Glycoconjugate Journal 20, 199–206, 2004

Sialoglycans in protozoal diseases: Their detection, modes of acquisition and emerging biological roles

Anil K. Chava[1], Sumi Bandyopadhyay[1], Mitali Chatterjee[2] and Chitra Mandal[1]*

[1] *Immunobiology Division, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Jadavpur, Kolkata 700 032, India,* [2] *Department of Pharmacology, Dr. BC Roy Postgraduate Institute of Basic Medical Sciences, I.P.G.M.E.R., 244B A.J.C. Bose Road, Kolkata 700 020, India*

Protozoan parasites including *Plasmodia*, *Leishmania*, *Trypanosoma*, *Entamoeba*, *Trichomonas* and others cause diseases in humans and domestic livestock having far-reaching socio-economic implications. They show remarkable propensity to survive within hostile environments encountered during their life cycle, and the identification of molecules that enable them to survive in such milieu is a subject of intense research. Currently available knowledge of the parasite cell surface architecture and biochemistry indicates that sialic acid and its principle derivatives are major components of the glycocalyx and assist the parasite to interact with its external environment through functions ranging from parasite survival, infectivity and host-cell recognition. This review highlights the present state of knowledge with regard to parasite sialobiology with an emphasis on its mode(s) of acquisition and their emerging biological roles, notably as an anti-recognition molecule thereby aiding the pathogen to evade host defense mechanisms.
Published in 2004.

Keywords: **sialic acid, *O*-acetylated sialic acid, parasite, trans-sialidases, UDP-GlcNAc2-epimerase**

Abbreviations: **PARPs: procyclic acidic repetitive proteins; GIP: glycosylphosphatidyl inositol; *O*-AcSGs: *O*-acetylated sialoglycoconjugates; SNA: *Sambucus nigra* agglutinin; MAA: *Maackia amurensis* agglutinin; GalNAc: *N*-acetylgalcatosamine; UDP-GlcNAc: Uridine diphosphate *N*-acetylglucosamine; CMP: Cytidine monophosphate; Neu5Ac: *N*-acetylneuraminic acid; ManNAc: *N*-acetylmannosamine; EBA: Erythrocyte binding antigen; Ssp-3: sialic acid specific protein; VL: Visceral Leishmaniasis.**

Introduction

Sialic acids are a structurally complex family of nine-carbon polyhydroxy amino ketoacid of *N*- and *O*-substituted derivatives of neuraminic acid, a monosaccharide commonly referred to as *N*-acetylneuraminic acid or Neu5Ac [1]. It is the most abundantly available monosaccharide present as the terminal residue of cell surface sugar chains. Its strategic terminal position provides it accessibility, reflected in its regulation of a multitude of cellular and molecular interactions [2]. Over 50 different modifications of sialic acid are generated following substitution of the amino group by an acetyl or glycolyl group and one or more hydroxyl groups by methylation or esterification with acetyl, lactyl, sulphate or phosphate groups [3]. However, the most frequently occurring modification (over 18) is *O*-acetylation at position C-7/8/9 to form *N*-acetyl-7/8/9-*O*-acetylneuraminic acid respectively generating a family of *O*-acetylated sialoglycoconjugates or *O*-AcSGs [1]. However, most parasitic protozoa differ from their mammalian and avian counterparts, in that neither sialic acid nor any of its structural derivatives are synthesized by the parasite. The parasite has instead devised ingenious methods of acquiring this sugar molecule.

To whom correspondence should be addressed: Dr. Chitra Mandal, Immunobiology Division, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Jadavpur, Kolkata 700 032, India. Tel: 91 33 2473 3493; Fax: 91 33 2473 5197 or 91 33 2472 3967; E-mail: cmandal@iicb.res.in, chitra_mandal@yahoo.com

Presence of sialoglycans on parasitic protozoa

Trypanosoma cruzi, the etiologic agent of Chagas disease or South American Trypanosomiasis shuttles between an intracellular dividing amastigote stage in mammalian host tissue and an extracellular nondividing bloodstream trypomastigote form responsible for propagation of infection [4]. These metacyclic trypomastigotes possess 35 and 50 kDa sialoglycoproteins that participate in parasite attachment to mammalian cells [5]. Neu5Ac is usually associated with mucin-like molecules,

a group of highly glycosylated GPI-anchored proteins rich in threonine, serine and proline residues [6,7].

The African trypanosome, *Trypanosoma brucei* responsible for sleeping sickness in humans and ngana in domestic animals, exhibits a digenetic life cycle that alternates between the tsetse fly vector (epimastigote form) and mammalian host (trypomastigote form) [4]. The cell surface coat of the procyclic form of *T. brucei* (present in tsetse flies) is composed largely of procyclic acidic repetitive proteins referred to as procyclins or PARPs. These PARPs contain an acidic Glu-Pro repetitive domain, a glycosylphosphatidyl inositol (GPI) membrane anchor, and a putative asparagine glycosylation site; analysis of the GPI anchor has revealed that Neu5Ac residues are major contributors to its negative charge [8].

The presence of Neu5Ac and its 9-*O*-acetylated derivative has been demonstrated on promastigotes of *Leishmania donovani*, the causative organism of Visceral Leishmaniasis [4] by fluorimetric high performance liquid chromatography. Their linkages were established using the sialic acid-binding lectins *Sambucus nigra* agglutinin (SNA), *Maackia amurensis* agglutinin (MAA), Siglecs 1, 2, 5, 7, 8 and 10 and indicated the presence of both α2,3- and α2,6-linked sialic acids [9]. *L. donovani* promastigotes also reacted with Achatinin-H, a lectin that preferentially identifies 9-*O*-acetylated sialic acid in α2,6 GalNAc linkage [10–12]. The binding of CD60b, a 9-*O*-acetyl GD3-specific monoclonal antibody, to promastigotes corroborated the presence of terminal 9-*O*-acetylated disialoglycans.

Entamoeba histolytica, the etiologic agent of amoebic dysentery, has a two-stage life cycle, the amoebic or trophozoite form and the infective cyst stage [4]. There is strong evidence that the encystation process is accompanied by the appearance of surface sialoglycans corresponding to 100 and 150 kDa [13]. Sialic acids are present in glycolipid on trophozoites and glycoproteins on encysting cells of *E. invadens* [14,15].

Crithidia are monogenetic members of the trypanosomatid family and colonize the digestive tract of flies. Indirect evidence was initially provided for the presence of sialoglycans as the major anionogenic group on the surface of *C. fasciculata* contributing toward the surface negative charge and was later substantiated using gold labeled *Limulus polyphemus* agglutinin [16]. Both *N*-acetyl and *N*-*O*-diacetylneuraminic acid have been identified on flagellates of wild and drug resistant mutants [17]. Subsequently, using sialic acid-binding lectins SNA and MAA and influenza virus C an increased presence of α2,6 linked sialic acid and the 9-*O*-acetylated derivative was identified in drug-resistant mutants suggesting that sialoglycans possibly influence mutation and cell growth [18].

Although Plasmodia, causative agents of malaria, do not contain or synthesize sialoglycans [19], Neu5Ac is an important determinant of parasite invasion as Miller *et al.* [20] reported that susceptibility of human erythrocytes to invasion by *Plasmodium falciparum* was blood-group dependent; this susceptibility decreased when erythrocytes were pretreated with trypsin or sialidase, stressing the importance of sialic acid-binding ligands. It is now firmly established that erythrocyte invasion of *P. falciparum* is facilitated by the binding of a parasite 175-kDa erythrocyte-binding antigen or EBA-175 [21]. Binding of EBA-175 to its erythrocyte determinant has a receptor-like specificity, is saturable, and is competitively inhibited only by Neu5Ac-α2,3-Gal containing oligosaccharides of glycophorin A. As selective cleavage of *O*-linked tetrasaccharides clustered at the NH2 terminus of glycophorin A markedly reduced binding, it was inferred that the Neu5Ac-α2-3-Gal-determinant on glycophorin A is the erythrocyte ligand for EBA-175 [21]. Another erythrocyte-binding antigen present on *P. falciparum* is EBA-140; it shares structural features and homology with EBA-175, similarly located on micronemes, and also binds to a sialic acid-containing receptor on human erythrocytes. The binding is sialidase sensitive and resistant to trypsin, proteinase K, and pronase [22]. However, as parasites that lack the EBA 140 gene can still invade erythrocytes, this suggests that unlike EBA-175, this protein is not vital for erythrocyte invasion [22].

Toxoplasma gondii, causative agent of Toxoplasmosis, is an obligate intracellular pathogen within the phylum *Apicomplexa*. Its life cycle includes three distinct stages namely sporozites, products of the sexual cycle within the cat, and the asexually dividing tachyzoites and bradyzoites within the mammalian host. Tachyzoites acquire a 15 kDa fetuin binding protein when harvested from P388D1 macrophage cultures. Notably, this was absent in tachyzoites obtained from peritoneal exudates of NMRI mice suggesting that these sialoglycans were acquired from the culture medium [23].

Trichomonas vaginalis and *Trichomonas foetus*, causative agents for non-viral sexually transmitted diseases, are flagellated parasites that colonize the urogenital lining of humans and cattle respectively. Chromatographic analysis of the carbohydrate profile in *T. foetus* showed Neu5Ac to be the principal sialoglycan present in flagellated cells at a density of 2.7×10^7 residues/cell [24]. A novel 728 kDa, sialic acid-binding lectin was isolated from culture supernatants of *T. foetus* that reacted equally with Neu5Ac and Neu5Gc either free or in α2-3/α2-6-linkage and a 7-fold weaker affinity to α2-8-linked Neu5Ac in colominic acid. Following purification by erythrocyte adsorption and fetuin-agarose affinity chromatography, the sialic acid binding lectin was shown to be different from two previously identified sialidases of 853 and 254 kDa, shed into the medium [25]. The cell agglutination and binding of wheat germ agglutinin displayed by a drug-susceptible strain was completely nullified by sialidase treatment confirming the presence of a surface sialic acid moiety on *T. vaginalis.* Sialylated glycoptopes were, however, absent in the drug-resistant strain that showed preferential binding to Con A [26].

Mode(s) of acquisition of sialic acids in parasitic protozoa

Trans-glycosylation in Trypanosoma

As Trypanosomes do not synthesize Neu5Ac *de novo* [27] they have the unusual ability of acquiring Neu5Ac from host

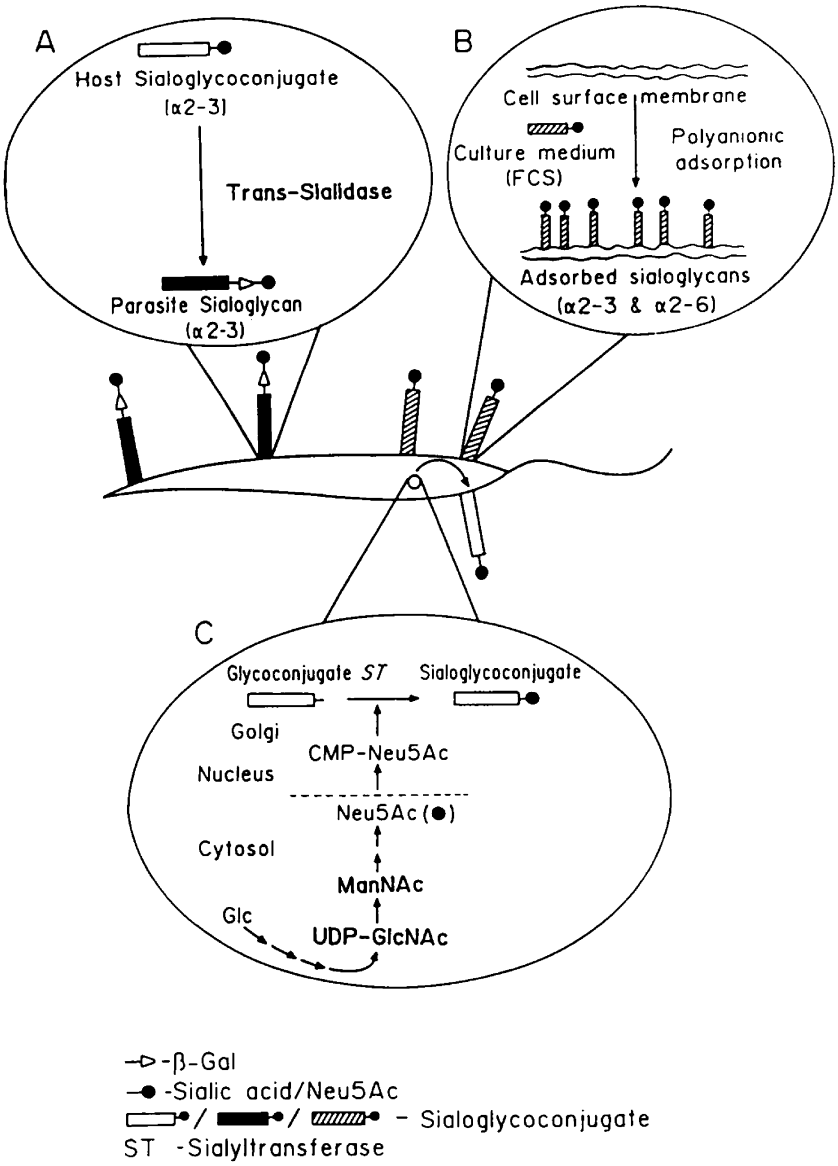

Figure 1. Schematic representation of acquisition of sialic acids by parasitic protozoa. (A) Sialic acid is acquired by Trans-sialidase in *Trypanosoma cruzi* [5,6] and *Trypanosoma brucei* [8,36]. (B) Polyanionic adsorption of serum sialoglycans by *Leishmania donovani* promastigotes [9]. (C) The enzymatic reactions [1] involved for *de novo* synthesis of sialic acids in *Entamoeba histolytica* [13], *Crithidia fasciculate* [18].

sialoglycoconjugates efficiently accomplished by a unique enzyme called trans-sialidase [28] that resides on the surface membrane of trypomastigotes (Figure 1A). *Trypanosoma cruzi* is a voracious sialic acid consumer that acquires sialoglycans from the surrounding medium via trans-sialidases that belong to a highly heterogeneous gene family of surface molecules sharing with each other and with bacterial sialidases variable degrees of nucleotide sequence homology and common motifs [28]. These trans-sialidases selectively catalyze the transfer of α2-3-linked sialic acid from extrinsic host sialoglycoconjugates to the terminal β-Gal residues of mucin-like molecules [5]. Unlike other sialyl transferases [29,30] that require cytidine monophosphate (CMP) Neu5Ac as a donor, trans-sialidases instead utilize α2,3 linked sialoglycans [31]. A ^{1}H NMR investigation has confirmed the transfer of Neu5Ac from sialic acid donor molecules to acceptors on the parasite using trans-sialidases in *T. cruzi* [32]. FAB-MS and NMR spectral data have provided evidence for the presence of O-linked sialylated oligosaccharides on mucin-like molecules [33]. Crystallographic analysis of the *T. cruzi* trans-sialidase, has demonstrated that the binding of sialic acid triggers a conformational switch on the parasite mucin molecules. This results in increased affinity for the acceptor substrate thus creating an amicable condition for efficient transglycosylation [34].

The African trypanosomes, *T. brucei* also possess similar trans-sialidase activity capable of transferring Neu5Ac from host sialoglycans onto the parasite surface. Sialylation in *T. brucei* occurs primarily in procyclins or procyclic acidic repetitive proteins (PARPs) of procyclic forms of *T. brucei* [35,36]. Like *T. cruzi*, the β-Gal termini of these parasites are substituted with Neu5Ac by trans-sialidases [37]. Genetically the *T. brucei* transsialidases are present at a small copy number, whereas *T. cruzi* have a large gene family [38]. The *N*-terminus of *T. brucei* transsialidase contains a region of 372 amino acids having 45% homology with the catalytic domain of *T. cruzi* transsialidase and contains the relevant residues for catalysis [38]. Despite *T. cruzi* and *T. brucei* transsialidases having remarkably similar donor and acceptor specificities, they are antigenically distinct [38]. More recently, two transsialidase forms from *T. congolense* having different sialic acids transfer and sialidase activities have been purified and characterized [39]. The catalytic domains of the two transsialidase forms TS1 and TS2 exhibited a significant degree of homology with *T. brucei* TS (56 and 46% respectively). Additionally, both the *T. congolense* TS sequences also showed 40–42% identity with the catalytic domain of *T. rangeli* sialidase. However, the sequence analysis of the *T. congolense* transsialidases revealed differences in their gene sequences, thus providing important information on the phylogenetic relationship of different TS enzymes [40].

However, not all trypanosome species across the board have transsialidase activity as *T. rangeli* contain a sialidase that hydrolyzes sialyl α2–3 lactose and much less efficiently, sialyl α2–6 lactose but not poly α2–8 Neu5Ac [41]. The *T. rangeli* and *T. cruzi* sialidase have been shown to differ antigenically and also differ in their pH optimum for hydrolase activity [41].

Adsorption of sialic acid in *Toxoplasma gondii* and *Leishmania donovani*

Lectin-binding studies have demonstrated the presence of a 68-kDa sialoglycoprotein in tachyzoites of *T. gondii* when harvested from P388D1 macrophage cell cultures, notably absent in tachyzoites maintained in peritoneal cavities of NMRI mice [23]. This was identified as fetuin, a regular component of fetal calf serum, present in the culture medium. Latex agglutination and immunofluorescence demonstrated its intracellular localization and its absence from the parasite surface. Using fetuin-agarose, the presence of a 15-kDa fetuin-binding protein was also detected in cytosolic fraction of tachyzoites [23].

Although Trypanosomes possess transsialidase activity and thus acquire Neu5Ac, another closely related kinetoplastid *Leishmania* does not possess such an enzyme. Lack of any detectable UDP-GlcNAc epimerase activity indicated that no endogenous biosynthesis of Neu5Ac exists in the *Leishmania* parasite [9]. In the light of such background information, we wished to investigate whether the sialoglycans on *Leishmania donovani* are obtained via adsorption of sialoglycans present in the culture medium (Figure 1B). Concomitant Western blotting of parasite membranes and culture medium with sialic acid-binding lectins indicating that α2,3 sialoglycans corresponding to 130, 117, and 70 kDa are adsorbed from the fetal calf serum present in the culture medium (Figure 2). Similarly α2,6-linked sialoglycans corresponding to 123, 90, and 70 kDa were acquired (Figure 2). A differential adsorption of sialoglycans on promastigotes was observed in virulent *vs.* avirulent strains highlighting the biological relevance of these glycotopes. Interestingly, amastigotes obtained from infected hamsters also demonstrate the presence of surface sialoglycans [42].

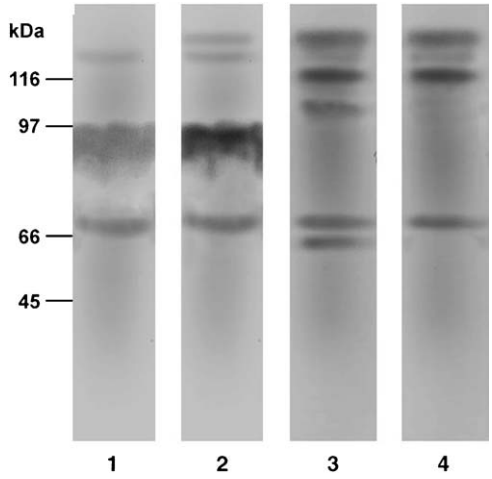

Figure 2. Adsorption of serum sialoglycans by *Leishmania donovani* promastigotes. Membrane proteins from promastigotes were electrophoresed (7.5% SDS PAGE) and following transfer onto nitrocellulose membranes were incubated with SNA (lane 1) or MAA (lane 3). To demonstrate the binding of SNA (lane 2) and MAA (lane 4) to medium M199 containing 10% fetal calf serum, Western blotting was similarly carried out. (Reproduced from [9] with permission of the publishers, Oxford University Press).

De-novo synthesis of sialic acid by Entamoeba histolytica, Crithidia fasciculate

The biosynthesis of Neu5Ac is a finely tuned phenomenon where UDP-GlcNAc2-epimerase plays a pivotal role by epimerizing UDP-GlcNAc to ManNAc (Figure 1C). From ManNAc, free Neu5Ac is synthesized in the cytosol and is then activated following its transfer to the nucleotide, CMP-glycoside (β-anomer) present in the nucleus. Resident sialyltransferases in the Golgi are next responsible for transferring CMP-Neu5Ac to the specific terminal non-reducing position on nascent glycoconjugate (protein or lipid) acceptors to generate sialoglyconjugates that then move to the membrane [1].

Metabolic labeling of the encysted form of *E. histolytica* with ^{3}H glucosamine has demonstrated labeling of a 150 kDa sialoglycoprotein amounting to 15 residues per molecule indicating the presence of sialoglycans, which are acquired through *de novo* synthesis [13]. The appearance of sialoglycoconjugates

Table 1. Biological importance of sialoglyconjugates in parasitic protozoa

Parasite	*Sialoglycoconjugates*	*Biological relevance*	*References*
Trypanosoma cruzi	Acquisition of sialict acids by mucins	(a) Invasive determinant (b) Modulation of host immune response (c) Protection against cytolytic agents.	[34,43–51]
Trypanosoma brucei	Acquisition of sialic acids by procyclic repetitive proteins (PARPs).	(a) Invasion of host cells (b) Survival within insect vector.	[8,35–37]
Entamoeba histolytica	Appearance of sialic acid during encystation and gangliosides in trophozoites.	(a) Decreases parasite adherence to target cells thereby reducing its cytolytic activity.	[13,66]
Plasmodium falciparum	Absence of Neu5Ac, instead sialic acid binding protein EBA-175 is present.	Ligands for EBA-175 are essential for erythrocyte invasion.	[21,63]
Trichomonas vaginalis and *Trichomonas foetus*	Sialic acid specific lectin identified in parasite culture supernatant	Enhances parasite adhesion to mucosal surfaces	[24–26,64,65]
Toxoplasma gondii	Uptake of fetuin from the culture medium.	Not known	[23]
Leishmania donovani	Polyanionic adsorption	(a) Determinant of virulence (?) (b) Complement mediated cell lysis (?)	[9,42,59]

on *C. fasciculate* has been shown to vary depending on the growth conditions. The parasites when grown in sialic acid-free chemically defined media also acquired surface sialglycoconjugates suggesting that these sialylated residues are synthesized *de novo* and not transferred by transsialidases as in case of trypanosomes [18].

Biological role

In the early 90s, Schenkman *et al.* [43] demonstrated that the invasive power of trypanosomes directly correlated with the sialyl transferring property of transsialidases. Amastigotes of *T. cruzi* contain little or no sialic acid on their surface but rapidly acquire the same upon transforming into infective trypomastigotes and concomitantly express a unique cell surface transsialidase enzyme. Further, evidence that Ssp-3 bearing sialylated glycotopes were critical determinants for parasite invasion and internalization was demonstrated using antibodies against Ssp-3 [44]. Time kinetic studies revealed that trans-sialidases have a stage-specific expression directly proportional with their infectivity [45]. These acquired sialoglycans help to form a negatively charged coat that protects the parasite from anti human galactosyl antibody mediated cytolysis (Table 1) [46]. Induction of protective immunity against *T. cruzi* infection occurred after administering the catalytic domain of Tran-sialidases as a DNA vaccine; upon challenge with bloodstream trypomastigotes, these immunized mice displayed reduced parasitemia and mortality indicating that transsialidases are responsible for fine tuning the degree of parasite infectivity [47]. Belen-Carrillo *et al.* [48] transfected transsialidases into another protozoan parasite *Leishmania major*, which resulted in induction of parasite virulence in BALB/c mice, evidenced by lesion progression and parasite numbers. Transsialidases also influence the host immune response as they induce apoptosis of cells of the immune system in the spleen, thymus and peripheral ganglia, thereby facilitating the parasite to evade the host-induced immune response [49]. Taken together, these findings strongly suggest that transsialidases possibly 'sabotage' the host immune system, conferring a growth advantage to the parasite. Inactive members of the transsialidase family also play a contributory role in host-parasite cell interaction as they physically interact with CD4+ cells via CD43 engagement. CD43 or leukosialin acts as a counter receptor on host CD4+ cells causing deactivation of T cell-induced cell death, corroborative evidence that transsialidases aid the parasite in eluding immune surveillance [50]. Mucins, namely TcMuc-e2 in *T. cruzi* are the main acceptors of Neu5Ac and participate in various host-parasite interactions, such as adhesion to macrophages, protection from complement lysis, and immunomodulation of the host immune response [51]. Transfection of *T cruzi* mucin gene (TcMuc-e2) into Vero cells diminishes T-cell proliferation and activation; this effect is reversed by pretreatment with O-sialoglycoprotease and sialidase corroborating that O-glycosylation and sialylation are essential for mediating this immunomodulation [51].

T. cruzi invades several vertebrate cells by a mechanism that involves recruitment and fusion of lysosomes at the site of invasion and is distinct from phagocytosis. Here again, transsialidases facilitate parasite survival by removal of Neu5Ac from lysosomal glycoproteins present on host cell lysosomes; the resultant lack of Neu5Ac on lysosomal glycoproteins facilitates membrane lysis and promotes its entry into host cells [52].

Tachyzoites of *T. gondii* tend to infect several cell types and the extent of infection was found to be proportionate to the

extent of sialylation of these host cells. Hypersialylation of host cells induced a higher degree of infectivity by *T. gondii* indicating that surface-exposed carbohydrate residues of the host cell regulate host cell recognition by *T. gondii* [53].

The enhanced presence of *O*-AcSGs has been reported on erythrocytes [54,55] and peripheral blood mononuclear cells [56] of patients with Visceral Leishmaniasis (VL). Ligands of the cell adhesion molecule CD22 can be masked by 9-*O*-acetylation of sialic acid and thereby hinder cell-cell adhesion. It is, therefore possible that that 9-*O*-AcSGs acquired on hematopoietic cells of VL patients reduces cell to cell adhesion. This may well be an approach adopted by the parasite to evade immune surveillance. The 9-*O*-AcSGs present on murine erythrocytes contribute toward their enhanced susceptibility to lysis by activation of the alternate pathway of complement [57,58]. Similarly, erythrocytes from patients with VL showed a significantly higher degree of complement-mediated hemolysis as compared to erythrocytes from normal healthy donors, reconfirming that linkage specific 9-*O*-AcSGs play a contributory role in complement-mediated hemolysis [59]. It remains to be investigated whether this enhanced presence of 9-*O*-AcSGs on these diseased erythrocytes plays a contributory role towards anemia, a common manifestation of the disease. As antibodies against *O*-AcSGs selectively mediate complement dependent cytolysis of lymphoblasts in patients with Acute Lymphoblastic Leukemia [60,61], it remains to be investigated whether the increased presence of antibodies against *O*-AcSGs demonstrated in VL patients can mediate parasite lysis [62].

Sialic acids play a critical role in the invasion of human erythrocytes by *P. falciparum* merozoites as erythrocytes that possess ligands for EBA 175. The 175-kDa erythrocyte-binding parasite antigen (EBA-175) are selectively invaded [21]. To assess whether Neu5Ac present on the erythrocyte functions simply by virtue of the negative charge it generates or is sialic acid a structural requirement for the merozoite receptor, the invasion of mouse erythrocytes by *P. falciparum* merozoites has been studied [63]. Murine erythrocytes were selected as they contain 9-*O*-AcSA besides Neu5Ac. It was observed that treatment of murine erythrocytes with esterase from influenza C virus enhanced the binding of merozoites. As these esterases cleave the 9-*O*-acetyl group thereby exposing Neu5Ac, it indicated that Neu5Ac is a structural requirement for invasion of erythrocytes.

Trichomonads also utilize sialic acid -binding lectins for adhesion to mucosal surfaces [64,65]. Sialic acids are not always beneficial to the parasites as observed in *E. histolytica.* By contributing to the negative charge of cysts of *E. histolytica,* Neu5Ac promotes their expulsion from the host's intestine, decreasing their adherence to target cells and reducing their cytolytic activity [66].

Perspective

Identification of invasive or evasive pathoantigenic determinants on parasitic protozoa, monitoring their mode of acquisition, and characterization of their complementary binding partners in host cells will provide insight into the etiopathogenesis of protozoal diseases. The emerging role of sialoglycoconjugates in parasitic protozoa is now a major focus of attention primarily due to their wide influence on biological functions, consequently having important therapeutic implications [67]. Therefore, identification of host receptors for parasite sialoglycans and probing the detailed mechanism of interaction is the challenge for the future. Molecular cloning and understanding the function of genes regulating enzymes such as trans-sialidases in *Trypanosoma* would have profound influence in developing new chemotherapeutic approaches to combat these protozoan infections. Continuing to further our understanding of sialoglycans in parasitic protozoa will help to foster innovative new strategies for diminishing the mortality and morbidity caused by these parasites.

Acknowledgments

The work was supported by the Department of Biotechnology and the Indian Council of Medical Research, Govt. of India. Anil Kumar Chava is a Senior Research Fellow of Council of Scientific and Industrial Research, Govt. of India.

References

1 Schauer R, Achievements and challenges of sialic acid research, *Glycoconj J* **17**, 485–99 (2000).
2 Kelm S, Schauer R, Sialic acids in molecular and cellular interactions, *Int Rev Cytol* **175**, 137–240 (1997).
3 Angata T, Varki A, Chemical diversity in the sialic acids and related-keto acids: An evolutionary perspective, *Chem Rev* **102**, 439–69 (2002).
4 Herwaldt BL, Infectious diseases, protozoal infections. In *Harrison's principles of internal medicine*, 14th edition, edited by Fauci S, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL (McGraw Hill Publishers, New York, 1998), pp. 1176–205.
5 Schenkman S, Eichinger D, Pereira ME, Nussenzweig V, Structural and functional properties of *Trypanosoma* trans-sialidase, *Annu Rev Microbiol* **48**, 499–23 (1994).
6 Frasch AC, Functional diversity in the trans-sialidase and mucin families in *Trypanosoma cruzi*, *Parasitol Today* **16**, 282–6 (2000).
7 Burleigh BA, Andrews NW, The mechanisms of *Trypanosoma cruzi* invasion of mammalian cells, *Annu Rev Microbiol* **49**, 175–200 (1995).
8 Ferguson MA, The structure, biosynthesis and functions of glycosylphosphatidylinositol anchors, and the contributions of trypanosome research, *J Cell Sci* **112**, 2799–809 Review (1999).
9 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, Identification and characterization of adsorbed serum sialoglycans on *Leishmania donovani* promastigotes, *Glycobiology* **13**, 351–61 (2003).
10 Mandal C, Basu S, A unique specificity of a sialic acid binding lectin Achatinin-H, from the haemolymph of *Achatina fulica* snail, *Biochem Biophys Res Commun* **148**, 795–801 (1987).

11 Mandal C, Basu S, Mandal C, Physicochemical studies on Achatinin-H, a novel sialic acid binding lectin, *Biochem J* **257**, 65–71 (1989).

12 Mandal C, Mandal C, Sialic acid binding lectins, *Experientia* **46**, 433–41 (1990).

13 Chayen A, Avron B, Nuchamowitz Y, Mirelman D, Appearance of sialoglycoproteins in encysting cells of *Entamoeba histolytica*, *Infect Immun* **56**, 673–81 (1988).

14 Ribeiro S, Alviano CS, Silva-Filho FC, da Silva EF, Angluster J, de Souza W, Sialic acid is a cell surface component of *Entamoeba invadens* trophozoites, *Microbios* **57**, 121–9 (1989).

15 Avron B, Chayen A, Stolarsky T, Schauer R, Reuter G, Mirelman D, A stage-specific sialoglycoprotein in encysting cells of *Entamoeba invadens*, *Mol Biochem Parasitol* **25**, 257–66 (1987).

16 Motta MC, Soares MJ, de Souza W, Intracellular lectin binding sites in symbiont-bearing *Crithidia* species, *Parasitol Res* **79**, 551–8 (1993).

17 Matta MA, Aleksitch V, Angluster J, Alviano CS, De Souza W, Andrade AF, Esteves MJ, Occurrence of *N*-acetyl- and *N*-*O*-diacetylneuraminic acid derivatives in wild and mutant *Crithidia fasciculate*, *Parasitol Res* **81**, 426–33 (1995).

18 do Valle Matta MA, Sales Alviano D, dos Santos Silva Couceiro JN, Nazareth M, Meirelles L, Sales Alviano C, Angluster J, Cell-surface sialoglycoconjugate structures in wild-type and mutant *Crithidia fasciculate*, *Parasitol Res* **85**, 293–99 (1999).

19 Schauer R, Wember M, Howard RJ, Malaria parasites do not contain or synthesize sialic acids, *Hoppe Seylers Z Physiolchem* **365**, 185–94 (1984).

20 Miller LH, Hudson D, Haynes JD, Identification of *Plasmodium knowlesi* erythrocyte binding proteins, *Mol Biochem Parasitol* **31**, 217–22 (1988).

21 Orlandi PA, Klotz FW, Haynes JD, A malaria invasion receptor, the 175-kilodalton erythrocyte-binding antigen of *Plasmodium falciparum* recognizes the terminal Neu5Ac (alpha 2-3)Gal-sequences of glycophorin A, *J Cell Biol* **116**, 901–9 (1992).

22 Thompson JK, Triglia T, Reed MB, Cowman AF, A novel ligand from *Plasmodium falciparum* that binds to a sialic acid-containing receptor on the surface of human erythrocytes, *Mol Microbiol* **41**, 47–58 (2001).

23 Gross U, Hambach C, Windeck T, Heesemann J, *Toxoplasma gondii*: Uptake of fetuin and identification of a 15-kDa fetuin-binding protein, *Parasitol Res* **79**, 191–4 (1993).

24 Dias Filho BP, Alviano CS, de Souza W, Angluster J, Polysaccharide and glycolipid composition in *Tritrichomonas foetus*, *Int J Biochem* **20**, 329–35 (1988).

25 Babal P, Pindak FF, Russell LC, Gardner WA Jr, Sialic acid-specific lectin from *Tritrichomonas foetus*, *Biochim Biophys Acta* **1428**, 106–16 (1999).

26 Dias Filho BP, Andrade AF, de Souza W, Esteves MJ, Angluster J, Cell surface saccharide differences in drug-susceptible and drug-resistant strains of *Trichomonas vaginalis, Microbios* **71**, 55–64 (1992).

27 Schauer R, Reuter G, Muhlpfordt H, Andrade AF, Pereira ME, The ocurrrence of *N*-acetyl and *N*-glycolylneuraminic acid in *Trypanosoma cruzi*, *Hoppe Seylers Z Physiol Chem* **364**, 1053–7 (1983).

28 Colli W, Trans-sialidase: A unique enzyme activity discovered in the protozoan *Trypanosoma cruzi*, *FASEB J* **7**, 1257–64 (1993).

29 Basu S, Basu M, Basu SS, Biological specificity of sialyltransferases. In *Biology of the sialic acid*, edited by Rosenberg A (Plenum Press, New York, 1995), pp. 68–94.

30 Basu S, Das K, Basu M, Glycosyltransferases in glycosphingolipid biosynthesis, In *Oligosaccharides in chemistry and biology-A comprehensive handbook*, edited by Ernst B, Sinay P, Hart G (Wiley-VCH Verlag GmbH, Germany, 2000), pp 329–47.

31 Vandekerckhove F, Schenkman S, Pontes de Carvalho L, Tomlinson S, Kiso M, Yoshida M, Hasegawa A, Nussenzweig V, Substrate specificity of the *Trypanosoma cruzi* trans-sialidase, *Glycobiology* **2**, 541–8 (1992).

32 Wilson JC, Kiefel MJ, Albouz-Abo S, von Itzstein M, Preliminary 1H NMR investigation of sialic acid transfer by the trans-sialidase from *Trypanosoma cruzi*, *Bioorg Med Chem Lett* **10**, 2791–4 (2000).

33 Todeschini AR, da Silveira EX, Jones C, Wait R, Previato JO, Mendonca-Previato L, Structure of *O*-glycosidically linked oligosaccharides from glycoproteins of *Trypanosoma cruzi* CL-Brener strain: Evidence for the presence of *O*-linked sialyl-oligosaccharides, *Glycobiology* **11**, 47–55 (2001).

34 Buschiazzo A, Amaya MF, Cremona ML, Frasch AC, Alzari PM, The crystal structure and mode of action of trans-sialidase, a key enzyme in *Trypanosoma cruzi* pathogenesis, *Mol Cell* **10**, 757–68 (2002).

35 Mehlert A, Zitzmann N, Richardson JM, Treumann A, Ferguson MA, The glycosylation of the variant surface glycoproteins and procyclic acidic repetitive proteins of *Trypanosoma brucei*, *Mol Biochem Parasitol* **91**, 145–52 (1998).

36 Ferguson MA, Murray P, Rutherford H, McConville MJ, A simple purification of procyclic acidic repetitive protein and demonstration of a sialylated glycosyl-phosphatidylinositol membrane anchor, *Biochem J* **291**, 51–5 (1993).

37 Engstler M, Reuter G, Schauer R, The developmentally regulated trans-sialidase from *Trypanosoma brucei* sialylates the procyclic acidic repetitive protein, *Mol Biochem Parasitol* **61**, 1–13 (1993).

38 Montagna G, Cremona ML, Paris G, Amaya MF, Buschiazzo A, Alzari PM, Frasch AC, The trans-sialidase from the african trypanosome *Trypanosoma brucei*, *Eur J Biochem* **269**, 2941–50 (2002).

39 Tiralongo E, Martensen I, Grotzinger J, Tiralongo J, Schauer R, Trans-sialidase-like sequences from *Trypanosoma congolense* conserve most of the critical active site residues found in other trans-sialidases, *Biol Chem* **384**, 1203–13 (2003).

40 Tiralongo E, Schrader S, Lange H, Lemke H, Tiralongo J, Schauer R, Two trans-sialidase forms with different sialic acid transfer and sialidase activities from *Trypanosoma congolense*, *J Biol Chem* **278**, 23301–10 (2003).

41 Pontes de Carvalho LC, Tomlinson S, Nussenzweig V, *Trypanosoma rangeli*; lacks trans-sialidase activity, *Mol Biochem Parasitol* **62**, 19–25 (1993).

42 Chava AK, Chatterjee M, Gerwig GJ, Kamerling JP, Mandal C, Identification of sialic acids on *Leishmania donovani* amastigotes, *Biol Chem* **385**, 59–66 (2004).

43 Schenkman S, Jiang MS, Hart GW, Nussenzweig V, A novel cell surface trans-sialidase of *Trypanosoma cruzi* generates a stage-specific epitope required for invasion of mammalian cells, *Cell* **65**, 1117–25 (1991).

44 Schenkman S, Kurosaki T, Ravetch JV, Nussenzweig V, Evidence for the participation of the Ssp-3 antigen in the invasion of non-phagocytic mammalian cells by *Trypanosoma cruzi*, *J Exp Med* **175**, 1635–41 (1992).
45 Frevert U, Schenkman S, Nussenzweig V, Stage-specific expression and intracellular shedding of the cell surface trans-sialidase of *Trypanosoma cruzi*, *Infect Immun* **60**, 2349–60 (1992).
46 Pereira-Chioccola VL, Acosta-Serrano A, Correia de Almeida I, Ferguson MA, Souto-Padron T, Rodrigues MM, Travassos LR, Schenkman S, 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**, 1299–307 (2000).
47 Costa F, Franchin G, Pereira-Chioccola VL, Ribeirao M, Schenkman S, Rodrigues MM, Immunization with a plasmid DNA containing the gene of trans-sialidase reduces *Trypanosoma cruzi* infection in mice, *Vaccine* **16**, 768–74 (1998).
48 Belen Carrillo M, Gao W, Herrera M, Alroy J, Moore JB, Beverley SM, Pereira MA, Heterologous expression of *Trypanosoma cruzi* trans-sialidase in *Leishmania major* enhances virulence, *Infect Immun* **68**, 2728–34 (2000).
49 Leguizamon MS, Mocetti E, Garcia Rivello H, Argibay P, Campetella O, Trans-sialidase from *Trypanosoma cruzi* induces apoptosis in cells from the immune system *in vivo*, *J Infect Dis* **180**, 1398–402 (1999).
50 Todeschini AR, Girard MF, Wieruszeski JM, Nunes MP, DosReis GA, Mendonca-Previato L, Previato JO, Trans-Sialidase from *Trypanosoma cruzi* binds host T-lymphocytes in a lectin manner, *J Biol Chem* **16**, 45962–8 (2002).
51 Argibay PF, Di Noia JM, Hidalgo A, Mocetti E, Barbich M, Lorenti AS, Bustos D, Tambutti M, Hyon SH, Frasch AC, Sanchez DO, *Trypanosoma cruzi* surface mucin TcMuc-e2 expressed on higher eukaryotic cells induces human T cell anergy, which is reversible, G *lycobiology* **12**, 25–32 (2002).
52 Andrews NW, Living dangerously: How *Trypanosoma cruzi* uses lysosomes to get inside host cells, and then escapes into the cytoplasm, *Biol Res* **26**, 65–7 (1993).
53 Monteiro VG, Soares CP, de Souza W, Host cell surface sialic acid residues are involved on the process of penetration of *Toxoplasma gondii* into mammalian cells, *FEMS Microbiol Lett* **164**, 323–27 (1998).
54 Sharma V, Chatterjee M, Mandal C, Basu D, Sen S, Rapid diagnosis of visceral leishmaniasis using Achatinin H, a 9-*O*-acetyl sialic acid binding lectin, *Am J Trop Med Hyg* **58**, 551–4 (1998).
55 Anil Kumar Ch, Chatterjee M, Sundar S, Mandal C, Development of an assay for quantification of linkage-specific *O*-acetylated sialoglycans on erythrocytes; its application in Indian visceral leishmaniasis, *J Immunol Methods* **270**, 1–10 (2002).
56 Bandyopadhyay S, Chatterjee M, Sundar S, Mandal C, Identification of 9-*O*-acetylated sialoglycans on peripheral blood mononuclear cells in Indian visceral leishmaniasis, *Glycoconj J* (2004) (in press).
57 Varki A, Kornfeld S, An autosomal dominant gene regulates the extent of 9-*O*-acetylation of murine erythrocyte sialic acids. A probable explanation for the variation in capacity to activate the human alternate complement pathway, *J Exp Med* **152**, 532–44 (1980).
58 Sharma V, Chatterjee M, Sen G, Ch. Anil Kumar, Mandal C, Role of linkage specific 9-*O*-acetylated sialoglycoconjugates in activation of the alternate complement pathway in mammalian erythrocytes, *Glycoconj J* **17**, 886–93 (2000).
59 Chava AK, Chatterjee M, Sharma V, Sundar S, Mandal C, Differential expression of *O*-acetylated sialoglycoconjugates induces a variable degree of complement-mediated hemolysis in Indian leishmaniasis, *J Infect Dis* (2004) (in press).
60 Mandal C, Chatterjee M, Sinha D, Investigation of 9-*O*-acetylated sialoglycoconjugates in childhood Acute Lymphoblastic leukaemia, *Br J Haematol* **110**, 801–12 (2000).
61 Pal S, Chatterjee M, Bhattacharya DK, Bandhyopadhyay S, Mandal C, Identification and purification of cytolytic antibodies directed against *O*-acetylated sialic acid in childhood acute lymphoblastic leukemia, *Glycobiology* **10**, 539–49 (2000).
62 Chatterjee M, Sharma V, Sundar S, Sen S, Mandal C, Identification of antibodies directed against *O*-acetylated sialic acids in visceral leishmaniasis: Its diagnostic and prognostic role, *Glycoconj J* **15**, 1141–7 (1998).
63 Klotz FW, Orlandi PA, Reuter G, Cohen SJ, Haynes JD, Schauer R, Howard RJ, Palese P, Miller LH, Binding of *Plasmodium falciparum* 175-kilodalton erythrocyte binding antigen and invasion of murine erythrocytes requires *N*-acetylneuraminic acid but not its *O*-acetylated form, *Mol Biochem Parasitol* **51**, 49–54 (1992).
64 Kneipp LF, Andrade AF, de Souza W, Angluster J, Alviano CS, Travassos LR, *Trichomonas vaginalis* and *Tritrichomonas foetus*: Expression of chitin at the cell surface, *Exp Parasitol* **89**, 195–204 (1998).
65 Babal P, Russell LC, Sialic acid-specific lectin-mediated adhesion of *Tritrichomonas foetus* and *Tritrichomonas mobilensis*, *J Parasitol* **85**, 33–40 (1999).
66 Leitch GJ, Dickey AD, Udezulu IA, Bailey GB, *Entamoeba histolytica* trophozoites in the lumen and mucus blanket of rat colons studied *in vivo*, *Infect Immun* **47**, 68–73 (1985).
67 Sinha D, Chatterjee M, Mandal C, *O*-acetylated sialic acids—Their detection biological significance and alteration in diseases, *TIGG* **12**, 17–33 (2000).