Sialoglycotherapeutics in Protozoal Diseases

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Abstract: The manipulation of glycosylation, mainly sialylation, holds enormous potential for understanding the biological functions of glycoproteins and glycolipids to treat many diseases. The existing knowledge in the field of glycobiology is exploited by glycotherapeutics for combating protozoan diseases. This review focuses on the development of novel glycobiological therapeutic strategies in the field of protozoan infections.

Key Words: Anti-*O*-acetylated sialoglycoprotein specific antibodies, anti-α-gal antibodies, carbohydrate engineering, drug delivery, engineering inhibitors of glycoproteins, glycotherapeutics, glycovaccine, immunotoxin, metabolite oligosaccharide engineering, oligosaccharide engineering, parasitic diseases, sialic acid engineering, sialoglycotherapeutics, trans-sialidase.

1. INTRODUCTION

According to the World Health Organization Tropical Disease Research 2005-report (http://www.who.int/tdr/publications/publications/pdf/pr17/pr17.pdf), protozoan diseases threaten a large section of the world's population. The growing number of newer drug resistant protozoal parasite strains is the biggest challenge in fighting these deadly infections. Therefore, the search for new drug targets along with synthesis of newer anti-infective agents for effective therapy of these infectious diseases is of primary research interest. Proteomics, genomics and glycomics have opened up a new era of parasitological research wherein the possible application of our knowledge could be used for the development of better and effective drugs for proper disease management.

Carbohydrates are the major constituents of the human cell surface. The structures of these cell surface carbohydrates display a dramatic change during host parasite interaction. Cell surface carbohydrates thus serve as a zip code for different types of infection. Considering the many functions of glycosylation, it is not surprising that abnormalities are intimately associated with many diseases and protozoal diseases are not an exception. Thus, manipulation of glycosylation holds enormous potential for understanding the biological functions of glycoproteins and glycolipids as well as for treating diseases. The contributions of cell surface oligosaccharides in critical biological processes are now being understood in significant molecular detail. These discoveries, at the forefront of biological research, have motivated the design of newer synthetic glycoconjugates as tools for the fundamental study of glycobiology and as candidates for future generations of therapeutic and pharmaceutical reagents [1].

Thus a new field of science, "Glycotherapeutics", has emerged which aims to apply this newly acquired information on the role of carbohydrates in parasitic infections and promises to be a potential strategy for combating such diseases more efficiently and effectively. The various approaches in glycotherapeutics include designing of (1) novel analogues to inhibit critical enzyme activities, (2) engineering of metabolite oligosaccharides, (3) antibodies against cell surface glycotopes crucial for establishment of infection and (4) the synthesis of unique analogues to inhibit host pathogen interactions.

Among the known carbohydrates, sialic acids due to their strategic terminal position can participate in critical cell recognition events and hence are potential target molecules. Thus, engineering of these molecules by introducing various analogues or modifying their metabolic pathways appears to be one of the ways of modulating their biological roles.

2. SIALIC ACID AND ITS BIOSYNTHESIS

Sialic acid, belong to a family of 9-carbon carboxylated monosaccharides and are the most abundant monosaccharide present as terminal residues of vertebrate cell surface sugar chains. Of the nine monosaccharides that constitute mammalian polysaccharides, sialic acid stands out as a major determinant. Sialic acid and its 50 different derivatives are known to play a significant role in the mediation of many biological phenomena involving cell-cell and cell-matrix interactions by either reacting with specific surface receptors or masking other carbohydrate recognition sites [2, 3].

N-acetylneuraminic acid (Neu5Ac) is the parent molecule of all the sialic acid derivatives. It is synthesized with the help of a crucial regulatory enzyme UDP-*N*-acetylglucosamine (UDP-GlcNAc) epimerase, which converts glucose to *N*-acetylmannosamine-6-phosphate in the cytosol. Further condensation of phosphoenol-pyruvate with *N*-acetylmannosamine-6-phosphate forms 5-*N*-acetyl 9-*O*-phosphoro neuraminic acid (Neu5Ac9P). After dephosphorylation, the free monosaccharide is activated to the CMP-glycoside in the nucleus. En route, CMP-Neu5Ac can be modified to CMP-Neu5Gc (glycolyl derivative of sialic acid) in the cytosol by CMP-Neu5Ac hydroxylase. CMP-Neu5Ac and CMP-Neu5Gc are then transported by a specific carrier into the Golgi appa-

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ratus, where they are transferred by sialyltransferases onto nascent glycoproteins and glycolipids [4]. The fine-tuning of expression of cell surface sialylation depends on the balance of sialyltransferases along with sialidases (Fig. **1**).

Subsequently, the sialoglycoconjugates generated may be further modified by *O*-acetyl transferases. These enzymes transfer the acetyl group from Coenzyme A onto sialoglycoconjugates at the C-7, 8, 9 positions, forming *O*-acetylated sialoglycoconjugates. Accordingly, the different derivatives of sialic acids may be designated as Neu5,9Ac_2 (9-*O*AcSA) or Neu5,4Ac_2 etc. Amongst over 40 – 50 diverse structural modifications of this sugar, the most common are *O*-acetyl substitutions [5]. As *O*-acetyl esters at the C-7 position are known to migrate to the C-9 position, 9-*O*acetylated sialic acids usually predominate on cell surface glycoproteins generating a family of 9-*O*acetylated sialoglycoproteins [6, 7]. Once attached these *O*-acetyl ester groups need to be removed at some point in the life cycle of the parent molecule. The enzymes involved in this process are the 9-*O*acetyl esterases followed by sialidases [8, 9]. Taken together, it appears that the expression of 9-*O*acetylation is dependant on the balance between sialyltransferases and *O*-acetyl tranferases at one end and the 9-*O*-acetyl esterases and sialidases at the other [10].

3. GLYCOCONJUGATES IN PROTOZOA

Glycotopes present on the protozoal surface have been demonstrated to play an important role during the establishment of infection (Table **1**). In protozoan parasites, glycosylation occurs through one of four major post-translational

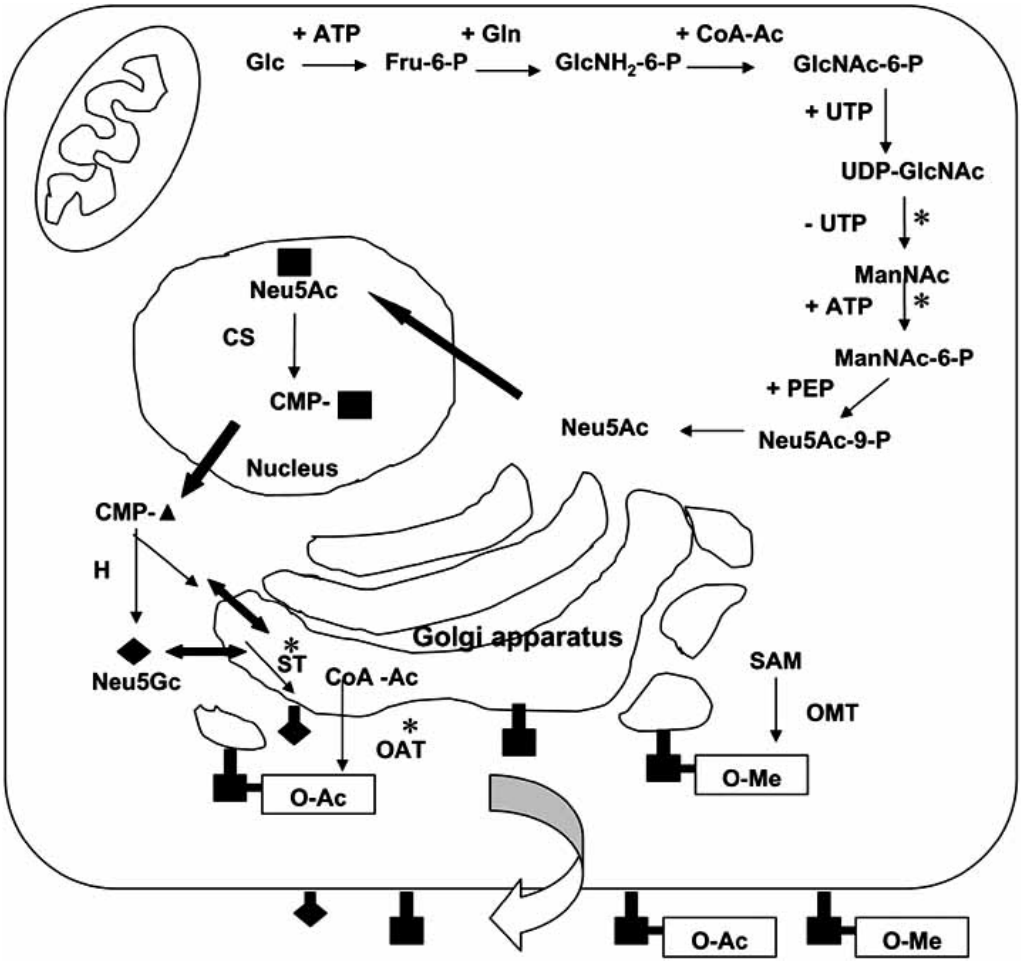


Fig. (1). A schemetic representation of sialic acid biosynthesis.

The enzymatic reactions involved in sialic acid (Neu5Ac) biosynthesis, activation, transfer and modification are shown with their intracellular localization [4]. The asterisk (*) shows probable sites for glycoengineering by suitable methods as mentioned in text.

The enzymes and co substrates are shown as: Adenosine triphosphate (**ATP**), cytidine monophosphate (**CMP**), uridine diphosphate (**UDP**), uridine triphosphate (**UTP**), CMP-Neu5Ac synthase (**CS**), CMP-Sia transporter (**CT**), sialyltransferase (**ST**), acetyl coenzyme A (**AcCoA**), S-adenosylmethionine (**SAM**), *O*-acetyltransferase (**OAT**), hydroxylase (**H**), *O*-methyltransferase (**OMT**).

The carbohydrate structures: glucose (Glc), glutamine (Gln), fructose-6-phosphate (Frc-6-P), glucosamine-6-phosphate ($GlcNH_2$-P), *N*-acetylglucose-6-phosphate (GlcNAc-6-P), UDP-*N*-acetylglucosamine (UDP-GlcNAc), *N*-acetylmannosamine (ManNAc), *N*-acetymannosamine-6-phosphate (ManNAc-6-P), phosphoenol pyruvate (PEP), 5-*N*-acetyl 9-*O*-phosphoro neuraminic acid (Neu5Ac-9-P), sialic acid (Neu5Ac, ■), CMP-*N*-acetylneuraminic acid (CMP-■), *N*-glycolylneuraminic acid (Neu5Gc, ◆), 8-*O*-methylated sialic acid (**O—**Me), *O*-acetylated sialic acid (**O—***O*-Ac), nascent glycoconjugate (▟).

Table 1. Protozoan-Specific Glycoconjugates: Nature, Characteristic Motif and Role

Protozoan parasite	Glycoconjugate/Glycotope	Characteristic motif (glycotope)	Role
Leishmania sp. [23, 24]	LPG	Glycan core/phosphorylated disaccharide repeat unit backbone	Virulence factor
	PPG	Manα1-PO_3-Ser	parasite attachment and invasion in macrophages
L. donovani Promastigotes [26]	Sialoglycoproteins (kDa) 123, 90 and 70 130, 117 and 70 123 and 109	Neu5Acα2-6Gal/GalNAc Neu5Acα2-3Galβ1-4GlcNAc Neu5,9$Ac_2\alpha$2-6GalNAc	possible virulence factor
Amastigotes [27]	164 and 150 188,162,136,137 and 124 158 and 150.	Neu5Acα2-6Gal/GalNAc Neu5Acα2-3Galβ1-4GlcNAc Neu5,9$Ac_2\alpha$2-6GalNAc	possible survival factor
Entamoeba histolytica [31-33]	Sialoglycoproteins (kDa) 100 and 150	Neu5Ac	helps in encystation
	PPG PPG	GPI containing α1,2Gal*p* Galα1-PO_3-Ser	virulence factor, prevents trophozoite adherence and cytolysis
	A lectin	Gal/GalNAc	regulates host cell adhesion and cytolysis
Plasmodia [30]	EBA-175	Neu5Acα2-3 Gal	establishes infection, binds to glycophorin on erythrocyte of host
Typanosoma cruzi [29]	GIPL	GPI containing 2-aminoethylphosphonate and galactofuranose substituents	Role in immunostimulatory action and survival
	sialoglycoprotein (kDa) 30 and 50 kDa	Neu5Ac	participate in attachment to mammalian cell
	sialoglycoprotein Mucin	*O*-glycan containing galacto furanose	parasite attachment to cells and alters immune cell function to enhance parasitism.

The references are given in square brackets

modifications like (i) *N*-glycosidic, between *N*-acetylglucosamine (GlcNAc) and the amide group of asparagine (ubiquitously expressed in nature); (ii) *O*-glycosidic, between GlcNAc and the hydroxyl group of threonine; (iii) phosphoglycosidic, through a phosphodiester bond between a galactose (Gal) or mannose residue; and (iv) the major protozoan protein post-translational modification, known as a glycosylphosphatidylinositol (GPI) anchor formed between the C-terminal residue of the protein (mediated by the hydroxyl group of serine *via* ethanolamine phosphate) and an oligosaccharide attached to phosphatidylinositol [11].

Distribution of various types of glycoconjugates in different parasites has been well documented that are intimately associated with the disease pathology. In some parasites, such as *Leishmania*, the dense glycocalyx is rich in GPI-linked carbohydrate and glycoinositolphospholipid (GIPL) [12, 13]. Additionally, lipophosphoglycan (LPG) constituting a species-dependent mixture of GIPL and members of a secreted family of heavily glycosylated proteins known as the proteophosphoglycans (PPG) are also essential components of the glycocalyx [14-23]. Although Leishmania LPG is an essential component of glycocalyx, and its role in virulence of *Leishmania mexicana* is still a subject of debate [24]. The composition of the glycocalyx varies with species and developmental stage of the parasite, which confer additional advantages for them.

A substantial amount of work in the field of sialoglycobiology has been reported from the authors' lab, which described the identification and characterization of different 9-*O*acetylated sialoglycoproteins in both promastigote and amastigote forms of *L. donovani* (Table **1**), [26, 27].

All African trypanosomes are covered by a GPI-anchored variant surface glycoprotein (VSG). This glycoprotein possesses *N*-linked carbohydrates containing oligomannose chains containing terminal α-galactopyranose (α-Galp) units [28, 29]. Protein glycosylation in *Plasmodium sp.* is exclusively *via* the attachment of a GPI anchor [30]. The surface of *E. histolytica* trophozoites expresses two major types of molecules: the Gal/GalNAc lectin and a PPG. The Gal/GalNAc lectin is a complex molecule involved in regulating host cell adhesion and cytolysis [31-33].

These essential glycoconjugates in parasites can thus possibly serve as candidates for drug targets or even for the development of diagnostic assays. As it is well established that glycotopes mediate adhesion between host cells and pathogens, synthetic analogues engineered to mimic such glycotopes could possibly be used to prevent this adhesion and subsequent infection.

4. BEGINNING OF A NEW ERA – ALTERING THE CELL SURFACE CARBOHYDRATES THROUGH ENGINEERING

The terminal position of sialic acid allows it to interact with the environment of cells and organisms. In general, protozoan infections show upregulation of glycosylation and sialylation in both host and the pathogen. These sugars greatly affect the disease pathology. This "battle" is fought in conjunction with carbohydrate moieties bearing sialic acid residues. Hence "carbohydrate engineering" or specifically sialic acid engineering has come in vogue as a future therapeutic and diagnostic application.

"Sialic acid engineering" refers to the strategy where cell surface carbohydrates are modified by the biosynthetic incorporation of metabolic intermediates. Current sialic acid engineering efforts are directed primarily at changing the bulk properties of cell surface carbohydrates in order to influence cellular responses. Bulk modification of surface sialic acids has also shown promise for use in biomedical applications such as inhibition of viral binding, modulation of the immune system and the selective delivery of diagnostic or therapeutic agents [34].

Carbohydrate or sialic acid engineering may broadly encompass (1) the development of enzyme inhibitors responsible for the fine tuning of sialic acid, (2) metabolite oligosaccharide engineering, (3) engineered monoclonal antibodies against specific parasite glycoproteins, (4) synthesis of unique inhibitors of glycoproteins to inhibit host pathogen interactions and (iv) glycoconjugates as new vaccine candidates, which are critically important for developing tools to investigate the cellular activity of glycans delineating the molecular basis for aberrant glycosylation in parasitic diseases.

4.1. Development of Enzyme Inhibitors

Expression of surface terminal sialic acid and its derivatives is due to the interplay between several enzymes, namely sialidase, sialyltransferase, *O*-acetyl transferase and *O*-acetyl esterase. The variation of sialic acid and its *O*-acetylation are of great importance in different pathological conditions [35]. The altered expression of *O*-acetylated sialoglyconjugates in several parasitic infections opens up new areas of research directed towards exploiting this expression as a disease index for evaluating disease status [35-41].

The status of several enzymes both in the pathogen and the host cells may be direct targets for development of suitable therapeutic inhibitors. As *O*-acetylation depends on *O*-acetyl transferases and 9-*O*-acetyl esterases, designing suitable inhibitors for the respective enzymes appears to be a novel proposition. These inhibitors would block the metabolic pathway for the synthesis of parasite specific disease-associated glycotopes, which would essentially be of help in parasitic diseases where *O*-acetylation of sialic acid serves as a tool for diagnosis and prognosis like visceral leishmaniasis [10, 21-23, 25-27 and 36-41]. Over expression of 9-*O*-acetylated sialoglycoproteins has been demonstrated on both erythrocytes and peripheral blood mononuclear cells of patients with visceral leishmaniasis. These novel molecules serve as important biomarkers of the disease and subsequently they have been applied for the development of novel diagnostic assays [25].

The *de novo* chemical synthesis of sialic acid analogues is difficult and their production often relies on the use of the enzyme *N*-acetylneuraminic acid lyase (NAL). The rationale for engineering this enzyme involves the reversible aldol condensation of pyruvate with the open-chain form of *N*-acetylmannosamine (Man*N*Ac) followed by cyclization, which produces *N*-acetylneuraminic acid (Neu5Ac). Engineered or synthetically evolved enzyme finally condenses the aldehyde with the pyruvate to yield the dipropylamides (Fig. **2a** & **b**), the precursors of sialidase inhibitors like dipropylcarboxamides (Fig. **2c**). These engineering efforts provide a scaffold for the further tailoring of *N*-acetylneuraminic acid lyase for the synthesis of sialic acid mimetics [42]. Such studies open up a future avenue of glycoengineering that may be used for the therapeutic purposes of different parasitic diseases where the marked up regulation of sialidase is used as a marker of pathogenesis.

The scenario is quite different in trypanosomiasis, where a unique enzyme namely the trans-sialidase is present, which appears to be an important virulence factor. This enzyme mediates sialic acid transfer from the host thereby increasing the virulence of the parasite. Therefore, inhibitors of trans sialidase may possibly be critical in reducing the parasite burden in the host and can effectively control the infection. More recently, this idea led to the development of inhibitors in the form of lactose derivatives that are indeed potent in blocking the activity of *Trypanosoma cruzi* trans-sialidase toward conventional substrates *in vitro* and *in vivo* [43]. The lactose open chain derivative lactitol was found to be a good acceptor of sialic acid. Lactitol, 4-*O*-β-D-galactopyranosyl-

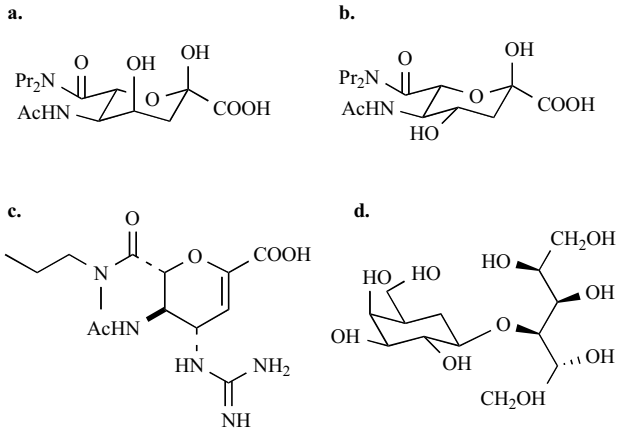
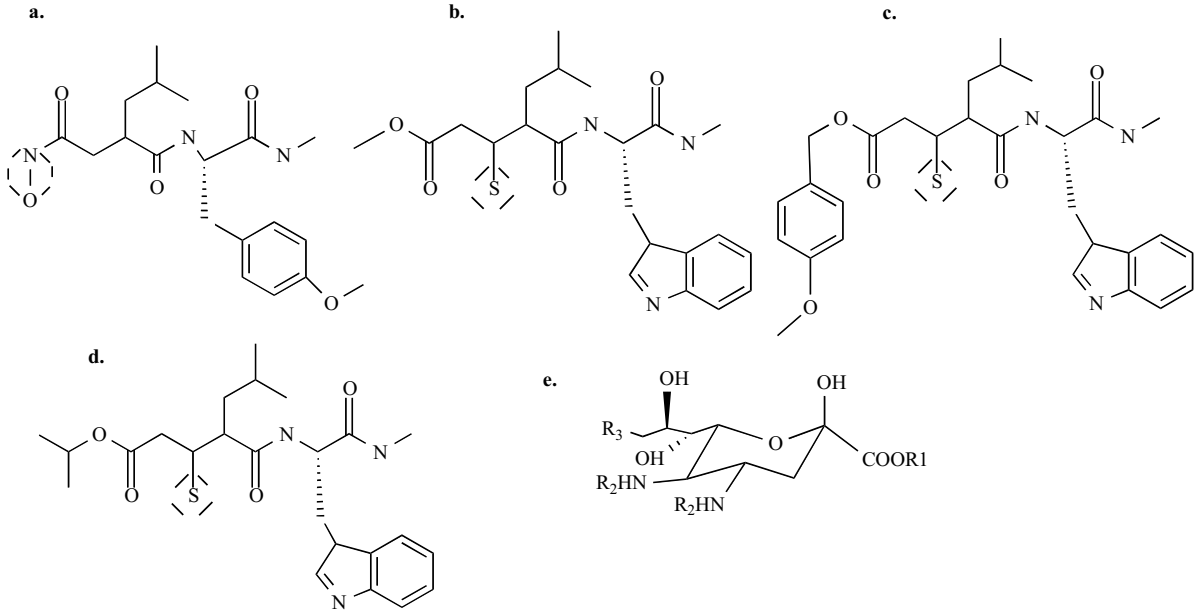


Fig. (2). Structures of axial **(a),** equatorial **(b)** isomers of dipropylamides, a sialidase inhibitor dipropylcarboxamide **(c)** and lactitol **(d),** an inhibitor of *Trypanosoma cruzi* trans-sialidase activity [42, 43].

D-glucitol, (Fig. **2d**) effectively inhibited the transfer of sialic acid to *N*-acetyllactosamine and repressed re-sialylation of parasite mucins when incubated with live trypanosomes and transialidase. Lactitol also diminished the *T. cruzi* infection in cultured cells by 20-27%.

Certain metalloprotease inhibitors are able to inhibit the release of VSG from cultured transgenic procyclic *Trypanosoma brucei*, confirming the identification of a cell surface zinc metalloprotease activity in this stage of the trypanosome lifecycle [44]. Four such compounds namely BRL29808, BRL49244, SB201140 and BRL57240 were found to have significant growth inhibitory activity in the low micromolar range (Fig. **3a**, **b**, **c** & **d**). Development of such higher affinity metalloprotease inhibitors may provide a novel avenue for the treatment of parasitic diseases.



f

Sialic acids	Substituents		
	R_1	R_2	R_3
NeuAc	H	CH_3CO	HO
9-deoxy-NeuAc	H	CH_3CO	H
9-amino-NeuAc	H	CH_3CO	H_2N
9-acetamido-NeuAc	H	CH_3CO	CH_3CO-NH
9-*N*-Gly-NeuAc	H	CH_3CO	H_2NCH_2CO-NH
9-*N*-Succ-NeuAc	H	CH_3CO	HOOC $(CH_2)_2$ CO-NH
9-iodo-NeuAc	H	CH_3CO	I
9-thio-NeuAc	H	CH_3CO	HS
9-SCH_3-NeuAc	H	CH_3CO	CH_3S
9-SO_2CH_3-NeuAc	H	CH_3CO	CH_3SO_2
5-*N*-fluoroac-Neu	H	FCH_2CO	HO
5-*N*-trifluoroac-Neu	H	CF_3CO	HO
5-*N*-Gly-Neu	H	H_2NCH_2CO	HO
5-*N*-Succ-Neu	H	HOOC $(CH_2)_2$ CO	HO
5-*N*-thioac-Neu	H	CH_3CS	HO
NeuAc-Me-ester	H_3C	CH_3CO	HO
NeuAc-Et-ester	H_5C_2	CH_3CO	HO

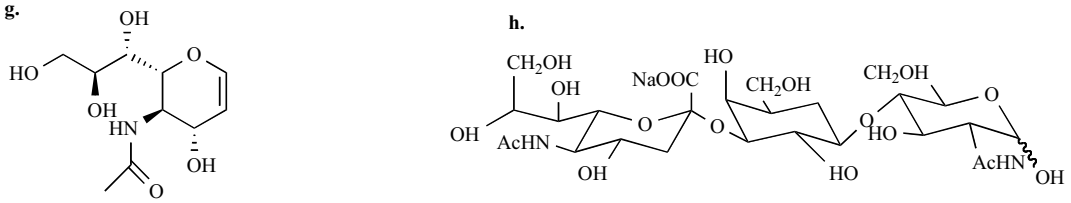


Fig. (3). Structures of different engineered inhibitors and synthetic sialic acid analogs.

Metalloprotease inhibitors [44] namely BRL29808 **(a)**, BRL49244 **(b)**, SB201140 **(c),** and BRL57240 **(d)** are shown. Stereochemistry has been indicated and dashed circles represent chelator moieties.

Structures of synthetic sialic acid analogues [54] used in the biosynthetic engineering of sialic acid is based on NeuAc **(e)** and all sialic acid analogs **(f)** used is substituted either in C-1 (R_1), C-5 (R_2), or C-9 (R_3) and chemical structure of **(g)** 2,3-didehydro-2-deoxy-*N*-acetyl neuraminic acid and **(h)** 3'-*N*-acetyl neuraminyl-*N*-acetyl lactosamine, the excellent inhibitors for the interaction between erythrocyte binding antigen 175 (EBA-175) with the erythrocyte receptor glycophorin A are shown [87].

4.2. Metabolite Oligosaccharide Engineering

Oligosaccharides influence the function of glycoproteins and glycolipids in many ways. Within the cell, oligosaccharides facilitate the folding of nascent proteins [45] and *O*-GlcNAc modification of cytoplasmic and nuclear proteins provides a dynamic signaling mechanism that complements phosphorylation [46]. On the cell surface, oligosaccharides usually extend through different branches to form a complex carbohydrate, which helps them to reach the other key molecules [47]. This 'carbohydrate code' helps to regulate many important functions in the health and disease [48-50]. Progress in manipulating carbohydrates began in the early 1980s when today's 'metabolic oligosaccharide engineering' methodology (Fig. **4**) originated from attempts to inhibit sialic acid production. These pioneering efforts were spurred by the recognition that altered glycosylation was a hallmark of malignancy with changes in sialic acid metabolism particularly implicated in carcinogenesis [51-53]. Most of the parasitic diseases are prevalent in the developing countries and are known as the 'diseases of the poor' and remain neglected. In diverse parasitic diseases glycosylation plays a significant role in the disease pathology hence, the application of oligosaccharide engineering would be an important tool in unraveling the disease biology. Metabolic oligosaccharide engineering thus presents a powerful method for imaging the localization, trafficking, and dynamics of glycans and isolating them for glyco-proteomic analysis.

4.2.1. Engineering of Sialic Acid

Biosynthetic engineering of sialic acid in living cells has been demonstrated using synthetic sialic acid analogues [54]. Sixteen synthetic sialic acid analogues carrying distinct C-1, C-5, or C-9 substitutions were added individually to cell cultures of which ten were readily taken up and incorporated. Uptake of C-5- and C-9-substituted sialic acids resulted in the structural modification of up to 95% of sialic acids on the cell surface (Fig. **3e** & **f**). It may be envisaged that similar analogs of sialic acid can also be used in case of parasitic

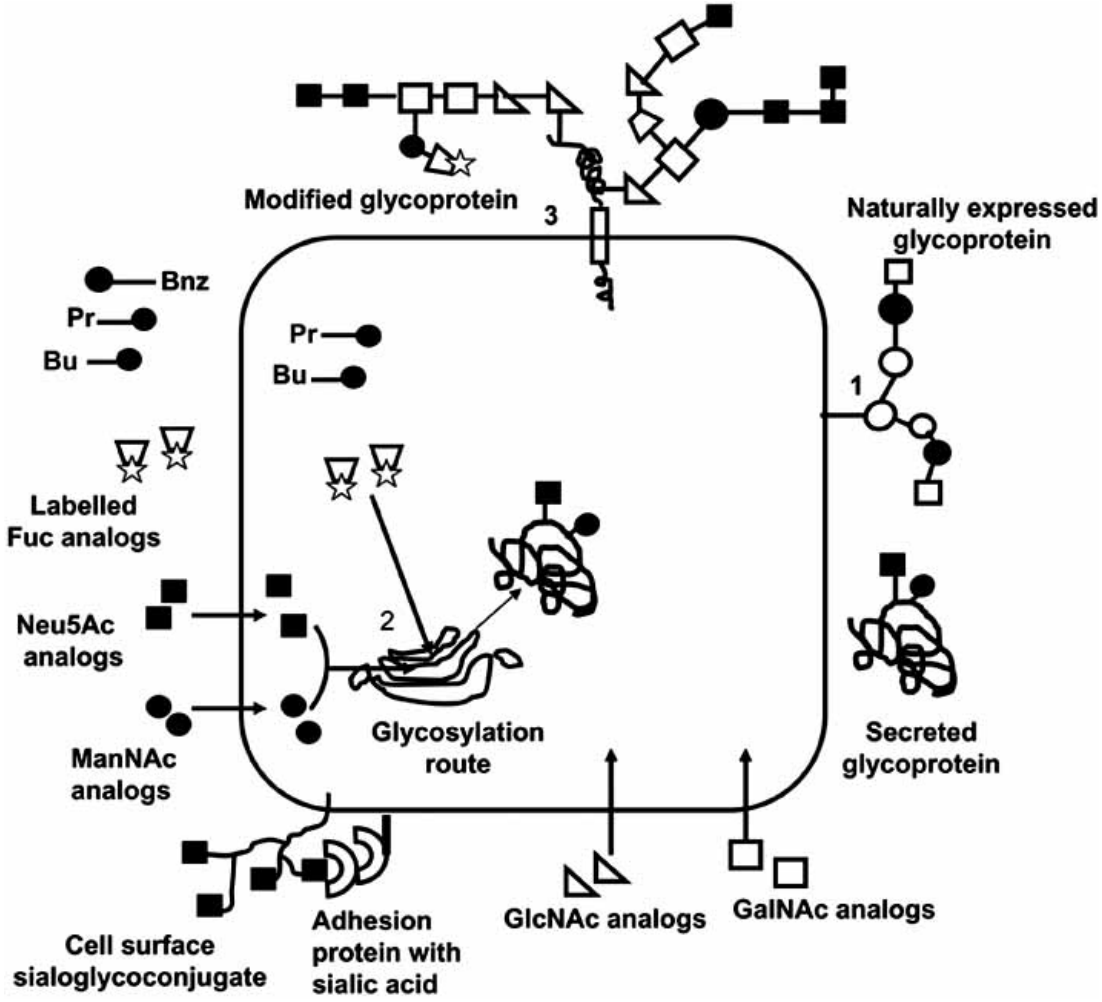


Fig. (4). Overview of cell surface oligosaccharide engineering.

Strategies for metabolic oligosaccharide engineering comprise monosaccharide analogs, including derivatives of sialic acid (Neu5Ac), *N*-acetylmannosamine (ManNAc), *N*-acetyl galactosamine (GalNAc), Fucose (Fuc), and *N*-acetylglucosamime (GlcNAc). Glycoengineering employs naturally expressed glycoproteins (1) to be modified by analogs through the different cellular glycosylation routes (2) as a result of which metabolically modified glycoproteins (3) are expressed on the cell surface. *N*-propionyl- (●—**Pr**) and *N*-butanoyl-mannosamine (●—**Bu**) and *N*-benzoyl mannosamine (●—**Bz**) are shown.

diseases to modify both host and pathogen glycoproteins that would probably affect the disease pathology.

4.2.2. Engineering of N-Acetylmannosamine Analogs

The precursor of most physiological sialic acids is *N*-acetyl-*D*-mannosamine. The ability to increase the repertoire of sialic acids on the cell surface *via N*-acetyl-*D*-mannosamine analogs, offers a means to modulate the natural biological functions of this sugar in novel ways. ManNAc analogs influence metabolic flux through the biosynthetic pathway and endow the cell surface with novel physical and chemical properties [55, 56].

4.2.3. Engineering of N-*Propanoyl and* N-*Butanoyl Mannosamine*

Novel *N*-propanoylmannosamine leads to the incorporation of new sialic acid, *N*-propanoylneuraminic acid; into cell surface glycoconjugates and has been applied to decrease polysialylation of the neural cell adhesion molecule [57]. *N*-propanoylmannosamine was taken up by the cells and efficiently metabolized to the respective *N*-acyl-modified sialic acid *in vitro* and *in vivo* [58]. Similarly, *N*-butanoylmannosamine was used to introduce unnatural modifications into cellular poly sialic acid. This has been applied in nervous system development and tumor vaccine studies [59].

This attractive protocol of incorporation of *N*-acetylmannosamine analogs can also be used in specific parasitic diseases where sugars are the principal gates for establishing infection and interiorization. In *T. cruzi,* the influence of carbohydrates on the adhesion to insect midgut was confirmed by inhibition of parasite attachment after midgut incubation with *N*-acetylgalactosamine, *N*-acetylmannosamine, *N*-acetylglucosamine, *D*-galactose, *D*-mannose or sialic acid [60].

4.2.4. Engineering of N-*Azidoacetylmannosamine*

Similarly, targeting of sialic acid as a host for azides by using *N*-azidoacetylmannosamine (ManNAz) as a biosynthetic precursor led to the metabolic conversion of ManNAz to *N*-azidoacetylsialic acid (SiaNAz) within membrane-bound and secreted glycoproteins (Fig. **5a**) as quantified in a variety of cell types [61].

Additionally, peracetylated *N*-α-azidoacetylmannosamine (Ac(4)ManNAz) is metabolized by cells to CMP-azidosialic acid. Exposure of cells to Ac(4)ManNAz allows *in vivo* chemical tagging of gangliosides based on the principle of the Staudinger ligation [62] which results in the formation of azidosialic acid-containing gangliosides [63].

4.2.5. Engineering of Alkynyl N-*Acetylmannosamine*

Chemoselectively labeled alkynyl sugar analogs based on sialic acid, fucose and *N*-acetylmannosamine have been used for visualization of glycoconjugates in cancer cells [64]. It may be envisaged that because these sugars generally exist as terminal glycan residues with a notably increased presence in both host and pathogen in parasitic infections, this approach would essentially promise to provide useful information about their roles in the disease pathology and ultimately can be of help for diagnostic and therapeutic purposes

4.2.6. Oligosaccharide Engineering and Modulation of Immune Response

Sialic acids are known to be involved in the differentiation and maturation of lymphocytes. Sialic acid engineering by application of *N*-propanoylmannosamine leads to an incorporation of Neu5Prop into human T-cells. These biochemically engineered T-cells show an increase in the peptidase-activity of CD26, a co stimulator of lymphocytes [65, 66]. Many parasitic diseases, like leishmaniasis, demonstrate severe immunosuppression. The successful recovery from infection due to *L. donovani* is associated with the development of life long immunity and resistance to re-infection [67]. The Th1/Th2 dichotomy, evidenced by interferon (IFN)-γ and interleukin (IL)-12, the signature cytokines for Th1 responses, are decreased during acute infection [68]. These responses persist at high levels after successful treatment and are accompanied by high IL-10 levels. Recently, IL-10 has been suggested to play a role in counterbalancing the exacerbated polarized response that may develop following cure [69]. The majority of people infected with *L. chagasi* or *L.donovani* have been shown to clear their infection spontaneously and develop protective immunity, characterized by lymphocyte proliferation and secretion of IFN-γ in response to antigen *in vitro*. Thus, it may be envisaged that stimulation of the immunosupressed T cells is possible by the aid of such biosynthetically modified sugars.

4.2.7. Oligosaccharide Engineering and Selective Drug Delivery

Cell surface oligosaccharides can be engineered to display unusual functional groups for the selective chemical remodeling of cell surfaces. An unnatural derivative of *N*-acetyl-mannosamine, *N*-levulinoyl mannosamine (ManLev) which has a ketone group, was converted to the corresponding sialic acid and incorporated into cell surface oligosaccharides metabolically, resulting in the cell surface display of ketone groups. The ketone group on the cell surface can then be covalently ligated under physiological conditions with molecules carrying a complementary reactive functional group such as the hydrazide (Fig. **5b** & **c**).

Cell surface reactions of this kind may have potential in the introduction of new recognition epitopes, such as peptides, oligosaccharides, or small organic molecules, onto cell surfaces and in the subsequent modulation of cell-cell or cell-small molecule binding events. The versatility of this technology has been demonstrated for selective drug delivery [70]. Cells were decorated with biotin through selective conjugation to ketone groups, and killed in the presence of a ricin A chain-avidin conjugate. It may be envisaged that similar kind of engineering may be possible for parasites for effective drug delivery.

4.3. ENGINEERED CARBOHYDRATE SPECIFIC ANTIBODIES IN PARASITIC DISEASES

The importance of antibodies is immense as they could be used as a tool to deliver toxins for killing parasites. Therefore, development of immunotoxins for targeted cytotoxic effects is an attractive alternative therapeutic concept. Hence, hybrid toxins may provide means of controlling dreadful parasitic diseases and counter morbidity as well as mortality.

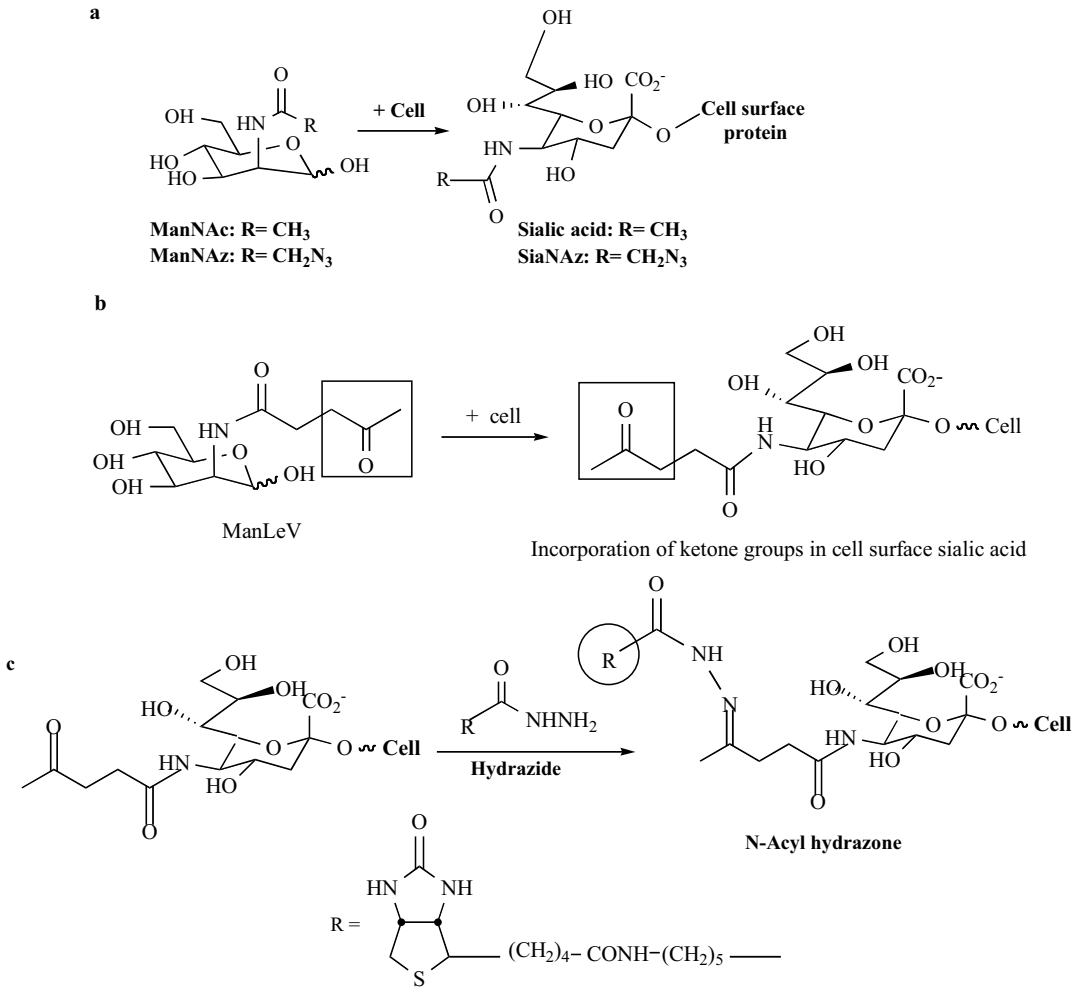


Fig. (5). Metabolic oligosaccharide engineering as a means to label glycoconjugates.

(a) ManNAc and its unnatural analog ManNAz converted into the corresponding sialic acids, and incorporated into cell surface glycoconjugates [61-63].

Biosynthetic incorporation of ketone groups into cell surface–associated sialic acid by **(b)** *N*-levulinoyl mannosamine (ManLev) that is metabolically converted to the corresponding cell surface sialoside and **(c)** display of ketone groups by chemoselectivel ligation to hydrazides through the formation of an acyl hydrazone [70].

The cytocidal effect of such hybrid molecules is expected to be highly specific and may require only minute doses. Therefore, this kind of approach may form a basis for the development of more effective therapeutic agents, as the need of new parasiticidal drugs is an urgent priority.

4.3.1. A Tool for Toxin Delivery

A single chain immunotoxin composed of a single chain antibody fragment directed to a protein on the surface of *Plasmodium berghei* ookinetes linked to a lytic peptide has been constructed [71]. The single-chain immunotoxin, expressed in *Escherichia coli*, was purified by a Ni-NTA column. This purified engineered immunotoxin exhibited optimum killing properties of *P. berghei* ookinetes demonstrating enhanced parasiticidal activity *in vitro*. This encouraging result may be extended by introducing genetically engineered bacteria into anopheline mosquitoes, which might offer a practical approach to the regulation of malaria transmission.

4.3.2. Anti-α-Galactosyl Antibodies

Sera of patients with chronic Chagas' disease (American trypanosomiasis) show elevated levels of anti-α-galactosyl antibodies and is used as a hallmark of active disease [72]. Antibodies to the core glycans of GIPL-2 are elevated in patients with *T. cruzi* and *T. rangeli*. As the antigen-antibody binding could be selectively blocked with Gal(α1-3)Gal for GIPL-2 antibodies, such reactive antibodies could efficiently be used in the development of serodiagnostic assay [73, 74].

The target molecules on cell-derived trypomastigotes react with this anti-α-galactosyl antibody. Moreover, these antibodies are lytic to metacyclic trypomastigotes in complement-mediated reactions. These results open the possibil-

ity of using anti-galactosyl antibodies for protection against infection by virulent *T. cruzi* [75].

4.3.3. Anti-O-Acetylated Sialoglycoprotein Specific Antibodies

An enhanced level of antibodies directed against *O*-acetylated sialoglycoproteins has been observed in patients suffering from visceral leishmaniasis. 9-*O*acetylated sialoglycoproteins have been identified and well characterized both in the promastigote (123 and 109 kDa) and amastigote (158 and 150 kDa) form of *L. donovani* [26, 27]. These sialoglycoproteins have the Neu5,9Ac_2GPα2-6GalNAc sialoglycotope. The enhanced levels of these carbohydrate specific antibodies have been exploited for the development of novel diagnostic assays for successful monitoring of patients. In contrast to antibodies against whole leishmania antigens, carbohydrate specific antibodies show a ray of hope, as a steady decrease of these antibodies is evidenced only after successful treatment and therefore can be used as an index of disease status [76-78].

Such strategies may pave the path for the extensive search of novel carbohydrate specific antibodies in other parasitic infectious diseases. Already an enormous pool of glycoconjugates are reported to be distributed on different parasites (Table **1**) which might serve as potent immunogens that could elicit a definite glyco specific humoral response.

These antibodies were further demonstrated to have anti-leishmanial activity as they triggered complement mediated lysis of the parasite [79]. Additionally, these antibodies may be engineered for selective killing of the parasite useful in therapeutic purposes.

4.3.4. Anti-Glycoprotein Monoclonal Antibodies

Two trypomastigote-specific monoclonal antibodies, namely H1A10 and 6A2, prevented *T. cruzi* invasion of LLC-MK2 cell monolayers. These antibodies recognized an 85kDa glycoprotein (Tc-85) of the trypomastigote surface, which contains *N*-acetyl-D-glucosamine and/or sialic acid [80].

Different monoclonal antibodies (SST-2, SST-3 and SST-4) specifically recognizing various glycoproteins of *Leishmania (Viannia) braziliensis* promastigotes. SST-2 recognizes a conformational epitope present in a 24-28 kDa doublet and 72 kDa components and is distributed homogeneously on the parasite surface. SST-3 recognizes a flagellar glycoprotein of 180 kDa. The reactivity of this monoclonal antibody was abolished by sodium meta-periodate treatment, indicating that SST-3 reacts with a carbohydrate epitope of the 180 kDa antigen. SST-4 recognizes a conformational epitope of a 98 kDa antigen. Fab fragments of SST-3 and SST-4 significantly inhibited the infectivity of *L. (V.) braziliensis* promastigotes to mouse peritoneal macrophages [81]. All these parasite glycotope specific antibodies, although successfully used in diagnosis, may be suitably engineered for further use that would widen the horizon of therapeutics.

4.3.5. Anti-Erythrocyte Binding Antigen-175 Antibodies

EBA-175 is a *P. falciparum* protein that binds its receptor glycophorin A, a heavily sialylated on human erythrocytes during invasion [82, 83]. Antibodies against EBA-175 inhibit binding to glycophorin A and block invasion of merozoites [84]. The crystal structure of the erythrocyte binding domain of EBA-175, RII, has been established as a potential vaccine candidate [85].

4.4. Engineering Inhibitors of Glycoproteins for Interfering with Host-Pathogen Interaction

The resistance of *Plasmodium falciparum* to chloroquine and mefloquine has become a worldwide problem resulting in the need to find new treatment strategies against multidrug-resistant strains. Fluoxetine, a P-glycoprotein inhibitor with antimalarial activity, is a promising candidate for reversing chloroquine/mefloquine resistance [86].

A panel of peptidomimetic compounds as inhibitors for mammalian zinc metalloproteases showed potential to inhibit purified GP63 from *L. major* [44]. The existence of such an untapped source of compounds designed as inhibitors of mammalian metalloproteases offers the potential to significantly streamline the search for compounds with efficacy against parasitic diseases. Inhibitors to such glycoproteins may lead to a successful therapy by gradual decrease of the parasite load and loss of virulence in parasites.

Compounds like 2,3-didehydro-2-deoxy-*N*-acetyl neuraminic acid and 3'-*N*-acetyl neuraminyl-*N*-acetyl lactosamine (Fig. **3g** & **h**) served as excellent inhibitors for the interaction between erythrocyte binding antigen 175 (EBA-175) with the erythrocyte receptor glycophorin A thereby significantly inhibiting the invasion of erythrocytes by *P. falciparum* [87]. Moderate levels of inhibition were also observed with monomers or oligomers of sialic acid.

5. GLYCOCONJUGATES AS NEW VACCINE CANDIDATES IN PARASITIC DISEASES

5.1. Targeting glycosylphosphtidylinositol

Glycosylphosphatidylinositols (GPIs) present in the micro domains of the host cell membrane account for 90% of the host glycoconjugates [88]. Importantly, these parasite GPIs have been targeted as dominant malarial toxins [89] and may be considered as a new vaccine candidate. A synthetic version of the malarial GPI, with multiple mannose residues, glucosamine, and 6-myoinositol-1,2-cyclic phosphate has been chemically synthesized and conjugated to keyhole limpet hemocyanin [90]. This conjugate generated high titers of IgG, which cross-reacted with the mature GPI of trophozoites and schizonts.

Because of differences in modification of the core GPI glycan between humans and malarial parasites, antibodies to the synthetic GPI-conjugate did not cross-react with mammalian GPI. Antibodies to the synthetic GPI-conjugate neutralized the pro-inflammatory effects on macrophages of GPI derived from *Plasmodium* extracts. Such strategies would open up newer avenues to exploit other glycoconjugates in different parasitic diseases.

5.2. Virosomal Formulations

Novel virosomal formulations of a synthetic oligosaccharide have been evaluated as a vaccine candidate against leishmaniasis [91]. A lipophosphoglycan-related synthetic tetrasaccharide antigen was conjugated to a phospholipid and

to the influenza virus coat protein hemagglutinin. These glycan conjugates were embedded into the lipid membrane of reconstituted influenza virus virosomes. These virosomal formulations elicited both IgM and IgG anti-glycan antibodies in mice, indicating an antibody isotype class switch to IgG. This antisera successfully recognized natural carbohydrate antigens expressed by leishmania cells. This finding supports the concept of using virosomes as universal antigen delivery platform for synthetic carbohydrate vaccines

5.3. Chemically Defined Synthetic Glycovaccine

Immunizations with *Leishmania mexicana* promastigote secretory gel or with a chemically defined synthetic glycovaccine containing the glycans showed protection against challenge by the bite of infected sand flies [92]. Designing newer synthetic glycovaccine strategies poses further hope for combating other parasitic diseases.

5.4. Trans-Sialidase as a Potential Vaccine Target

Generation of effective vaccines, which interfere with glycan biosynthesis of the parasite, has become another important approach. *T. cruzi* does not synthesize sialic acid, but expresses a unique enzyme named *trans*-sialidase that catalyzes the transfer of sialic acid from host glycoconjugates to the parasite surface [93]. This trans-sialidase is a virulence factor required for successful infection. Immunization of mice with a plasmid DNA containing a gene encoding the catalytic domain of the trans-sialidase generated antibody and T-cell-mediated immune responses. These antibodies recognized the native enzyme and inhibited its activity *in vitro*. The immunized animals displayed reduced parasitemia and mortality upon challenge with bloodstream trypomastigotes [94]. The recombinant genetically engineered form of the *trans*-sialidase showed protective immunity in vaccinated susceptible mice [95]. Thus, the engineered *trans*-sialidase may be considered as an important vaccine target for trypanosomiasis.

5.5. GDP-Mannose Pyrophosphorylase Mutant Parasites, as a Live Attenuated Vaccine

Leishmania parasites synthesize a range of mannose-containing glycoconjugates thought to be essential for virulence in the mammalian host and sandfly vector. A prerequisite for the synthesis of these molecules is the availability of the activated mannose donor, GDP-mannose, the product of the catalysis of mannose-1-phosphate and GTP by GDP-mannose pyrophosphorylase. Unlike other eukaryotes, where deletion of GDP-mannose pyrophosphorylase is lethal, the deletion of GDP-mannose pyrophosphorylase gene in *Leishmania mexicana* did not affect parasite viability but led to a total loss of virulence, making it an ideal target for anti-*Leishmania* drug development [96]. Moreover, in view of the lack of several known host-protective antigens, injection of the mutant parasites into BALB/c mice confered significant and long lasting protection against infection, suggesting that these temperature sensitive mutants are an attractive candidate for a live attenuated vaccine [97].

6. PERSPECTIVE

In light of the growing interest in the field of carbohydrates, various approaches have been taken for their applications in diagnosis, prognosis and therapy. Modulation, identification and characterization of glycotopes both on the host and the parasite in the disease conditions appear to be an important area of investigation. This is of help in unraveling important biomarkers, which can subsequently be exploited in the development of new reliable, cheap, easy and rapid assays. Newly induced disease associated carbohydrate molecules may also serve as important tools in the development of carbohydrate-based therapy in the field of infectious diseases. Development of both glyco and sialoengineering is in its infancy. However, the progress is relatively steady in the field of cancer but the negligence towards parasitic diseases has decelerated its pace for these infections. Due to the increase in emergence and drug resistance, this approach is in great demand in the present scenario of parasitic diseases.

The review highlights the several instances of glycoengineering including an array of strategies from engineering of enzyme inhibitors, metabolite oligosaccharides, antibodies immunotoxins and vaccines. Use of engineered carbohydrates may provide a ray of hope in the therapy of protozoan diseases. We probably only see the tip of the iceberg at present, especially when it is considered that the ligand function of the sialic acid residues in mammalian cells was discovered only a few years ago.

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ABBREVIATIONS

Ac(4)ManNAz	=	Peracetylated *N*-α-azidoacetylmannosamine
EBA	=	Erythrocyte binding antigen
Gal	=	Galactose
GDP-MP	=	GDP-mannose pyrophosphorylase
GlcNAc	=	*N*-acetylglucosamine
GPI	=	Glycosylphosphatidylinositol
GIPL	=	Glycosylinositolphospholipid
LPG	=	Lipophosphoglycan
ManNAz	=	*N*-azidoacetylmannosamine
ManNAc	=	*N*-acetylmannosamine
Neu5NAc	=	*N*-acetylneuraminic acid or sialic acid
kDa	=	Kilodalton
PPG	=	Proteophosphoglycan
VSG	=	Variant surface glycoprotein
9-*O*AcSGP	=	9-*O*-acetylated sialoglycoprotein

REFERENCES

[1] Yarema, K.J.; Bertozzi, C.R. *Curr. Opin. Chem. Biol.,* **1998**, *2*, 49.
[2] Kelm, S.; Schauer, R. *Int. Rev. Cytol.*, **1997**, *175*, 137.

[3] Angata, T.; Varki, A. *Chem. Rev*., **2002**, *102*, 439.
[4] Schauer, R. In *Glycobiology,* Sansom, C.; Markman, O., Ed.; Scion Publishing group:Oxfordshire, **2007**, pp.135 (In press).
[5] Kamerling, J.P.; Schauer, R.; Shukla, A.K.; Stoll, S.; Van Halbeek, H.; Vliegenthart, J.F. *Eur. J. Biochem*., **1987**, *162*, 601.
[6] Varki, A. *Glycobiology*, **1992**, *2*, 25.
[7] Schauer, R. *Glycoconj. J*., **2000**, *17*, 485.
[8] Diaz, S.; Higa, H.H.; Hayes, B.K.; Varki, A.. *J. Biol. Chem.,* **1989**, *264*, 19416.
[9] Higa, H.H.; Manzi, A.; Varki, A.. *J. Biol. Chem.,* **1989**, *264*, 19435.
[10] Mandal, C.; Chatterjee, M.; Sinha, D. *British J. Haematol.* **2000**, *110*, 801.
[11] Mendonca-Previato, L.; Todeschini, A.R.; Heise, N.; Previato, J.O. *Current Opinion in Struc. Biol.,* **2005**, *15*, 499.
[12] McNeely, T.B.; Rosen, G.; Londner, M.V.; Turco, S.J. *Biochem. J.,* **1989**, *259*, 601.
[13] Ilgoutz, S.C.; Zawadzki, J.L.; Ralton, J.E.; McConville, M.J. *EMBO J.,* **1999**, *18*, 2746.
[14] Turco, S.J.; Descoteaux, A. *Annu. Rev. Microbiol.,* **1992**, *46*, 65.
[15] Descoteaux, A.; Turco, S.J.; *Biochim. Biophys. Acta,* **1999**, *1455*, 341.
[16] McConville, M.J.; Schnur, L.F.; Jaffe, C.; Schneider, P. *Biochem. J.,* **1995**, *310*, 807.
[17] Ilg, T.; Stierhof, Y.D.; Wiese, M.; McConville, M.J.; Overath, P. *Parasitology*, **1994**, *108*, Suppl:S63-71.
[18] Ilg, T.; Stierhof.; Y.D.; Craik, D.; Simpson, R.; Handman, E; Bacic, A. *J. Biol. Chem.,* **1996**, *271*, 21583.
[19] Ilg, T.; Handman, E.; Stierhof.; Y.D. *Biochem. Soc. Trans*., **1999**, *27*, 518.
[20] Ilg, T.; Montgomery, J.; Stierhof, Y.D.; Handman, E. *J. Biol. Chem*., **1999**, *274*, 31410.
[21] Mukhopadhyay, S.; Mandal, C. *Indian J. Med. Res.,* **2006**, *123*, 203.
[22] Chava, A.K.; Bandyopadhyay, S.; Chatterjee, M.; Mandal C. *Glycoconj. J*., **2004**, *20*, 199.
[23] Mukhopadhyay, S.; Mandal, C. In *Drug targets in kinetoplastid parasite*; H.K. Mazumdar, Ed.; Landes Biosciences: Austin, TX, **2007** (In press).
[24] Ilg, T. *EMBO J.,* **2000**, *19*, 1953.
[25] Chava, A.K.; Chatterjee, M.; Sundar, S.; Mandal, C. *J. Immunol. Methods*., **2002**, *270*, 1.
[26] Chatterjee, M.; Chava, A.K.; Kohla, G.; Pal, S.; Merling, A.; Hinderlich, S.; Unger, U.; Strasser, P.; Gerwig, G.J.; Kamerling, J.P.; Vlasak, R.; Crocker, P.R.; Schauer, R.; Schwartz- Albiez, R.; Mandal.; C. *Glycobiology,* **2003**, *13*, 351.
[27] Chava, A.K.; Chatterjee, M.; Gerwig, G.J.; Kamerling, J.P.; Mandal, C. *Biol. Chem.,* **2004**, *385*, 59.
[28] Cros, G.A.M. *Phil. Trans. R. Soc. Lond.* **1984**, *B307*, 3.
[29] DosReis, G.A.; Peçanha, L.M.; Bellio, M.; Previato, J.O.; Mendonça-Previato' L. *Microbes Infect*., **2002**, *4*, 1007.
[30] Davidson, E.A.; Gowda, D.C. *Biochimie.,* **2001**, *83*, 601.
[31] Stauffer, W.; Ravdin, J.I. *Curr. Opin. Infect. Dis.,* **2003**, *16*, 479.
[32] Petri, W.A Jr.; Haque, R.; Mann, B.J. *Annu. Rev. Microbiol.,* **2002**, *56*, 39.
[33] Moody-Haupt, S.; Patterson, J.H.; Mirelman, D.; McConville, M.J. *J. Mol. Biol.,* **2000**, *297*, 409.
[34] Chava, A.K.; Chatterjee, M.; Mandal. C. In *Hand book of carbohydrate engineering*; K.J. Yarema, Ed.; Taylor and Francis Group, book division: USA, **2005**, Chap. 3, pp. 71-98.
[35] Sinha, D.; Chatterjee, M.; Mandal, C. *Trends Glycosci. Glycotechnol.,* **2000**, *12*, 17.
[36] Chava, A.K.; Chatterjee, M.; Sundar, S.; Mandal, C. In *Trends and Research in leishmaniasis.* Raghunath, D., Nayak, R., Eds.; Tata McGraw-Hill:New Delhi, **2005**, *Vol. 5*, pp. 223-243.
[37] Bandyopadhyay, S.; Chatterjee, M.; Sundar, S.; Mandal, C. *Glycoconjugate J.,* **2004**, *20*, 531.
[38] Chatterjee, M.; Baneth, G.; Jaffe, C.L.; Sharma, V.; Mandal, C. *Veterinary Immunol. Immunopathol.,* **1999**, *70*, 55.
[39] Chatterjee, M.; Jaffe, C.L.; Shyam, S.; Basu, D.; Sen, S.; Mandal, C. *Clinic. Diagnos. Lab. Immunol*., **1999**, *6*, 550.
[40] Sharma, V.; Chatterjee, M.; Mandal, C.; Sen, S.; Basu, D. *Amer. J. Trop. Med. Hyg*., **1998**, *58*, 551.
[41] Sharma, V.; Chatterjee, M.; Sen, G.; Chava, A.K.; Mandal, C. *Glycoconj. J.,* **2000**, *17*, 22.
[42] Williams, G.J.; Woodhall, T.; Nelson, A.; Berry, A. *Protein Eng. Des. Sel*., **2005**, *18*, 239.
[43] Agusti, R.; Paris, G.; Ratier, L.; Frasch, A.C.; de Lederkremer, R.M. *Glycobiology*, **2004**, *14*, 659.
[44] Bangs, J.D.; Ransom, D.A.; Nimick, M.; Christie, G.; Hooper, N.M. *Mol. Biochem. Parasitol.,* **2001**, *114*, 111.
[45] Helenius, A.; Aebi, M.; *Science*, **2001**, *291*, 2364.
[46] Zachara, N.E.; Hart, G.W. *Chem. Rev*., **2002**, *102*, 431.
[47] Gabius, H.J.; Siebert, H.C.; Andre, S.; Jimenez-Barbero, J.; Rudiger, H. *Chembiochem,* **2004***, 5,* 740.
[48] Rudd, P.M.; Elliott, T.; Cresswell, P.; Wilson, I.A.; Dwek, R.A. *Science*, **2001**, *291*, 2370.
[49] Daniels, M.A.; Hogquist, K.A.; Jameson, S.C. *Nat. Immunol*., **2002**, *3*, 903.
[50] Kannagi, R. *Curr. Opin. Struct. Biol*., **2002**, *12*, 599.
[51] Fuster, M.M.; Esko, J.D.; *Nat. Rev. Cancer*, **2005**, *5*, 526.
[52] Hakomori, S.I. *Cancer Res*., **1985**, *45*, 2405.
[53] Dennis, J.W.; Granovsky, M.; Warren, C.E. *Biochim. Biophys. Acta,* **1999**, *1473*, 21.
[54] Oetke, C.; Brossmer, R.; Mantey, L.R.; Hinderlich, S.; Isecke, R.; Reutter, W.; Keppler, O.T.; Pawlita, M. *J. Biol. Chem*., **2002**, *277*, 6688.
[55] Jones, M.B.; Teng, H.; Rhee, J.K.; Lahar, N.; Baskaran, G.; Yarema, K.J. *Biotechnol. Bioeng*., **2004**, *85*, 394.
[56] Campbell, T.C.; Sampathkumar, S.G.; Yarema, K.J. *Mol. Biosyst*., **2007**, *3*, 187.
[57] Buttner, B.; Kannicht, C.; Schmidt, C.; Loster, K.; Reutter, W.; Lee, H.Y.; Nohring, S.; Horstkorte, R. *J. Neurosci.,* **2002**, *22*, 8869.
[58] Charter, N.W.; Mahal, L.K.; Koshland, D.E. Jr.; Bertozzi, C.R. *J. Biol. Chem.,* **2002**, *277*, 9255.
[59] Pon, R.A.; Biggs, N.J.; Jennings, H.J. *Glycobiology,* **2007**, *17*, 249.
[60] Alves, C.R.; Albuquerque-Cunha, J.M.; Mello, C.B.; Garcia, E.S.; Nogueira, N.F.; Bourguingnon, S.C.; de Souza, W.; Azambuja, P.; Gonzalez, M.S. *Exp. Parasitol.,* **2007**, *116*, 44.
[61] Luchansky, S.J.; Argade, S.; Hayes, B.K.; Bertozzi, C.R. *Biochemistry,* **2004**, *43*, 12358.
[62] Saxon, E.; Bertozzi, C.R. *Science,* **2000**, *287*, 2007.
[63] Bussink, A.P.; van Swieten.; P.F.; Ghauharali, K.; Scheij, S.; van Eijk, M.; Wennekes, T.; van der Marel, G.A.; Boot R.G.; Aerts, J.M.; Overkleeft.; H.S. *J. Lipid Res.,* **2007**, *48*, 1417.
[64] Hsu, T.L.; Hanson, S.R.; Kishikawa, K.; Wang, S.K.; Sawa, M.; Wong, C.H. *Proc. Natl. Acad. Sci.,* **2007**, *104*, 2614.
[65] Horstkorte, R.; Rau, K.; Laabs, S.; Danker, K.; Reutter W. *FEBS Lett*., **2004**, *571*, 99.
[66] Villavicencio-Lorini, P.; Laabs, S.; Danker, K.; Reutter, W.; Horstkorte, R. *J. Mol. Med*., **2002**, *80*, 671.
[67] Locksley, R.M.; Louis, J.A. *Curr. Opin. Immunol.,* **1992**, *4*, 213.
[68] Pirmez, C. ; Yamamura, M.; Uyemura, K. *J. Clin. Invest*., **1993**, *91*, 1390.
[69] Trinchieri, G. *J. Exp.Med*., **2001**, *194*, F53.
[70] Mahal, L.K.; Yarema, K.J.; Bertozzi, C.R. *Science*, **1997**, *276*, 1125.
[71] Yoshida, S.; Ioka, D.; Matsuoka, H.; Endo, H.; Ishii, A. *Mol. Biochem. Parasitol.,* **2001**, *113*, 89.
[72] Almeida, I.C.; Ferguson, M.A.; Schenkmen, S.; Travassos, L.R. *Biochem. J.,* **1994**, *304*, 793.
[73] Avila, J.L.; Rojas, M.; Acosta.; A.. *J. Clin. Microbiol.,* **1991**, *29*, 2305.
[74] Avila, J.L.; Rojas, M. *„J Clin. Microbiol*., **1990**, *7*, 1530.
[75] Milani, S.R.; Travassos, L.R.; *Braz. J. Med. Res.,* **1988***, 21*, 1275.
[76] Chatterjee, M., Sharma, V., Mandal, C.; Sundar, S.; Sen, S**.** *Glycoconj. J.,* **1998**, *15*, 1141.
[77] Chatterjee, M.; Basu, K.; Basu, D.; Banerjee, D.; Pramanik, N.; Guha, S.K.; Goswami, R.P.; Saha, S.K.; Mandal, C. *Clin. Exp. Immunol*., **1998**, *114*, 408.
[78] Bandyopadhyay, S.; Chatterjee, M.; Pal, S.; Waller, R.F.; Sundar, S.; McConville, M.J.; Mandal, C. *Diagn. Microbiol. Infect. Dis*., **2004**, *49*, 15.
[79] Bandyopadhyay S, Chatterjee M, Das T, Bandyopadhyay S, Sundar S, Mandal C. *J. Infect. Dis*., **2004**, *190*, 2010.
[80] Pereira-Chioccola, V.L.; Acosta-Serrano, A.; Correia de, Almeida, I.; Ferguson, M.A.; Souto-Padron, T.; Rodrigues, M.M.; Travassos, L.R.; Schenkman, S*.J. Cell Sci.,* **2000**, *113*, 1299.

[81] Silveira, T.G.; Suzuki, E.; Takahashi, H.K.; Straus, A.H. *Int. J. Parasitol*., **2001**, *31*, 1451.
[82] Klotz, F.W.; Orlandi, P.A.; Reuter, G.; Cohen, S.J.; Haynes, J.D.; Schauer, R.; Howard, R.J.; Palese, P.; Miller, L.H. *Mol. Biochem. Parasitol*., **1992**; *51*, 49.
[83] Orlandi, P.A.; Klotz.; F.W.; Haynes, J.D. *J. Cell Biol.,* **1992**; *116*, 901.
[84] Sim, B.K, .; Orlandi, P.A.; Haynes, J.D.; Klotz , F.W.; Carter.; J.M.; Camus, D.; Zegans , M.E.; Chulay , J.D. *J. Cell Biol.,* **1990**, *111*, 1877.
[85] Tolia, N.H.; Enemark, E.J.; Sim, B.K.; Joshua-Tor, L. *Cell*, **2005**; *122*, 183.
[86] Khairul, M.F.; Min, T.H.; Low, J.H.; Nasriyyah, C.H.; A'shikin, A.N.; Norazmi, M.; Ravichandran, M.; Raju., S.S. *Jpn. J. Infect. Dis.,* **2006**, *59*, 329.
[87] Bharara, R.; Singh, S.; Pattnaik, P.; Chitnis, C.E.; Sharma, A. *Mol. Biochem. Parasitol.*, **2004**, *138*, 123.
[88] Lauer S.; VanWye J.; Harrison, T.; McManus, H.; Samuel, B.U.; Hiller, N.L.; Mohandas, N.; Haldar, K. *EMBO J.,* **2000**, *19*, 3556.
[89] Clark, I.A.; Schofield.; L. *Parasitol. Today,* 2000, *16*, 451.
[90] Schofield, L.; Hewitt, M.C.; Evans, K.; Siomos, M.A.; Seeberger, P.H. *Nature*, **2002**, *418*, 785.
[91] Liu, X.; Siegrist, S.; Amacker, M.; Zurbriggen, R.; Pluschke, G.; Seeberger, P.H. *ACS Chem. Biol.*, **2006**, *1*, 161.
[92] Rogers, M.E.; Sizova, O.V.; Ferguson, M.A.; Nikolaev, A.V.; Bates, P.A. *J. Infect. Dis.,* **2006**, *194*, 512.
[93] Schenkman, S.; Jiang, M.S.; Hart, G.W.; Nussenzweig, V. *Cell,* **1991**, *65*, 1117.
[94] Costa, F.; Franchin, G.; Pereira-Chioccola, V.L.; Ribeirão, M.; Schenkman, S.; Rodrigues, M.M. *Vaccine,* **1998**, *16*, 768.
[95] Pereira-Chioccola, V.L.; Costa, F.; Ribeirao, M.; Soares, I.S.; Arena, F.; Schenkman, S.; Rodrigues, M.M. *Parasite Immunol.,* **1999**, *21*, 103.
[96] Davis, A.J.; Perugini, M.A.; Smith, B.J.; Stewart, J.D.; Ilg .T.; Hodder, A.N.; Handman, E. *J. Biol. Chem.,* **2004**, *279,* 12462.
[97] Stewart, J.; Curtis, J.; Spurck, T.P.; Ilg, T.; Garami, A.; Baldwin, T.; Courret, N.; McFadden, G.I.; Davis, A.; Handman, E. *Int. J. Parasitol*., **2005**, *35*, 861.

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