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REVIEW

The interplay between Siglecs and sialylated pathogens

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Siglecs are mammalian sialic acid (Sia) recognizing immunoglobulin-like receptors expressed across the major leukocyte lineages, and function to recognize ubiquitous Sia epitopes on cell surface glycoconjugates and regulate immunological and inflammatory activities of these cells. A large subset referred to as CD33-related Siglecs are inhibitory receptors that limit leukocyte activation, and recent research has shown that the pathogen group B *Streptococcus* (GBS) binds to these Siglecs in Sia- and protein-dependent fashion to downregulate leukocyte bactericidal capacity. Conversely, sialoadhesin is a macrophage phagocytic receptor that engages GBS and other sialylated pathogens to promote effective phagocytosis and antigen presentation for the adaptive immune response. A variety of other important Siglec interactions with bacterial, viral and protozoan pathogens are beginning to be recognized. Siglec genes and binding specificities are rapidly evolving among primates, with key extant polymorphisms in human populations that may influence susceptibility to infection-associated disorders including chronic obstructive pulmonary disease and premature birth. This review summarizes current understanding of interactions between pathogens and Siglecs, a field of investigation that is likely to continue expanding in scope and medical importance.

Keywords: bacterial pathogenesis / innate immunity / molecular mimicry, sialic acid / Siglec

Introduction

Just as glycans are major components of the outermost surface of all animal cells, so too are polysaccharides found on the surface of all bacterial species. Thus, most (if not all) interactions of bacterial pathogens with their hosts are influenced by the pattern of glycans and glycan-binding receptors (lectins/adhesins/agglutinins) that each expresses. In a complex environment with many microbial threats, higher organisms have evolved systems of immunity that can discriminate between potential pathogens and mount appropriate antimicrobial responses to block systemic spread and limit damage to their cells and tissues.

Although our understanding of host–pathogen interactions is ever expanding with new discoveries and insights, knowledge regarding glycan–receptor interactions in bacterial pathogenesis is still in its early stages. Sialic acids (Sias) are nine-carbon backbone monosaccharides primarily expressed by vertebrates as well as by some microbial pathogens. Sias and its related nonulosonates are unique in nature, representing the only nine-carbon sugars found in prokaryotes. In addition, Sias are among the most prevalent monosaccharides at the host–pathogen interface by virtue of their usual terminal positioning in glycan structures. This review will focus on how Sia-decorated pathogens complicate microbial pattern recognition and modulate immune reactions of hosts by interacting with a Sia-recognizing receptor family, the Siglecs (Sia-binding immunoglobulin (Ig)-like lectins).

In mammalian cells, Sia is usually the terminal 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). Bacterial Sia was first discovered in 1950s in the culture supernatant of *Escherichia coli* as the repeating subunit of a capsular polysaccharide (CPS) (Barry and Goebel 1957; Barry 1958). Since its discovery, Sia produced by de novo biosynthesis or via metabolic scavenging pathways has been detected in a growing list of other bacterial, fungal and protozoan species (Vimr and Lichtensteiger 2002). Several medically important pathogens displaying Sias on their surface are thought to use Sia as an anti-recognition molecule, allowing the microbe to masquerade as "self" and thereby elude or subvert host immune responses. This understanding has spurred interest in exploring the mechanisms by which sialylated pathogens can exploit host receptor systems to modulate immune responsiveness.

An important facet of Sia biology is the function of Siglecs, Sia-recognizing receptors largely expressed across the major leukocyte lineages, which have been shown to carry out important innate and adaptive immune functions (Crocker et al. 2007; Varki 2007; Cao and Crocker 2011; Pillai et al. 2012). Siglecs can be grouped into two subsets on the basis of their sequence similarity and evolutionary conservation: (i) Siglecs common to mammals, including sialoadhesin, Siglec-2, -4 and -15 and (ii) the CD33-related Siglecs (CD33rSiglecs), most of which possess a cytoplasmic domain containing both a membrane-proximal immunoreceptor tyrosine-based inhibitory motif (ITIM) and a membrane-distal ITIM-like motif (Varki and Angata 2006;

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Crocker et al. 2007). Negative regulation of immune functions by Siglecs with ITIMs has been reported in the realms of cell expansion, cytokine production, cellular activation and induction of apoptosis (Crocker et al. 2007). Sia can act as self-associated molecular patterns (Varki 2011), which are recognized by the inhibitory CD33rSiglecs and serve to maintain a baseline non-activated state of innate immune cells, and help to counter-regulate inflammatory responses activated upon sensing of danger-associated molecular patterns or pathogen-associated molecular patterns (Cao and Crocker 2011).

Pretreatment of Siglec transfectants with sialidase has been reported to potentiate their *trans* ligand-binding ability (Razi and Varki 1998, 1999; Jones et al. 2003). Unlike most of Siglecs which have one or more ITIMs in their cytosolic tails, sialoadhesin lacks known signaling domains and possesses a unique extended 17 Ig-like extracellular domain structure. The extended extracellular length of sialoadhesin makes it standing out of the surface glycocalyx to prevent potential *cis*-ligand masking of its Sia-binding pocket. Therefore, sialoadhesin is believed to mediate critical initial contacts with sialylated pathogens through direct phagocytosis/endocytosis, or in coordination with other patter recognition receptors to promote efficient uptake or activate responses to counteract sialylated pathogen infection.

Increasing evidence suggests that several pathogenic microbes have evolved mechanisms to interact with numerous Siglecs. Our work on group B *Streptococcus* (GBS)–Siglec interaction and its impact on the modulation of leukocyte activation will be described first, followed by the concise summary for the published interaction between Siglec and other sialylated pathogens.

Interaction between Siglecs and group B *Streptococcus*

GBS is a leading cause of neonatal pneumonia, septicemia and meningitis (Edwards 2006; Heath and Schuchat 2007; Thigpen et al. 2011), and GBS colonization during pregnancy increases the incidence of preterm rupture of membranes and premature delivery (Ferrieri et al. 1977; Galask et al. 1984). GBS expresses a α2-3-linked sialylated CPS that is a major virulence factor contributing to evasion of host immune defense mechanisms (Rubens et al. 1987) and promoting GBS survival in vivo (Shigeoka et al. 1983; Wessels et al. 1989). In addition to interfering with host complement functions by blocking C3b deposition and limiting C5a production (Marques et al. 1992; Takahashi et al. 1999), we found that GBS can engage multiple inhibitory CD33rSiglecs via its sialylated CPS, with the potential to dampen host immune response and provide a survival advantage to the pathogen (Carlin et al. 2007). Carlin, Uchiyama, et al. (2009) provided the first in vitro evidence that GBS blunts neutrophil activation by engaging the inhibitory human Siglec-9, resulting in impaired oxidative burst and neutrophil extracellular trap (NET) formation, thereby reducing neutrophil bactericidal activity. Adding complexity to this dynamic, GBS of all serotypes tested so far display partial O-acetylation of Sias on their native CPS (Lewis et al. 2004). While GBS Sia O-acetylation level does not significantly affect C3b complement deposition, studies with isogenic mutants differing in the O-acetylation phenotype showed that the modification markedly reduced binding of the pathogen to human Siglec-9 (Weiman et al. 2009). CPS Sia O-acetylation was later found to attenuate GBS Sia-mediated neutrophil suppression and animal virulence which may be partially if not all attributable to the impaired GBS Sia-mediated Siglec-9 engagement (Weiman et al. 2010). Parenthetically, O-acetylation on CPS Sia blocked the removal of GBS CPS Sia by bacterial sialidases, which may help GBS to gain an advantage in niche competition when co-inhabiting with sialidase-expressing microbes on the gastrointestinal or vaginal mucosa (Weiman et al. 2009).

The direct impact of Sia-Siglec engagement in the context of in vivo infection was addressed in our recent publication in the murine model. Because of the rapid evolution of CD33rSiglecs in primates, it is understood that the composition of the CD33rSiglec family varies significantly between primates and rodents. However, we examined mice deficient in Siglec-E, a functional paralog of human Siglec-9, with similar cellular expression pattern on innate immune cells of the myelomonocytic lineage. Like Sia-mediated GBS-Siglec-9 interaction, GBS also bound to Siglec-E in a Sia-dependent manner. In addition, Sia-expressing GBS triggered greater proinflammatory and reduced anti-inflammatory cytokine responses in Siglec-E deficient mice, whereas GBS Sia-deficient mutants induced similar cytokine responses (Chang, Olson, Beasley, et al. 2014). The exaggerated proinflammatory cytokine release in Siglec-E deficient mice was associated with exacerbated mortality upon lethal challenge. Intriguingly, mice showed reduced GBS brain dissemination in a sublethal intravenous challenge model, possibly benefiting from increased proinflammatory and decreased anti-inflammatory cytokine IL-10 production (Chang, Olson, Beasley, et al. 2014).

Sialoadhesin was first identified as a receptor interacting with red blood cell receptors (Crocker and Gordon 1986) to modulate host immune responses through its regulation of cell–cell interactions (Wu et al. 2009). However, sialoadhesin also functions as a critical host defense receptor to restrict invasive bacterial infection since it recognizes the very same Sia epitope utilized by the sialylated pathogens to dampen host innate immune responses. In support of this hypothesis, we found that GBS bound sialoadhesin even stronger than it did to human Siglec-9 or the murine CD33-related inhibitory Siglec-E (Chang, Olson, Louie, et al. 2014), and that the GBS–sialoadhesin interaction facilitated the phagocytic and bactericidal activity of macrophages in vitro and the efficient capture and control GBS dissemination in vivo upon systemic intravenous challenge. In contrast, sialoadhesin does not mediate the phagocytic uptake of host cells including red blood cells. Sialoadhesin was found to be key in control of invasive GBS bacterial infection even within the in vivo milieu in which the host expresses an array of pattern recognition and scavenger receptors that can also sample an invading pathogen. In addition, we found that production of specific anti-GBS IgM antibodies responses were impaired in sialoadhesin-deficient mice after GBS challenge (Chang, Olson, Louie, et al. 2014). These findings indicate that sialoadhesin is critical for innate recognition and serves as a bridge to subsequent adaptive immune defenses against the invasive sialylated pathogen.

In addition to the Sia-dependent Siglec engagement, we unexpectedly discovered that certain GBS strains can use a protein, the surface-anchored β protein, to bind human Siglec-5, an inhibitory receptor expressed on macrophages and neutrophils (Carlin, Chang, et al. 2009). The site of Siglec-5 binding mapped

to the N-terminal domain of GBS β protein, a site distinct from a previously characterized IgA-binding property (Nordstrom et al. 2011). This protein-dependent interaction with Siglec-5 promoted bacterial attachment to the macrophage surface but impaired the cell's phagocytic activity. In addition, engagement of Siglec-5 by β protein increased SHP phosphatase recruitment to Siglec-5 and blunted neutrophil oxidative burst, NET formation and bactericidal activity (Carlin, Chang, et al. 2009). These observations provided the first example of a protein-mediated Siglec interaction, and suggested an evolutionary selective advantage for GBS to express a protein ligand capable of engaging an inhibitory Siglec to gain the best fitness advantage for survival within the host.

An activating Siglec, Siglec-14, has been identified that is nearly identical to Siglec-5 in its ligand-binding domain, but rather associates with activating DNAX-activating protein of 12 kDa (DAP12) bearing ITAM-motif instead of the inhibitory ITIM motif on the cytosolic side (Angata et al. 2006). Since neutrophils and monocytes express Siglec-14 and Siglec-5, we recently explored the possibility that these function as paired Siglec receptors to balance immune responses to GBS. β-Protein-expressing GBS bound both Siglec-5 and -14 on neutrophils, and the latter engagement countered pathogen-induced host immune suppression by activating p38 mitogen-activated protein kinase and AKT signaling pathways (Ali et al. 2014). Interestingly, Siglec-14 is absent in some humans because of a *SIGLEC14*-null polymorphism, and we showed that homozygous *SIGLEC14*-null neutrophils were the most prone to GBS immune subversion. An unexpected human-specific expression of Siglec-5 and -14 was also discovered on amniotic epithelium, the site of initial contact of invading GBS with the fetus, and GBS amnion immune activation was similarly influenced by the *SIGLEC14*-null polymorphism. A limited genetic survey suggested that this polymorphism could influence the risk of prematurity among human fetuses of mothers colonized with GBS (Ali et al. 2014). The demonstration of a paired receptor system in the Siglec family has implications for regulation of host immunity, and future research might explore whether other Siglecs expressing similar extracellular-binding motifs but differential ITIM- vs. ITAM-containing intracellular signaling domains (e.g. human Siglec-11 and 1-6) could also function as paired receptors in mediating host–pathogen interactions (Figure 1).

Interactions between Siglecs and other sialylated pathogens

Campylobacter jejuni

Human infections caused by *C. jejuni* are a leading cause of foodborne enteritis, usually transmitted by the ingestion of undercooked poultry or contact with farm animals. Moreover, *C. jejuni* strains with sialylated lipooligosaccharides (LOS) are a likely immunological trigger for some cases of the neurological disease Guillain–Barre' syndrome, occurring subsequent to *C. jejuni* enteritis. This disease is an acute peripheral neuropathy caused by autoantibodies elicited to recognize *C. jejuni* LOS but that aberrantly target peripheral nerve gangliosides that share identical oligosaccharide structures (Hughes and Cornblath 2005; Yuki 2005). Both Siglec-7 and sialoadhesin can recognize *C. jejuni* LOS expressing a terminal α2,8-linked Sia or an α2,3-linked Sia, respectively (Avril et al. 2006; Heikema et al. 2010). Importantly, sialoadhesin preferentially binds to Guillain–Barré syndrome-associated *C. jejuni* strains over simple enteritis-associated *C. jejuni* strains, and those α2,3-linked Sias on the *C. jejuni* LOS recognized by sialoadhesin indeed share similar structure with neuronal gangliosides GD1a, GM1b and GM3 (Heikema et al. 2010). These observations point out a potential functional consequence of linkage-dependent Siglec engagement and its relevancy to development of postinfectious autoimmune neuropathy. GD1a/GM1a mimics of *C. jejuni* LOS expressing terminal α2,3-linked Sias are reported to be associated with pure motor forms of Guillain–Barré syndrome (Jacobs et al. 1996), whereas GD1c mimics of LOS exposing terminal α2,8-linked Sias are associated with Guillain–Barré syndrome with ophthalmoplegia (Godschalk et al. 2007).

Loss of Sia expression on *C. jejuni* LOS in the sialyltransferase *cst-II* mutant strains has been reported to cause reduced dendritic cell activation, an important step to initiate and differentiate adaptive immune responses and subsequent B-cell proliferation (Kuijf et al. 2010). In addition, GD1a/GM1a LOS mimics and GD1c LOS mimics preferentially induced Th2 and Th1 responses, respectively, potentially through induction of different cytokine profile of dendritic cells (Bax et al. 2011). Sialoadhesin on the macrophages was shown to play a key role in capturing sialylated *C. jejuni* and promoting rapid proinflammatory cytokines and type I interferon responses in a Sia- and sialoadhesin-dependent in vitro and in vivo (Klaas et al. 2012). These observations suggest that the linkage of the terminal of Sia on the *C. jejuni* LOS and Siglec engagement may affect the functional properties of dendritic cells and macrophages and subsequent antigen presentation and cell-mediated T helper cell polarization.

Neisseria meningiditis

Neisseria meningitidis is an exclusively human pathogen that causes significant morbidity and mortality in children and young adults worldwide, infecting up to 1.2 million people with a death toll of ~135,000 (Rouphael and Stephens 2012). The Sias expressed by *N. meningitidis* on its CPS and outer membrane LOS contribute to resistance to the bactericidal activity of normal human serum (Vogel et al. 1996, 1997; Estabrook et al. 1997; Kahler et al. 1998). Sialoadhesin was shown to function directly or in synergy with other phagocytic receptors to enhance macrophage phagocytosis by binding to sialylated LOS on the surface of *N. meningitidis* (Jones et al. 2003). A moderate interaction between *N. meningitidis* and Siglec-5 was also reported by the same group (Jones et al. 2003), and interaction with multiple Siglecs raises the possibility that sialylated LOS on *N. meningitidis* may influence macrophage, neutrophil and monocyte functions through engaging sialoadhesin and/or Siglec-5 naturally expressed on those cells.

Pseudomonas aeruginosa

Pseudomonas aeruginosa rarely infects healthy people without an underlying defect in immunity or disruption of the mechanical epithelial barrier; instead, the opportunistic pathogen preferentially colonizes immunocompromised patients and is classically associated with disease in cystic fibrosis, neutropenic cancer chemotherapy patients or victims of third-degree burns (Williams et al. 2010). *P. aeruginosa*, lacking a defined sialylation machinery, was found to acquire Sias when cultured

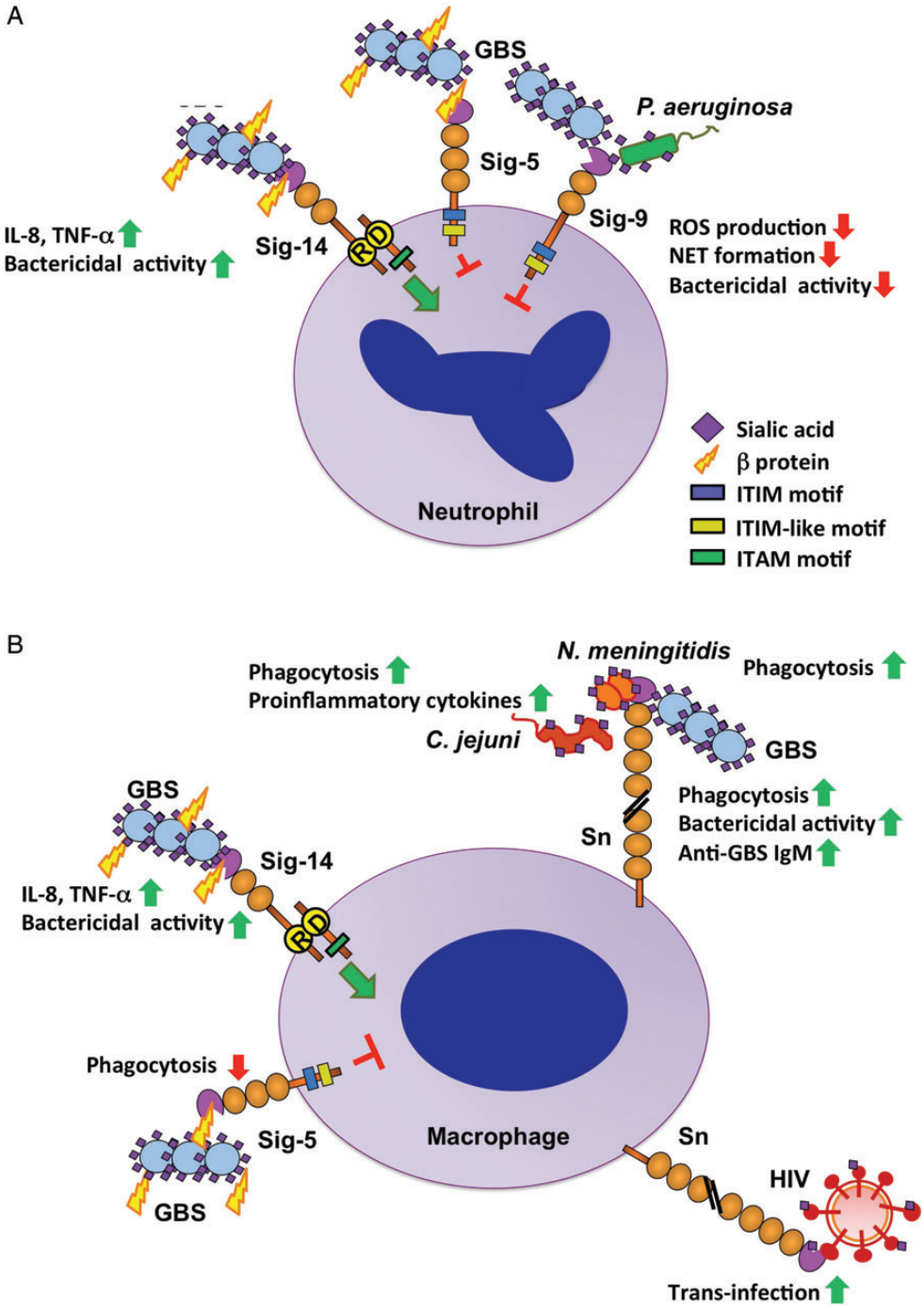

Fig. 1. The interplay of sialylated bacterial pathogens with Siglecs expressed on neutrophils and macrophages. (**A**) Group B *Streptococcus* (GBS) and *P. aeruginosa* utilize surface sialic acids to engage the inhibitory Siglec-9, blunting neutrophil activation and bactericidal activity. GBS β-protein triggers inhibitory and activating signals on individuals expressing Siglec-5 and Siglec-14, respectively. (**B**) Sialoadhesin on macrophages recognizes multiple sialylated pathogens to promote phagocytosis and killing, inflammatory cytokine secretion and enhanced antibody responses. However, HIV uses sialoadhesin-mediated sialic acid recognition to facilitate its *trans*-infection. Like neutrophils, GBS β-protein triggers inhibitory and activating signals on individuals expressing Siglec-5 and -14, respectively.

in exogenous Sia-supplemented medium. Although the mechanism for how *P. aeruginosa* acquires those Sias remains unclear, the adsorbed Sias showed a clear ability to reduce C3b complement deposition on the *P. aeruginosa* surface. In addition, Sia-acquired by *P. aeruginosa* bound various human CD33rSiglecs, including Siglec-3, -5, -7, -9 and -10 (Khatua et al. 2010). This same group of investigators further demonstrated that Sia-acquired by *P. aeruginosa* could downregulate neutrophil activation though Siglec-9 engagement to blunt neutrophil oxidative burst, elastase secretion and NET formation, thereby impairing innate bactericidal responses (Khatua et al. 2012). It is interesting to contemplate whether thick and sticky

mucins, rich sources of Sias, present in the lungs of cystic fibrosis patients provide *P. aeruginosa* a unique niche for Sia acquisition, allowing *P. aeruginosa* to downregulate host immunity to establish persistent colonization.

Haemophilus influenzae

Chronic obstructive pulmonary disease (COPD) is a leading cause of mortality in many countries. COPD exacerbation, an episodic worsening of symptoms, is associated with frequent hospitalization and increased mortality rates. Bacterial airway infections, particularly those caused by nontypeable *Haemophilus influenzae* (NTHi), are a major trigger for COPD exacerbation. NTHi expresses LOS containing Sias, and can interact with the paired Siglec-5 and -14 receptors. NTHi interacts with Siglec-14 to enhance macrophage proinflammatory cytokine production in vitro through a Syk tyrosine kinase-dependent pathway (Angata et al. 2013). Loss of Siglec-14, due to SIGLEC14-null allele homozygosity, was associated with reduced risk of COPD exacerbation in a Japanese patient cohort. Thus, the interaction of the sialylated NTHi pathogen with Siglec-14 and its downstream signaling pathway facilitate an "infection–inflammation–exacerbation" axis of COPD disease progression, and may be a target for therapeutic intervention, e.g. by Syk tyrosine kinase inhibition (Angata et al. 2013).

Porcine reproductive and respiratory syndrome virus

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of porcine reproductive and respiratory syndrome, which can produce economic devastation in the pig industry with an estimated annual loss of $560 million for US swine producers (Collins et al. 1992; Neumann et al. 2005). Porcine sialoadhesin (pSn) was identified as the critical entry receptor expressed on alveolar macrophages mediating PRRSV internalization through the Sia-binding N-terminal V-set domain of pSn (Duan et al. 1998; Vanderheijden et al. 2003; Delputte et al. 2007). The interaction of pSn and PRRSV is dependent on Sias expressed on the PRRSV virions; removal of Sias on the virions or addition of sialyllactose acid and Sia-containing glycoconjugates reduced PRRSV infection and attachment to alveolar macrophages (Delputte and Nauwynck 2004). Sias on the viral envelope structural protein M/GP5 heterodimer was shown to be the binding target of pSn (Van Breedam et al. 2010). Interaction of European genotype PRRSV with pSn on alveolar macrophages caused a reduction in macrophage phagocytic capacity (De Baere et al. 2012); however, a more recent study showed that ablation of pSn expression in pigs had no measurable differences on the clinical course and histopathology upon PRRSV (Prather et al. 2013).

Human immunodeficiency virus type I

The human immunodeficiency virus (HIV) is the causative agent of the human acquired immunodeficiency syndrome in which a progressive failure of the immune system can lead to life-threatening opportunistic infections and cancer development. HIV binds to sialoadhesin-transfected THP-1 cells, and this interaction can be reduced by a sialoadhesin-neutralizing monoclonal antibody or by enzymatic removal of Sias on HIV-1 (Rempel et al. 2008). Moreover, expression of sialoadhesin has been demonstrated to effectively facilitate *trans*-infection of permissive cells by HIV-1 (Rempel et al. 2008). Sias on the viral envelope protein gp120 are the sialoadhesin-interacting ligands of HIV-1. Neuraminidase treatment to remove Sias from gp120 or mild sodium periodate oxidation or truncate the side chain of those Sias, significantly reduced HIV-1 binding to sialoadhesin (Zou et al. 2011). HIV-1 was recently reported to incorporate the host cell-derived α2,3 sialylated glycosphingolipids GM1 and GM3 into the viral particles (Izquierdo, Lorizate, Contreras, et al. 2012; Puryear et al. 2012). Recognition of α2,3 sialylated glycosphingolipid on retroviral particles by sialoadhesin on mature dendritic cells (DCs) is essential and sufficient for the DC-mediated retrovirus *trans*-infection pathway. Furthermore, DCs limit antigen presentation by downregulating MHC class II expression and avoid allowing captured HIV-1 to reach the endolysosomal compartment (Izquierdo-Useros, Lorizate, Puertas, et al. 2012; Puryear et al. 2013). Since sialoadhesin expression on the $CD14^+$ monocytes is upregulated after HIV-1 infection and is directly correlated to the viral load detected in patients (van der Kuyl et al. 2007), HIV-1 may utilize this Sn–GM1/3 interaction as a subversion mechanism to facilitate viral spread from DCs to $CD4^+$ T cells through *trans*-infection. In addition, interaction of Siglec-3, -5, -7 and -9 with HIV gp120 was confirmed by surface plasmon resonance analysis; Siglec-9 showed the highest binding to gp120 among the tested CD33rSiglecs. Finally, R5-pseudovirus infection of macrophages was partially inhibited by soluble recombinant proteins for Siglec-7 and -9 and blocking antibody for Siglec-3, respectively, suggesting that CD33rSiglec recognition of viral sialylated glycans may also be involved in HIV-1 attachment and entry into macrophages (Zou et al. 2011).

Trypanosoma cruzi

The protozoan pathogen *T. cruzi* is the causative agent of Chagas disease, which affects ~18 million people in Latin America (Barrett et al. 2003). *Trypanosoma cruzi* can acquire up to 10^7 Sia residues on its surface, mostly incorporating these into mucin-like molecules anchored on the parasite membrane as catalyzed by its *trans*-sialidase (Schenkman et al. 1994). Macrophages have an important early role in reacting to infection and in carrying parasites to other sites within the body, and macrophages express various Siglecs that may potentially contribute to *T. cruzi* pathogenesis through interactions with its surface sialoglycoproteins. Monteiro et al. (2005) provided the first evidence showing that the association of *T. cruzi* with mouse macrophages could be correlated to the expression level of sialoadhesin induced by autologous serum; this interaction could be reduced by addition of a sialoadhesin-neutralizing mAb or by sialidase treatment of the parasites. In addition, murine Siglec-E was shown to bind more avidly to the pathogenic *T. cruzi* Tulahuen strain, which has higher *trans*-sialidase activity and higher sialylation than the non-pathogenic Tehuantepec strain. Interestingly, incubation of DCs with heat-inactivated pathogenic *T. cruzi* Tulahuen reduced LPS-induced secretion of the proinflammatory cytokine IL-12, and instead induced the production of the anti-inflammatory cytokine IL-10; sialidase-treated parasites did not exhibit the same modulatory effects (Erdmann et al. 2009). Although the observed immune modulation may potentially be triggered by sialylated *T. cruzi*-mediated Siglec-E engagement on DCs, direct evidence

proving Siglec-E-dependency of this phenomenon has yet to be addressed in a Siglec-E knockout or blocking experimental paradigm.

Sialidase expression by Streptococcus pneumoniae

The major human bacterial pathogen *S. pneumoniae* causes pneumonia, sepsis and meningitis, often accompanied by strong inflammatory responses. *S. pneumoniae* expresses a surface-anchored sialidase (NanA) that contributes to nasal colonization and blood–brain barrier penetration. Using wild-type and isogenic NanA-deficient mutant strains, we showed that *S. pneumoniae* NanA can desialylate the surface of human monocytes, leading to increased ERK phosphorylation, NF-κB activation and proinflammatory cytokine release, and also desialylate the surface of human neutrophils, stimulating interleukin-8 release and NET formation (Chang et al. 2012). Decreased SHP-2 recruitment to the Siglec-5 intracellular domain upon NanA treatment suggests a mechanistic contribution of "unmasking" of inhibitory Siglec-5 from *cis* Sia interactions to the proinflammatory effect of NanA. Increased production of proinflammatory cytokines by NanA was further corroborate in vivo in a murine intranasal challenge model of pneumococcal pneumonia (Chang et al. 2012). In a murine polybacterial sepsis model, mice treated with sialidase inhibitors showed reduced mortality by maintaining the CD24–Siglec-G interaction to counteract overwhelming inflammation (Chen et al. 2011). In addition, the same group demonstrated that wild-type *S. pneumoniae* induced greater inflammatory cytokine secretion and higher mortality than a NanA-/NanB-double sialidase mutant, even though similar bacterial counts were recovered in the bloodstream after intraperitoneal infection (Chen et al. 2011). Together with our findings, the stronger inflammatory responses triggered by unmasking of Siglec-*cis*-ligand interactions represent a double-edged sword to the host, depending on the site, stage and magnitude of infection.

Some evolutionary considerations

Comparative analysis of major CD33rSiglec (Siglec-3, -5 and -9) orthologs in humans, chimpanzees and baboons has revealed marked quantitative and qualitative interspecies differences in interactions with different GBS strains and with sialoglycans presented as gangliosides or in the form of sialoglycan microarrays, including variations such as *N*-glycolyl and *O*-acetyl groups (Padler-Karavani et al. 2014). Primate Siglecs also show marked quantitative and qualitative intra- and interspecies variations in expression patterns on their leukocytes. It appears the CD33rSiglec-encoding gene cluster is undergoing rapid evolution via multiple mechanisms, driven by the need to maintain self-recognition by innate immune cells while simultaneously escaping mechanisms of pathogen subversion (Padler-Karavani et al. 2014). Indeed, two primate Siglecs that were rendered nonfunctional by single genetic events during hominin evolution after our common ancestor with the chimpanzee: *SIGLEC13* was deleted by an Alu-mediated recombination event, and a single base pair deletion disrupted the ORF of *SIGLEC17*. Siglec-13 is expressed on chimpanzee monocytes and the human *SIGLEC17P* pseudogene mRNA is still expressed at high levels in human natural killer cells. As both resulting pseudogenes are homozygous in all human populations, we recently resurrected the originally encoded proteins to examine their functions (Wang et al. 2012). Both Siglec-13 and -17 possess a single positively charged residue within their membrane-spanning region that is characteristic of immune cell proteins that interact with DAP12. Co-transfection of DAP12 together with Siglec-13 or -17 in cells stabilized their surface expression and altered inflammatory cytokine secretion in response to Toll-like receptor-4 stimulation. In addition, both Siglecs could be engaged by the sialylated pathogens GBS and *E. coli* K1; reduced inflammatory cytokine secretion was observed for *E. coli*-infected cells expressing Siglec-13. These data suggested that genetic elimination of Siglec-13 and/or -17 could represent signatures of infectious selective processes that contributed to population restrictions during hominin origins (Wang et al. 2012).

Conclusion

It is known that many pathogens incorporate Sias into their surface glycoconjugates, including CPS and LOS, through various mechanisms (Vimr and Lichtensteiger 2002). Mounting evidence has emerged to support the notion that Sia molecular mimicry can be exploited as a virulence mechanism to subvert host immune systems or to infect permissive target cells through an interplaying with various Siglecs. Conversely, the host can use the Sia-binding phagocytic receptor sialoadhesin or ITAM-bearing activating Siglecs to better recognize and respond to sialylated pathogens and support immune defense. Continued investigations to understand the biological interactions of microbial sialylation with host Siglecs are likely expand this paradigm to additional pathogens and infectious disease conditions.

Abbreviations

CD33rSiglecs, CD33-related Siglecs COPD, chronic obstructive pulmonary disease; CPS, capsular polysaccharide; DAP12, DNAX-activating protein of 12 kDa; DCs, dendritic cells; GBS, group B *Streptococcus*; HIV, human immunodeficiency virus; Ig, immunoglobulin; ITIM, immunoreceptor tyrosine-based inhibitory motif; LOS, lipooligosaccharides; NET, neutrophil extracellular trap; NTHi, nontypeable *Haemophilus influenzae*; PRRSV, porcine reproductive and respiratory syndrome virus; pSn, porcine sialoadhesin; Sia, sialic acid; Sn, sialoadhesin

Conflict of interest statement

None declared.

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