS, Varki A, Marth JD, Nizet V (2019) Dual actions of group B *Streptococcus* capsular sialic acid provide resistance to platelet-mediated antimicrobial killing. *Proc Natl Acad Sci U S A* 116(15):7465–7470
- 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(1):e1000730
- van den Berg TK, Nath D, Ziltener HJ, Vestweber D, Fukuda M, van Die I, Crocker PR (2001) Cutting edge: CD43 functions as a T cell counterreceptor for the macrophage adhesion receptor sialoadhesin (Siglec-1). *J Immunol* 166(6):3637–3640
- Van Reeth K, Labarque G, Nauwynck H, Pensaert M (1999) Differential production of proinflammatory cytokines in the pig lung during different respiratory virus infections: correlations with pathogenicity. *Res Vet Sci* 67(1):47–52
- 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 macrophages. *J Virol* 77(15):8207–8215
- Varchetta S, Lusso P, Hudspeth K, Mikulak J, Mele D, Paolucci S, Cimbro R, Malnati M, Riva A, Maserati R, Mondelli MU, Mavilio D (2013) Sialic acid-binding Ig-like lectin-7 interacts with HIV-1 gp120 and facilitates infection of CD4<sup>pos</sup> T cells and macrophages. *Retrovirology* 10:154
- Varki A (1993) Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* 3(2):97–130
- Wang Y, Neumann H (2010) Alleviation of neurotoxicity by microglial human Siglec-11. *J Neurosci* 30(9):3482–3488

- Weiman S, Dahesh S, Carlin AF, Varki A, Nizet V, Lewis AL (2009) Genetic and biochemical modulation of sialic acid O-acetylation on group B *Streptococcus*: phenotypic and functional impact. *Glycobiology* 19(11):1204–1213
- Weiman S, Uchiyama S, Lin FY, Chaffin D, Varki A, Nizet V, Lewis AL (2010) O-Acetylation of sialic acid on Group B *Streptococcus* inhibits neutrophil suppression and virulence. *Biochem J* 428(2):163–168
- Wessels MR, Rubens CE, Benedi VJ, Kasper DL (1989) Definition of a bacterial virulence factor: sialylation of the group B streptococcal capsule. *Proc Natl Acad Sci U S A* 86(22):8983–8987
- Wessels MR, Goldberg JB, Moses AE, DiCesare TJ (1994) Effects on virulence of mutations in a locus essential for hyaluronic acid capsule expression in group A streptococci. *Infect Immun* 62(2):433–441
- Willison HJ, Yuki N (2002) Peripheral neuropathies and anti-glycolipid antibodies. *Brain* 125(Pt 12):2591–2625
- Willison HJ, Jacobs BC, van Doorn PA (2016) Guillain-Barre syndrome. *Lancet* 388(10045):717–727
- Xia W, Wu Z, Guo C, Zhu S, Zhang X, Xia X, Sun H (2018) Recombinant adenovirus-delivered soluble CD163 and sialoadhesin receptors protected pigs from porcine reproductive and respiratory syndrome virus infection. *Vet Microbiol* 219:1–7
- Xie J, Christiaens I, Yang B, Breedam WV, Cui T, Nauwynck HJ (2017) Molecular cloning of porcine Siglec-3, Siglec-5 and Siglec-10, and identification of Siglec-10 as an alternative receptor for porcine reproductive and respiratory syndrome virus (PRRSV). *J Gen Virol* 98(8):2030–2042
- Yamanaka M, Kato Y, Angata T, Narimatsu H (2009) Deletion polymorphism of SIGLEC14 and its functional implications. *Glycobiology* 19(8):841–846
- Yu X, Feizpour A, Ramirez NG, Wu L, Akiyama H, Xu F, Gummuluru S, Reinhard BM (2014) Glycosphingolipid-functionalized nanoparticles recapitulate CD169-dependent HIV-1 uptake and trafficking in dendritic cells. *Nat Commun* 5:4136
- Zheng Q, Hou J, Zhou Y, Yang Y, Xie B, Cao X (2015) Siglec1 suppresses antiviral innate immune response by inducing TBK1 degradation via the ubiquitin ligase TRIM27. *Cell Res* 25(10):1121–1136
- Zhu L, Song H, Zhang X, Xia X, Sun H (2014) Inhibition of porcine reproductive and respiratory syndrome virus infection by recombinant adenovirus- and/or exosome-delivered the artificial microRNAs targeting sialoadhesin and CD163 receptors. *Virology* 461:219–225
- 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 macrophages through adhesion with viral sialic acids. *PLoS ONE* 6(9):e24559