Review Article

Sialic acids siglec interaction: A unique strategy to circumvent innate immune response by pathogens

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Sialic acids (Sias) are nine-carbon keto sugars primarily present on the terminal residue of cell surface glycans. Sialic acid binding immunoglobulins (Ig)-like lectins (siglecs) are generally expressed on various immune cells. They selectively recognize different linkage-specific sialic acids and undertake a variety of cellular functions. Many pathogens either synthesize or acquire sialic acids from the host. Sialylated pathogens generally use siglecs to manipulate the host immune response. The present review mainly deals with the newly developed information regarding mechanism of acquisition of sialic acids by pathogens and their biological relevance especially in the establishment of successful infection by impairing host innate immunity. The pathogens which are unable to synthesize sialic acids might adsorb these from the host as a way to engage the inhibitory siglecs. They promote association with the immune cells through sialic acids-siglec dependent manner. Such an association plays an important role to subvert host's immunity. Detailed investigation of these pathways has been discussed in this review. Particular attention has been focused on *Pseudomonas aeruginosa* (PA) and *Leishmania donovani*.

Key words Elastase - innate immunity - *Leishmaniasis* - neutrophil extracellular traps (NETs) - *Pseudomonas aeruginosa* - reactive oxygen species - Sialic acids - Siglecs

Introduction

Structural diversity and unique strategic location of sialic acids (Sias) on the cells make them one of the most important molecules in life and set the challenges for sialoglycobiologist[1]. Sialic acids are nine carbon acidic sugars typically found as the terminal residue of cell surface sugar chains as well as on secreted glycoproteins and in the extracellular matrix. About 50 different modifications exist for sialic acids in nature[2].

Sialic acids possess contrasting character of working as masking element of an array of cell surface receptors whereas on the other side these function as a recognition site for various lectins and antibodies, indicating the unique nature of the molecule[2,3-5]. Many immunological functions are attributed to sialic acids such as formation of negatively charged barrier for host to reduce interaction with pathogen and dampening the classical or alternative pathways of complement by selective deposition on pathogen surface, thus functioning as both beneficial as well as harmful for the system[6-9].

Sialic acid binding immunoglobulins (Ig)-like lectins (siglecs) belong to I-type lectin with a selective

expression on the haematopoetic cell lineages. These have amazing structural diversity to recognize and interact with an array of linkage-specific sialic acids on a glycan structure express on host cells as well as pathogen[10]. Fourteen human and nine murine siglecs have already been identified and the list is still increasing[11].

Based on the inter-species sequence similarity and conservation in mammals, siglecs are mainly classified into two families. Siglec-1 (sialoadhesin or CD169), siglec-2 (CD22), siglec-4 (myelin-associated glycoprotein, MAG) and siglec-15 are evolutionary conserved with poor inter-species sequence similarity. Most of the mammalian species contain definite orthologue of all these siglecs. Other family of siglecs, mainly siglec-3/CD33 related siglecs has encountered rapid evolution indicated by variable interspecies sequence homology making it difficult to find orthologue among species[12,13]. These show 50-99 per cent sequence homology. It comprises the largest family containing 10 human (siglec-3, -5, -6, -7, -8, -9, -10, -11, -14, -16) and five rodent siglecs (siglec-3, -E, -F, -G, -H)[12].

Siglec-3/CD33 related-siglecs express mostly on haematopoetic cell lineages. Siglec-9 is expressed on neutrophils, monocytes, fraction of natural killer (NK) cells, B cells *etc.*, while siglec-8 appearance is restricted on the circulating eosinophils and negligible on besophils[14-16]. Several siglecs can be present on the same cells, such as monocytes express siglec-3, -5, -7, -9 and -10 indicating the extent of functional redundancy at cellular level[17-20]. However, siglec-4 is expressed only on neuronal cells.

After binding with terminally sialylated glycoconjugates, siglecs undertake various functions such as internalization of sialylated pathogens, attenuation of inflammation, restraining cellular activation, attenuation of damage-associated molecular pattern-mediated inflammation along with inhibition of NK cell activation[21,22].

Innate immune system is the first line of defense evolved during the years of evolution. This is responsible for controlling and clearing invading pathogens by non specific means such as toll like receptors (TLRs), complement or by selectively activating the adaptive immune response[23-25]. Polymorphonuclear neutrophils (PMN) are crucial innate immune cells that protect host from invading bacterial and fungal infections by using different defense strategies. Neutrophils exhibit phagocytic activity against pathogenic bacteria and release antimicrobial molecules. Several potent antimicrobial molecules like cationic peptides, proteases, lactoferrin, reactive oxygen species (ROS) and reactive nitrogen intermediates are produced by the neutrophils to serve as potent innate immune cells against pathogens[26]. Neutrophils can also combat severe infection by neutrophil extracellular traps (NETs) formation, a mechanism by which activated neutrophils eliminate pathogenic bacteria. Macrophages, another innate immune cells, are the professional antigen presenting cells (APCs) found nearly in all tissues, to effectively present antigen to the T cells[27].

Although siglecs were identified in early eighties, not much work has been done to explore the consequences of sialic acids acquisition by various pathogens and their interactions with immune system in sialic acids-siglec dependent manner in promoting infection or mediating immune activation.

This review highlights the sialic acids-siglec interaction-mediated innate immune escape of pathogens with special reference to an opportunistic Gram-negative bacteria *Pseudomonas aeruginosa* (PA). Our group has demonstrated the status of sialic acids on PA and their role in host recognition through involvement of human siglecs present on the immune cells[28]. Sialic acids-mediated interactions of PA with siglec-9 present on neutrophils dampen the innate immune functions[29]. Such an interaction during *Leishmania donovani* infection has also been discussed briefly.

Detection of sialic acids on pathogens

Considering the vast microbial kingdom, assessment of the sialoglycan profile remains a relatively unexplored domain of microbial sialobiology. Although a few sialylated microorganisms have been reported (Table I)[28-56], sialylation status on PA remains untouched. Our group has recently demonstrated the status of sialic acids on PA by several analytical, biochemical and immunological methods[28,57]. Sialic acid (Neu5Ac), its glycolyl derivative (Neu5Gc) and *O*-acetylated form (Neu5,9Ac$_2$) on the surface of PA are detected by thin layer chromatography and fluorimetric-HPLC (Fig. 1A). The chromatogram exhibited well-resolved intense peaks corresponding to Neu5Ac, Neu5Gc and Neu5,9Ac$_2$. Additionally, matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF-MS) of purified Neu5Ac, Neu5Gc and Neu5,9Ac$_2$ matched the expected signal having m/z at 448.7, 464.8 and 490.6, respectively confirming their

Table I. Status of sialic acids in different microorganisms

Category	Pathogens	Status of sialic acids	Mode of acquisition	Mode of detection	References
Gram-positive bacteria	Group B *Streptococcus*	α2,3- and α2,6-linked Neu5Ac, Neu5,7/9Ac$_2$		a - d, f - h	30, 31
	Streptococcus pneumoniae	α2,3- and α2,6-linked Neu5Ac	Synthesized	a, h, g, j	32
	Neisseria meningitidis	α2,3- and α2,6-linked Neu5Ac		a, b, d, i, n	33, 34
	Escherichia coli K1	α2,8- and α2,9-linked Neu5Ac		n	35
	Campylobacter jejuni	α2,3- and α2,8-linked Neu5Ac		a, b, j, l	36, 37
Gram-negative bacteria	*Pseudomonas aeruginosa*	α2,3- and α2,6-linked Neu5Ac, Neu5Gc	Adsorbed from host	a - d, g, h	28, 29
	Haemophilus influenzae	α2,3-linked Neu5Ac, Neu5Gc		f, g, m, o	38
	Haemophilus ducreyi	α2,3-linked Neu5Ac	Acquired	e, h	39
	Pasteurella multocida	α2,3-linked Neu5Ac, Neu5Gc		n	40
Parasites	*Leishmania donovani*	α2,3- and α2,6-linked Neu5Ac, Neu5Gc, Neu5,9Ac$_2$	Adsorbed from host	a, b, f, g, h	41-50
	Trypanosoma cruzi	α2,3- and α2,6-linked Neu5Ac	Acquired by trans-sialidase	a, b, j, m	51-53
Viruses	HIV-1	α2,3-linked Neu5Ac		a, b	54
	Porcine Reproductive and Respiratory Syndrome Virus	α2,3-linked Neu5Ac	Synthesized	a, j	55, 56

a. Siglec binding assay; b. Lectin binding assay; c. Thin layer chromatography; d. Gas liquid chromatography; e. Ion-exchange chromatography; f. Fluorimetric-high performance liquid chromatography; g. MALDI-TOF-MS; h. Electro-spray ionization mass spectroscopy; i. Capillary zone electrophoresis mass spectroscopy; j. Fluorescence microscopy; k. Confocal microscopy; l. Live cell video microscopy; m. Scanning electron microscopy; n. Nuclear magnetic resonance; o. Isothermal titration calorimetry

occurrence on PA (Fig. 1B). Binding with *Sambucus nigra* agglutinin (SNA) and *Maackia amurensis* agglutinin (MAA) having specificity towards α2,6- and α2,3-linked sialic acids, respectively confirm the presence of linkage-specific sialic acids on PA[58,59] (Fig. 2). The bacterial membrane fraction showed stronger binding with SNA compared to *Galanthus nivalis* agglutinin, specific for terminal mannose (1,3), (1,6) and (1,2) mannose, *Datura stramonium* agglutinin, specific for galactose (1,4) *N*-acetyl glucosamine and peanut agglutinin, specific for galactose (1,3) *N*-acetyl galactosamine[60]. Further evidence for the presence of sialic acids is obtained using different siglecs where siglec-9 shows maximal binding with this bacteria[33].

PA also possesses α2,6-linked Neu5,9Ac$_2$ on its surface as confirmed through binding with a lectin, Achatinin-H having preferential affinity towards Neu5, 9Ac$_2$α2, 6GalNAc sialoglycotope[42,43,61,62]. A comparable amount of 9-*O*-acetylated sialic acids as a percentage of total sialic acids was detected on intact bacteria and its membrane fraction by fluorimetric estimation[63,64]. To demonstrate this binding specificity toward the 9-*O*-acetyl moiety, bacteria were incubated with a recombinant acetylesterase to remove the *O*-acetyl group[65,66]. The resultant de-*O*-acetylation caused a near total abolition of Achatinin-H binding, confirming the presence of linkage specific 9-*O*-acetylated sialic acids on PA.

By using similar analytical methods and probes, our group has also reported that both *L. donovani* promastigotes and amastigotes contain α2,6- and α2,3-linked sialic acids (Neu5Ac, Neu5Gc, Neu5,9Ac$_2$) on their cell surface[43-48] (Fig. 2). Trypanosoma, another major genus of kinetoplastida, possesses highly sialylated and glycosylphosphatidylinositol (GPI) anchored dense mucin layer on its surface[67].

Acquisition of sialic acids by pathogens

Detection of these sialic acids raises the obvious question regarding the mechanism(s) adopted by the
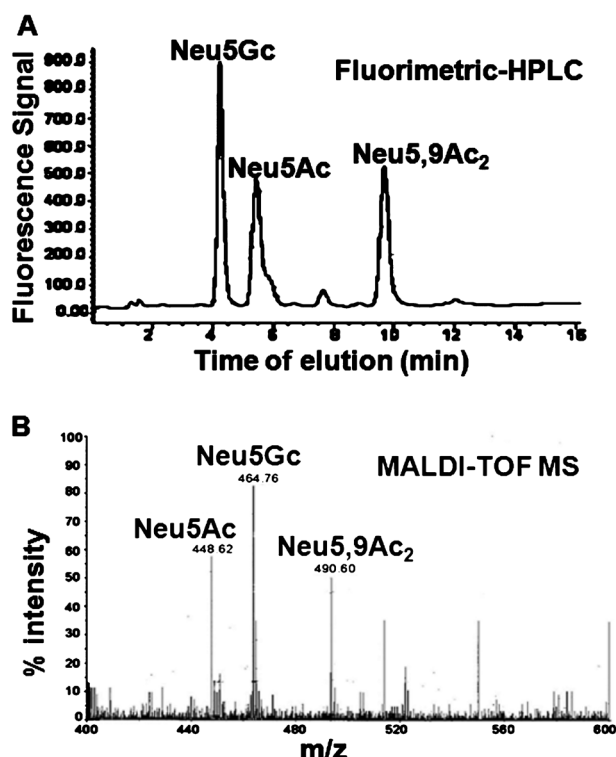
Fig. 1. Identification of sialic acids of *Pseudomonas aeruginosa* (PA) by analytical methods. **(A)** *Fluorimetric-HPLC of 1,2-diamino-4,5-methyleneoxybenzene (DMB) derivatized sialic acids.* A representative fluorimetric reverse phase HPLC profile of DMB derivatized sialic acids purified from PA. Three intense peaks were observed corresponding to Neu5Ac, Neu5Gc and Neu5,9Ac$_2$. Reproduced with permission from John Wiley and Sons, Oxford, UK (*FEBS Lett* 2010; *584* : 555-61). **B**. *MALDI-TOF-MS spectra of the total sialic acids from PA*. Sialic acids were purified from PA[+Sias] and derivatized with 1,2-diamino-4,5-methyleneoxybenzene (DMB). These were analyzed by MALDI-TOF MS on a target with an equal volume of matrix (2,5-dihydroxybenzoic acid). All mass spectra were recorded in the positive ion mode. Sodium cationized molecular ions of Neu5Ac, Neu5Gc and Neu5,9Ac$_2$ peaks having m/z at 448.7, 464.8 and 490.6, respectively are shown. Reproduced with permission from John Wiley and Sons, Oxford, UK (*FEBS Lett* 2010; *584* : 555-61).

pathogens to acquire these terminal sugar molecules. More than 20 pathogenic organisms either synthesize or acquire sialic acids from the host (Table I)[28-56,68]. A few Gram-negative bacteria like *Haemophilus influenza*, *Pasteurella multocida* and *H. ducreyi* use their growth medium as a source of sialic acids whereas GBS, a Gram-positive bacteria and some others Gram-negative bacteria such as *Escherichia coli* K1, *Neisseria meningitidis* and *Campylobacter jejuni* have capability to synthesize sialic acids[30-43]. The mechanism by which

these sialic acids present in human serum are absorbed by PA remains to be explored.

Bioinformatic searches through the genome of PA indicate the absence of a defined endogenous biosynthetic pathway that prompted us to examine whether the sialic acids on the PA surface are derived from the growth medium. The medium (TSB) with 10 per cent heat inactivated normal human serum used for culturing PA (PA[+Sias]) showed the presence of Neu5Ac and Neu5Gc whereas the spent media demonstrated reduced levels of these sialic acids, suggesting possible adsorption of sialic acids from environment[29]. The decrease in the amount of sialic acids on PA[+Sias] with decreasing concentration of human serum in growth medium further suggested adsorption of sialic acids from culture medium (Fig. 3)[29]. We have not found any key enzyme in the sialic acids biosynthesis pathway in *Leishmania*. Moreover, trans-sialidase is also absent in this parasite. We have demonstrated that the sialic acids are adsorbed by promastigotes and amastigotes from the host[49,50].

In this scenario, it may be envisaged that these sialic acids may initially be fragmented or degraded fully or partially by cellular enzymes and subsequently may be available for adsorption by pathogens. Adsorption of sialic acids from culture medium may be either by transglycosylation or by integration of sialic acids to the polyanionic lipophosphoglycan (LPG), LPG/proteophosphoglycan rich cell surface of
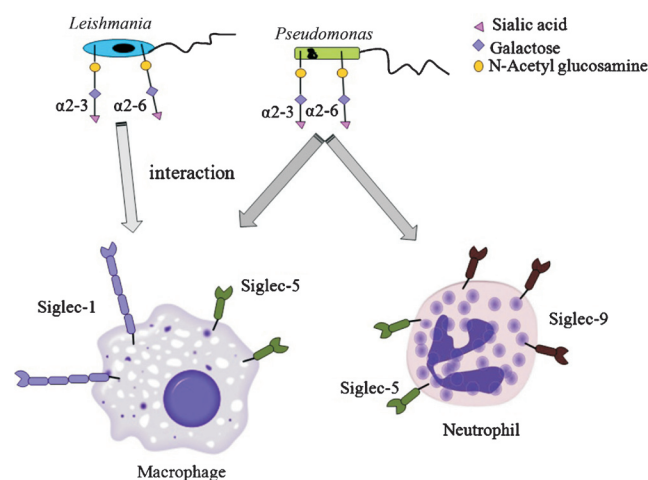

Fig. 2. Interaction of pathogens through linkage-specific sialic acids with different siglecs present on neutrophil and macrophages. The two pathogens namely *L.donovani* and *P. aeruginosa* contain α2,3 and α2,6 linked sialic acids on their surface. These linkage-specific sialic acids bind with siglec-1 and siglec-5 present on macrophages whereas siglec-5 and siglec-9 present on neutrophils.
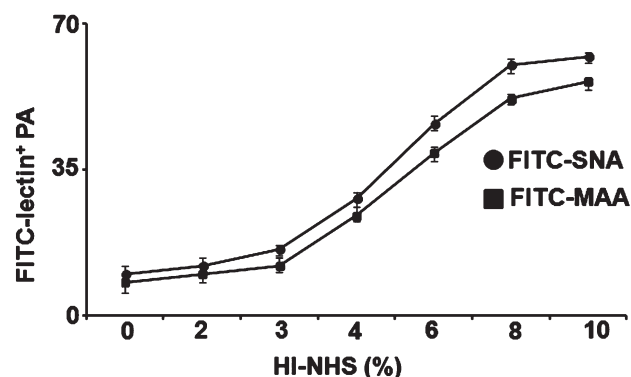
Fig. 3. Adsorption of sialic acids by PA from normal human serum, used as a source of sialic acids. PA was cultured in tryptic soy broth (TSB) containing increasing concentrations of heat inactivated normal human serum (HI-NHS, 2-10%). Flow cytometric analysis showed adsorption of α2,6-linked sialic acids [*Sambucus nigra* agglutinin (SNA) positivity, filled square] and α2,3-linked sialic acids [*Maackia amurensis* agglutinin (MAA) positivity, filled circle] from HI-NHS. Data were presented as per cent of FITC-lectins positive PA cells. Reproduced with permission from Society for Leukocyte Biology, Bethesda, USA (*J Leukoc Biol* 2012; *91* : 641-55).

pathogens. Another option is catalytic transfer of sialic acids from the nucleotide sugar donor CMP-Neu5Ac onto acceptor glycoconjugates on pathogens by serum sialyltransferase.

The presence of a unique enzyme trans-sialidase (TS) in trypanosome facilitates the transfer of glycosidically bound sialic acids from serum sialoglycoconjugates. Although *Trypanosoma* is devoid of any indigenous sialic acids biosynthesis machinery, trans-sialidase accomplishes the function of sialic acids acquisition. Trans-sialidase cleaves the sialic acids containing glycoconjugates from the host cell and transfers it to the parasite surface[69]. The uniqueness of this enzyme lies in that unlike sialyltransferase instead of transferring activated CMP-sialic acid, trans-sialidase transfers carbohydrate-linked sialic acids to the glycan structure and forms a new α2,3-glycosidic linkage to galactose or *N*-acetylgalactosamine[70-72].

Biological relevance of sialic acids on pathogens

The major immunological advantage of sialic acids acquisition by pathogens is to subvert the host immunity by acting as a molecular mimic as this sugar is the indispensable factor for the host[73]. One of the major functions of trans-sialidase is to subvert the complement deposition by acquiring sialic acids on parasite surface[70]. C3 is the central component of the complement system.

C3b being a product of C3 activation, binds covalently through a reactive intramolecular thioester in the C3d domain to bacterial surfaces[74]. Due to the presence of sialylated lipooligosaccharide on the surface, both *N. meningitidis* and *N. gonorrhoeae* show limited C3-deposition on their surface[75]. In parallel, high sialic acids content of type III GBS strains also inhibits C5a production[76]. Interestingly, PA^{+Sias} also shows lower anti-C3 binding compared to PA^{-Sias} indicating a direct relationship between the sialic acids levels and C3-deposition on PA, suggesting a way of escaping from the host serum[28]. In contrast, enhanced expression of 9-*O*-acetylated sialoglycoproteins on erythrocytes of patients with visceral leishmaniais[77-82], increases the activation of alternative complement pathway-mediated haemolysis which leads to anaemia[41]. Along with this, sialic acids acquired by the parasite act as a virulent factor to help in dampening the immune response during infection[49,50].

Immunological relevance of siglecs

Innate immunity is body's first line of defense containing both humoral and cellular immune component. The major disadvantage in this process is the collateral damage of the host cells in due course. To minimize the damage of self, nature has evolved some host-specific ligands that engage some inhibitory receptors present on immune effector cells such as NK cells, neutrophils, monocytes and macrophages to restrict the magnitude of immune reaction. Siglecs mainly act as a negative regulator in immune cells by constraining the magnitude of immune reaction[12,24,25].

Siglec-mediated regulation of cellular activation

Most of the CD33-related siglecs posses a membrane proximal immunoreceptor tyrosine based inhibitory motif (ITIM) and a membrane-distal ITIM like motif[12]. ITIM motif is more dominant than ITIM like motif for recruitment of phosphatase and the phosphatase-mediated inhibitory functions[83]. When a sialylated ligand binds with the siglec, ITIMs carry out the signal to the downstream molecule. After phophorylation by Src-family kineses, ITIMs act as a docking site for binding of either Src homology 2 domain-containing inositol polyphosphate 5' phosphatase (SHIP) or Src homology 2 domain-containing protein tyrosine phosphatases SHP-1 and SHP-2. These phosphates get activated and dephosphorylate an array of phosphorylated molecules, thus play a major role in balancing cellular response or controlling over-activation by antagonizing with the signals coming from immunereceptor tyrosine-based

activation motifs (ITAMs)[84]. This pathway mainly has been explored in B- and NK cells[84]. Human siglec-14,-15, murine CD33 and siglec-H lack ITIM motif interact with DNAX activating protein of 12 kD (DAP12) which subsequently carry out the downstream signaling[85]. Siglec-H does not bind to sialic acids, thus cannot be designated as orthodox siglec[86].

Cis-trans interaction

Sialic acids-siglec interaction is one of the crucial factors in balancing the positive and negative signals in a cell. Linkage specificity of sialic acids with the penultimate sugars, nature of side chain derivatives, structure of distant sugar residues and their modifications, *etc.* regulate the nature of the sialic acids-siglec interaction[24]. Siglecs on a cell make bonding with its own cell surface sialic acids named as *cis* interaction whereas during *trans* interaction, siglecs undergo molecular interaction with the sialic acids present on other cells[12]. Due to the high local concentration (~100 mM in B cells) of sialic acids on the immune cell surface, most of the siglecs except sialoadhesin are masked by the sialic acids of its own cell in *cis* interaction[87,88]. Protruded structure of sialoadhesin help them to stay much way out of cell surface and therefore making it unable to form cis interaction with the membrane sialic acids[89,90]. During physiological condition, *cis* conformation of siglecs in a cell undergoes renovation into *trans* conformation in consequence of cellular activation or in vicinity with the sialylated pathogens. Presence of membrane sialidase or change in glycosylation pattern or reorganization of plasma membrane domain also regulate the conversion of *cis* interaction into *trans*[91].

In resting B cell, inhibitory receptor siglec 2 (CD22) remains mostly in masked condition, and undergoes unmasking during its activation[92-94]. *Cis* interaction has significant impact on modulating the cellular functions by inhibiting the non specific interaction of siglecs with any unintruded cell or sialylated molecules that can initiate unwanted signaling. However, *cis* and *trans* interaction in a cell is a dynamic process, *trans* interaction cannot totally preclude the *cis* interaction of a cell[95,96]. NK cell inhibitory receptor namely siglec-7 having specificity for the Neu5Acα2,8Neu5Ac bearing glycotope shows little cytotoxicity on target cell due to masking effect caused by c*is* interaction. However, pre-treatment of NK cells with sialidase increase its cytotoxic activity due to the unmasking of siglec-7 from *cis* interaction[97,98].

Sialic acids-siglec interaction subverts innate immunity

The persistence and successful establishment of pathogenic infection requires close interactions with host cells. The molecular determinants require for such interactions often include glycoproteins or glycolipids of the membrane. Siglecs on immune cells act as specific ligands for recognition mediated via sialic acids on several pathogenic bacteria[33,36,37]. Porcine reproductive and respiratory syndrome virus uses sialic acids present on envelope for infecting pig alveolar macrophages through siglec-1[55,99]. *N. meningitidis, C. jejuni*, group B *Streptococcus* and *T. cruzi* also exhibit sialic acids-mediated interactions with siglec-1 and several CD33-related siglecs on host cell (Table II)[28-30,32,33,36,52-54,56,100-102].

Sialylated capsular polysaccharide (CPS) of various GBS serotypes specifically recognizes siglec-5,-7,-9 present on human leukocytes for establishment of successful infections[30]. Engagement of sialylated glycan of GBS serotype III through the siglec-9 of neutrophils subverts the host innate immune response by transmitting negative regulatory signals[100]. GBS Ia strains can also bind to human siglec-5 in a sialic acids-independent manner through a specific protein anchored to the bacterial cell wall and impairs immune evasion[101]. The specific engagement of sialylated lypo-oligosaccharides on *C. jejuni* with siglec-1 plays a crucial role in the pathogenesis of Guillain-Barré syndrome[102]. Similarly, *C. jejuni* also interacts with siglec-7 and modulates inflammatory as well as immune responses of host (Table II)[28-30,32,33,36,52-54,56,100-102].

We have investigated the role of adsorbed sialic acids on PA in microbial pathogenesis through association with host cells. Binding of PA[+Sias] with recombinant soluble siglec-7/9 as well as CHO-siglec-7 and CHO-siglec-9 is considerably higher indicating the association of bacteria with innate immune cells through siglecs[28,29].

Co-infection is one of the major problems in most of the immune suppressive diseases. Co-infection with PA in some of the visceral leishmaniasis patients led us to carry forward the study of sialic acids-siglec interaction in the immune cells of these patients. Siglec-7 and siglec-9 expressing on CD56^{+} (NK cells) and CD14^{+} (monocytes) cells isolated from peripheral blood mononuclear cells of active visceral leishmaniasis patient showed higher binding with PA[+Sias] than those cells isolated from normal PBMC[28]. Thus, it may be

Table II. Role of siglecs in different diseases			
Pathogens	Disease	Siglecs	Role
Group B *Streptococcus*	Sepsis and meningitis in human newborns	Siglec-9	Sialoglycan of GBS interacts with siglec-9 expressed on neutrophils in *trans*. Such interaction dampens the innate immune response of hosts by transmitting negative regulatory signals[100].
		Siglec-5	GBS beta-protein which anchored to the bacterial cell wall, binds to siglec-5 expressed on the surface of leukocytes. Such binding promotes GBS survival by impairing human leukocyte function[101].
		Siglec-5,7,9	Capsular polysaccharides (CPS) sialic acids of various GBS serotypes play an important role in interaction with human leukocytes through siglec-5,7,9 to control infections[30].
Streptococcus pneumoniae	*Pneumonia*, sepsis and meningitis	Siglec-5	Sialidase of *S. pneumoniae* bacteria can enhance unmasking of inhibitory siglec-5 on leukocyte and enhance the inflammatory response[32].
Neisseria meningitidis	Septicaemia and meningitis of human	Siglec-1,5	Siglecs can interact with sialylated lypopolysaccharide of *N. meningitidis* and play a potentially protective role during infections[33].
Campylobacter jejuni	Guillain-Barré Syndrome (GBS), Diarrhoea	Siglec-7	Sialylated lipo-oligosaccharides of *C. jejuni* are recognized by siglec-7 on monocytes, NK cells and dendritic cells in a siglec-Sias dependent manner and this interaction modulates the host immune response[36].
		Siglec-1	Siglec-1 on macrophages binds to sialylated lipooligosaccharides leads to formation of cross-reactive antibodies in GBS disease[102].
Pseudomonas aeruginosa	Cystic fibrosis, Urinary tract infection, Skin and soft tissue infections *etc*.	Siglec-9	PA adsorbs glycosidically bound sialoglycoconjugates from the host serum. Due to the association of PA with neutrophils via the Sias-siglec-9 interaction, it can establish persistent infection by reducing the host innate immune response[28,29].
Trypanosoma cruzi	Chagas disease	Siglec-E	Sialylated *T. cruzi* interacts with siglec-E present on neutrophils, macrophages and dendritic cells and modulate leukocyte function to establish infections[52,53].
HIV-1	Acquired immune deficiency syndrome (AIDS)	Siglec-1	Sialylated HIV-1 binds to siglec-1 on monocytes and primary macrophages to facilitate viral infection[54].
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)	Porcine reproductive and respiratory syndrome	Siglec-1	PRRSV binds to alveolar macrophages through siglec-1 and internalizes to establish infection[56].

envisaged that sialic acids-siglec interaction plays a significant role in establishing bacterial infection in immune compromised hosts.

Neutrophils express more siglec-9 and to some extent siglec-5 on its surface (Fig. 2). PA^{+Sias} shows enhanced binding with neutrophils and sialidase-treated neutrophil compared to PA^{-Sias}. Due to blocking of siglec-9 with anti-siglec-9 antibody on neutrophils, the binding of PA^{+Sias} is reduced indicating direct involvement of siglec-9. In contrast, antibody against siglec-5-treated neutrophils shows no such decrease in binding recommending adhesion of PA with neutrophils maximally through sialic acids-siglec-9 interaction[29].

Attenuation of oxidative burst of neutrophils by PA^{+Sias}

During ingestion of microorganisms, neutrophils undergo "respiratory burst" leading to striking increase in oxygen consumption[103]. Neutrophils possess NADPH oxidase which is a complex of membrane-bound multi-component enzyme. Different bacteria/immune complexes after association with host cell-surface receptors release soluble mediators (chemoattractant peptides and chemokines) which can activate NADPH oxidase quickly. The activated enzyme releases electron from NADPH. The major intermediate for such reaction is superoxide anions $O^{2-\bullet}$. Dismutation of superoxide

subsequently produces hydrogen peroxide (H_2O_2). H_2O_2 converts nontoxic compounds into highly reactive, toxic metabolites and then these become antimicrobial. Additionally, hydroxyl radical ($^\bullet OH$) and hypochlorous acids (HOCl) are also formed[88]. Myeloperoxidase, a granular enzyme, catalyses to produce HOCl from Cl^-. This enzyme is mainly expressed in immature and mature neutrophils and also in monocytes[104]. Thus activated NADPH oxidase creates large quantities of ROS in the phagosome of neutrophil which combat with invading pathogens.

Due to interaction with PA^{+Sias} through sialic acids-siglec-9 neutrophil produces comparable amount of ROS as that of untreated neutrophil suggesting functional impairment of host's first line of defense. In contrast, due to absence of sialic acids in PA^{-Sias}, the interaction of sialic acids-siglec-9 is inhibited. As a result, higher amount of ROS is generated by the neutrophils thereby inhibiting bacteria to establish infection in host[29].

Due to reduced or absence of oxidative burst of neutrophil, chronic granulomatous disease patients are frequently infected with an array of microbial pathogens including *Staphylococcus*, *Salmonella*, *Aspergillus*, and *Candida* species to spread out lymphadenitis, skin abscesses and pneumonia[105,106].

Interaction of siglec-9 with sialic acids on PA^{+Sias} facilitates reduced release of elastase from neutrophil

Leukocyte granule–associated proteinase such as neutrophil elastase is a 30-kD glycoprotein with potent catalytic activity dictated by a catalytic triad that consists of histidine, asparagine and serine residues by forming a charge-relay system[107]. Elastase presents at high concentrations in the neutrophil granules. It acts as an antimicrobial agent by disrupting the structural integrity of invading microbes. Toxins of *Shigella*, *Salmonella*, and *Yersinia* are efficiently degraded by neutrophil elastase. This enzyme effectively kills them in its phagosomes[108].

Due to the engagement of PA^{+Sias} to neutrophil through sialic acids-siglec-9 interaction, the release of elastase is significantly reduced with respect to PA^{-Sias}. Blocking of sialic acids-siglec-9 interaction either by specific antibody or removal of sialic acids, innate immune cells secrete significantly higher amount of elastase signifying importance of this interaction in impairment of the neutrophil's function[29]. Outer membrane protein A of *E. coli, K. pneumonia* and PA

strain (H103) degrade neutrophil elastase to establish their infection. Moreover, ecotin, a serine protease inhibitor of *E. coli* shows a protective activity against neutrophil elastase[109].

Engagement of sialic acids and siglec-9 attenuates the neutrophils extracellular traps formation by neutrophil

Neutrophils extracellular traps (NETs) are composed of extracellularly released chromatin material, serine proteases and cytoplasmic proteins[27]. Elastase, cathepsin G, azurocidin and proteinase 3 are the main serine proteases present in neutrophils and play essential role in acute infections and inflammation. Due to these high serine proteases, both Gram-negative as well as Gram-positive bacteria and their virulent proteins are degraded extracellularly by NETs[110].

Production of NETs as shown by clear network of extracellular fibers is attenuated after engagement of neutrophils with PA^{+Sias} through siglec-9-sialic acids association (Fig. 4). Thus PA^{+Sias} shows resistance towards NETs-mediated killing by neutrophils demonstrating impairment of the power of innate immune cells for the effective clearance of bacteria due to Sias-siglec-9 interaction. In contrast, enhancement of such extracellular fibers is observed after engagement of neutrophil with PA^{-Sias} or sialidase-treated PA^{+Sias} due to minimal siglec-9-sialic acids interaction to eliminate the bacteria[29].

DNAse a glycosylated polypeptide, catalyzes the degradation of phosphodiester linkages of single- and double-stranded DNA. To address the question concerning the formation of NETs for killing PA^{-Sias}, neutrophils are treated with DNAse. As a result, although there is no sialic acids-siglec-9 interaction of PA^{-Sias} with DNAse-treated neutrophils, NETs formation is inhibited[29]. This is clearly reflected in higher survivability of PA^{-Sias} confirming direct involvement of NETs for killing of PA^{-Sias}.

In several vertebrates, different pathogenic microorganisms like *S. aureus, Shigella flexneri, Streptococcus pyogenes, Bacillus anthracis, Mycobacterium tuberculosis, Candida albicans,* and *L. amazonensis* are effectively killed through NETs formation[26]. Cathepsin G deficient-mice are more prone to *S. aureus* infections[111]. Similarly, neutrophil-elastase deficient mice are more susceptible to various bacteria like *E. coli*, *K. pneumoniae* and many enterobacterial infections[108,112]. *Staphylococcus aureus* expressing nuclease, *S. pneumoniae* expressing capsule and
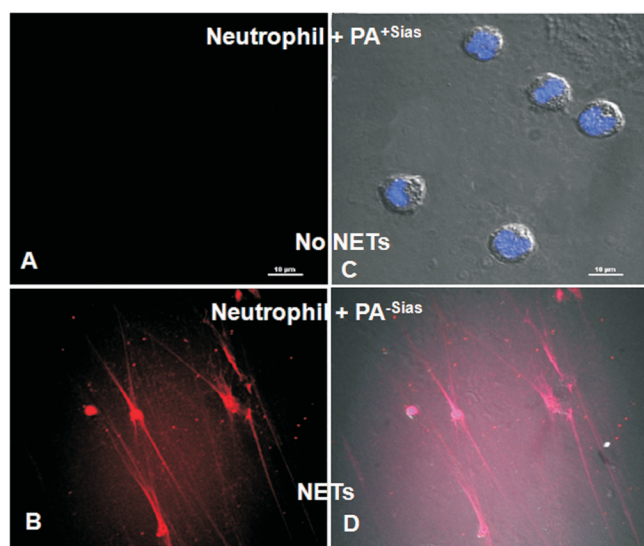
Fig. 4. Visualization of neutrophil extracellular traps (NETs) by confocal microscopy. PA was incubated with neutrophils. Neutrophil extracellular traps (NETs) were visualized under confocal microscopy after stained with Sytox orange (DNA stain for dead cell) only as well as both with DAPI (nuclear stain) and Sytox orange. No NET formation was observed when PA^{+Sias} was incubated with neutrophils (A-C). In contrast, NETs formation was visualised after incubating PA^{-Sias} with neutrophils followed by staining with Sytox orange only (B) as well as DAPI and Sytox orange together (D). Reproduced with permission from Society for Leukocyte Biology, Bethesda, USA (*J Leukoc Biol* 2012; *91* : 641-55).

D-alanylated lipoteichoic acid show resistance towards NETs-mediated killing by neutrophils resulting in increased pathogenesis *in vivo*[26,27].

Siglec-sialic acid interaction during Leishmania infection

During its life cycle *Leishmania* encounters a hostile environment and an array of immune effector cells. In due course of evolution, *Leishmania* has evolved mechanisms to survive and subvert the host immune system. Our recent findings indicate that *Leishmania* promastigotes interact more with siglec-1 and siglec-5 than other siglecs, indicating a specific sialic acids-siglec interaction might exist during infection (our unpublished data). Moreover, differential sialic acids-siglec interaction is observed among virulent and avirulent strain of *L. donovani* with different macrophage cell lines (unpublished data).

We further try to investigate whether sialic acids-siglec interaction modulate the immune response during leishmania infection. Preliminary findings reveal the phosphatase-mediated deactivation of various signaling pathways is differentially regulated in presence or absence of sialic acids-siglec interaction (Fig. 5, unpublished data).

Siglec-sialic acids interaction in Trypanosoma

Siglec-E, murine orthologue of human siglec-9 presents on neutrophils, macrophages and dendritic cells acts as a first line of defense against *T.cruzi*[113]. Pathogenic strain of *T. cruzi* exhibits enhanced binding with siglec-E-Fc chimera compared to non-pathogenic strain[52]. *T. cruzi* also contains the modified structure of sulphated glycan which is the ligand for siglec-E[114]. Siglec-E transfected CHO cells show increased association of parasites and rapid mobilization to the contact zone. However, control CHO cells and siglec-E transfected CHO cells exhibit same percentage of infection with *T. cruzi*, indicating that siglec-E is not an indispensable factor for infection[53]. *T. cruzi* interacts with tissue macrophages through sialic acids-siglec-1 dependent manner[115]. Dendritic cells reduce MHC (major histocompatibility complex) class-I antigen presentation *in vitro*[116] and produce interleukin (IL)-12 which plays a major part against this infection[113]. Accordingly, there might be siglec-1-mediated association, phagocytosis of *T. cruzi* by macrophages and regulation of immune cells by ITIM bearing CD33-related siglecs.

Proposed mechanism of survival of PA in host

Taken together, we have demonstrated that pathogens adsorb sialic acids from the host serum and use these to inhibit C3-deposition[28]. Additionally, these also promote association with the innate immune cells through siglec-dependent recognition to persist within the host. Here, we have shown that a representative pathogen, PA, not only adsorbs sialic acids from host but also associates with neutrophils via the sialic acids-siglec-9 interaction. Due to such interaction, the neutrophils produce less ROS and elastase and thereby NETs formation is dramatically reduced[29]. Consequently, the neutrophil-mediated killing activity is impaired. Therefore, it may be envisaged that pathogens which are unable to synthesize sialic acids adsorb host sialic acids as a way to engage the inhibitory siglec-9 and thus subvert immunity (Fig. 6)[29]. This may be one of the general mechanisms by which other pathogens either synthesize or acquire sialic acids from host can establish infection by reducing the host innate immune response.
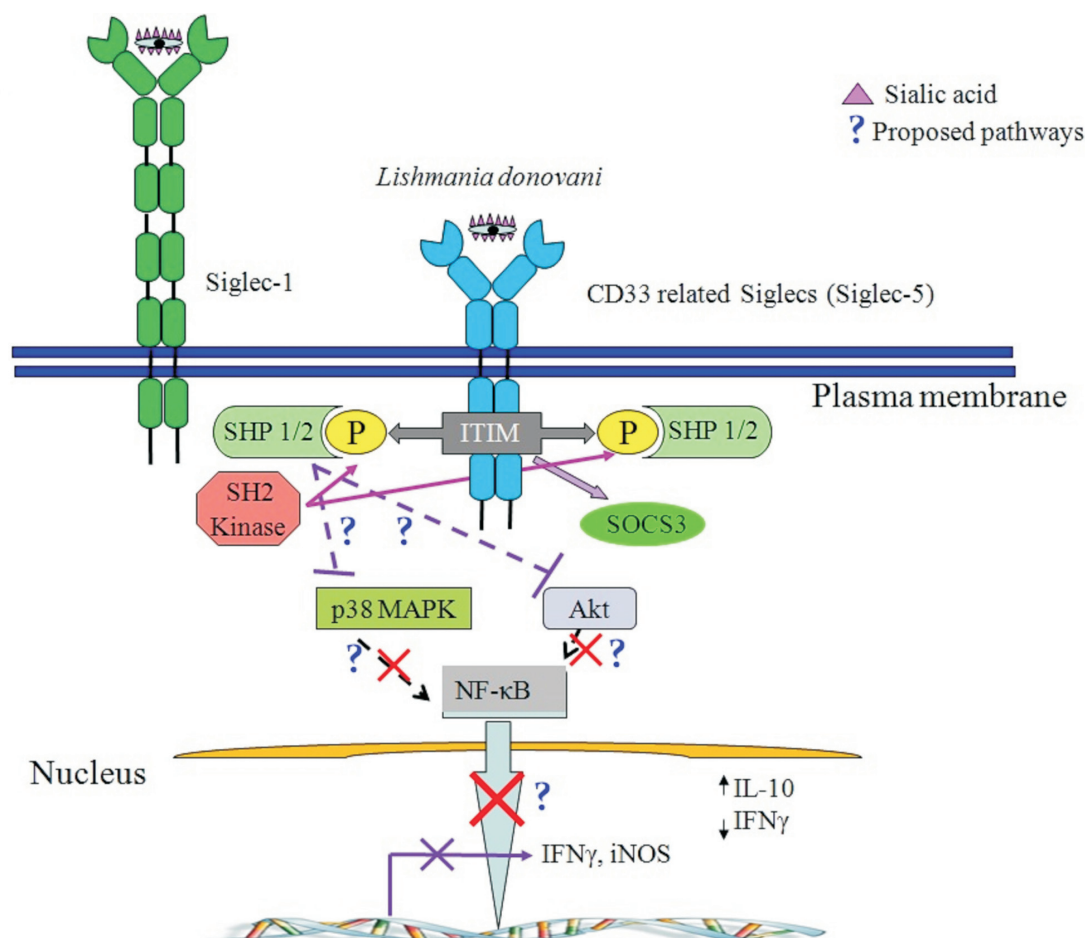
Fig. 5. Schematic diagram of the proposed pathway of CD33 siglec-mediated suppression of cellular function in *Leishmania* infection. On macrophage, after ligand (present on various pathogen surface) binding with CD33 related siglecs, immunoreceptor tyrosine-based inhibition motif (ITIM) present on the cytosolic portion of siglecs get activated by phosphorylation with SH2 family kinases (Lyn, Syk). Then Src homology 2 domain-containing phosphatases 1 and 2 (SHP1 and SHP2) bind with the ITIM and get activated. These activated SHPs further dephosphorylate various signaling molecules and suppress cellular activation. The target of SHPs might be p38 mitogen-activated protein kinases (p38 MAPK) or Akt or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kβ) and ultimately downregulate the effector functions of the cell. In macrophages this siglec-mediated pathway might regulate the polarization of macrophage function towards anti-inflammatory type by reducing Th1 cytokines (IFNγ) and increasing Th2 cytokines (IL-10) along with downregulating inducible nitric oxide synthase (iNOS) gene expression for nitric oxide (NO) secretion.

Future perspective

In recent years, a great advancement has been made in the field of sialoglycobiology of human pathogens, still it is the tip of iceberg. The sialylation pattern and the mode of acquisition of sialic acids on several bacterial and viral families are yet to be explored. Although pathogens stimulate an array of inflammatory responses, the specific role of these sialylated molecules in evading the host defense remains to be fully characterized and appears to be a complex phenomenon. Even though siglec-mediated

interaction has been established in a few pathogens, the role of siglecs in recognition and pathogenesis in other sialylated pathogens might open up a new avenue towards understanding the disease biology in near future. PA along with other important bacteria is becoming more and more antibiotic resistant day by day. Subsequently, the role of surface sialylation of pathogens and their siglec-mediated interaction with host immune cells might be helpful and possibly this information could be used as a potential therapeutic target as an alternative of antibiotics. The importance of this sialic acids-siglec interaction is not only restricted
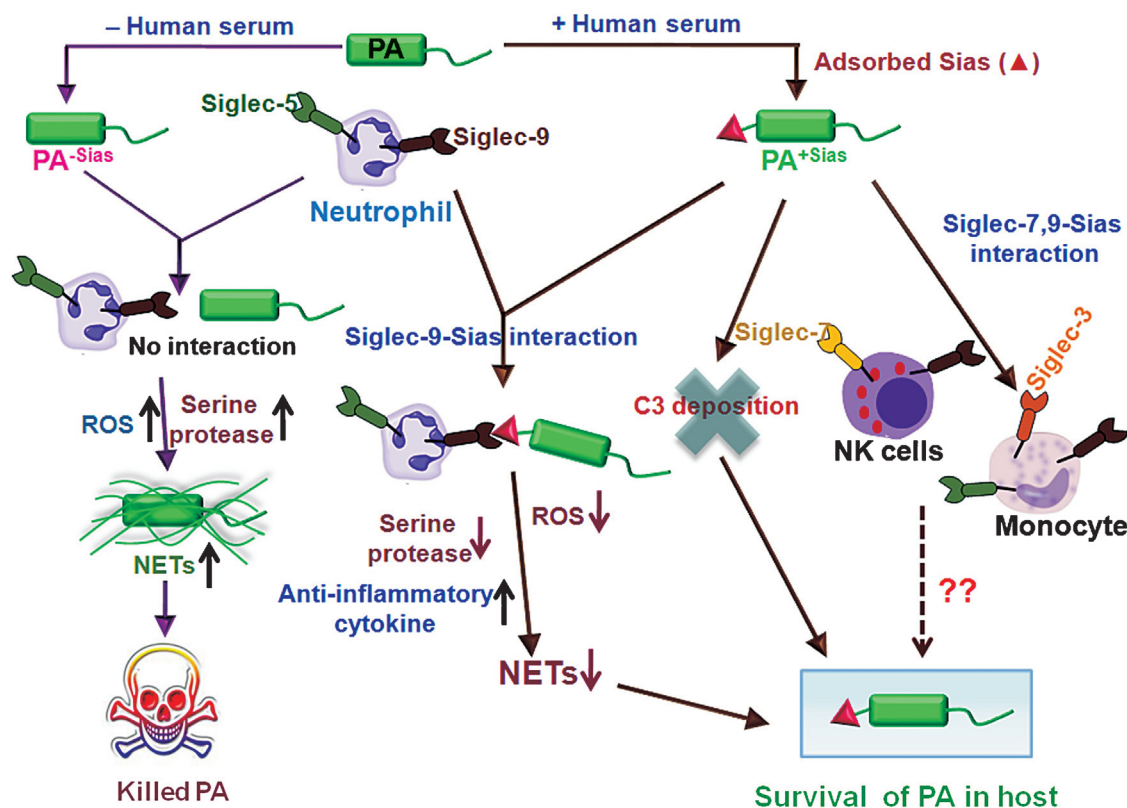
Fig. 6. Schematic diagram of proposed mechanism for survival of PA[+Sias] in hostile environment. Schematic representation of interaction of PA[+Sias] with immune cells through siglecs and their survival in host by dampening host innate immune response is shown. PA adsorbed sialic acids from host serum (PA[+Sias]). PA[+Sias] associated with immune cells (NK-cells, monocytes and neutrophils) through sialic acids-siglecs (siglec-7 and siglec-9, respectively) interaction. Neutrophils produced reduced amount of reactive oxygen species, serine proteases (elastase) and neutrophil extracellular traps (NETs) through siglec-9-sialic acids interaction with PA[+Sias]. At the same time, due to such interaction neutrophils enhanced anti-inflammatory cytokines production leading to survival of PA[+Sias] within host. Sialic acids on PA resist complement deposition directing survival of PA[+Sias]. On the other hand, PA cultured in sialic acids free medium (PA[-Sias]) exhibited no interaction with neutrophils. Therefore, neutrophils produced increased secretion of reactive oxygen species, serine proteases (elastase) followed by neutrophil extracellular traps (NETs) formation leading to effective clearance of PA. Reproduced with permission from Society for Leukocyte Biology, Bethesda, USA (*J Leukoc Biol* 2012; *91* : 641-55).

to the bacteria or in viruses, it is true even in parasitic infections such as leishmaniasis and trypanosomiasis. Such mechanisms used by the parasites to survive in the hostile environment are not well investigated till now, whereas more and more drug-resistant strains are arising. In this context, a detailed mechanistic approach is of utmost importance to explore sialic acids-siglec pathways for identifying future drug targets as sialic acids acquisition has become almost an indispensable factor for most of the human pathogens.

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