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CHAPTER 1

Sialic acid and biology of life: An introduction

1 Introduction

Biomolecules including monosaccharides of carbohydrates, amino acids of proteins, fatty acid of lipids, and nucleic acids including DNA and RNA play a significant role in the growth, development, and proper function of the body. Although proteins, nucleic acids, lipids, and small molecules form the major constituents of human cell, the last decade has evidenced considerable progress in the study of glycans on human cells and their role in cell-cell interaction, signaling, host-pathogen interaction, and carbohydrates contributing to important biological functions in cells. Attached to lipids and proteins, carbohydrates comprise glycoproteins and glycolipids, respectively, and play diverse myriads of roles in development, signaling, host-parasite interaction, and immune system in different organisms [1–5]. The study of sialylation in bacteria is a relatively new domain which is of immense importance in understanding and targeting host-parasite interaction in infectious diseases.

Plants and insects are being exploited for the production of human recombinant glycoconjugated proteins of therapeutic importance and therefore construction of recombinant organisms capable of synthesis of glycosylated proteins as therapeutic agents in humans holds importance.

Altered glycosylation and sialylation have been associated with several diseases in humans including cancer and finds importance in disease targeting. Although with the advent of new technology, it has become possible to study the complex glycans and decipher their biological role in health and disease in greater details [1–6], a lot remains unknown.

Sialic acids or *N*-acetylneuraminic acids (Neu5Ac) are a diverse group of 9-carbon carboxylated monosaccharides synthesized in animals, present at the outermost end of *N*-linked and *O*-linked carbohydrate chains and in lipid-associated glycoconjugates (Fig. 1, 1–6) and lack in plants [6]. Some bacterial species can de novo synthesize sialic acids while some can acquire sialic acid from host and therefore finds relevance from the point of view

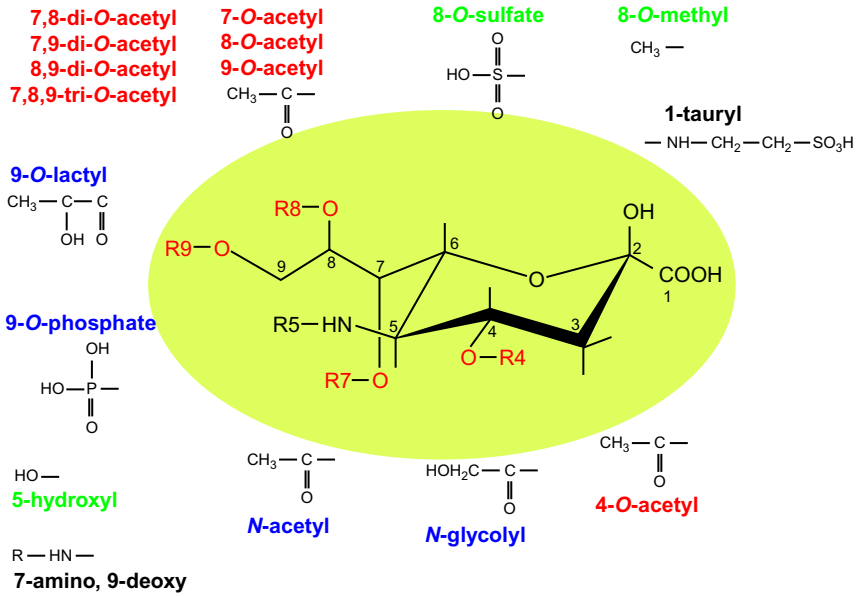


Fig. 1 The family of naturally occurring sialic acid. (Image adapted with permission from Schauer R., Srinivasan G.V., Wipfler D., Kniep B., Schwartz-Albiez R. *O-acetylated sialic acids and their role in immune defense*. In: Wu A. (editor) *The molecular immunology of complex carbohydrates-3. Advances in experimental medicine and biology*, vol 705. 2011, Springer, Boston, MA.)

of host-parasite interactions revealing evolutionary relationship [7, 8]. Sialic acid-like saccharides termed as legionaminic acid have been reported to occur in Archaea [9].

The negative charge and hydrophilic properties of sialic acid enable its role in different normal and pathological processes, acting as binding sites for various pathogens and toxins wherein pathogen-binding protein recognizes sialic acids present in specific linkages which are thought to have evolved in vertebrates through evolution. Molecular mimicry exhibited by pathogens, by decorating with sialic acids, has been known to enable them to evade the host immune system [1–6, 10–12].

Sialic acid content of the human brain is the highest among other organisms, and may be associated with evolutionary advancement of an organism [1–6, 13–18]. In Neu5Gc or N-glycolylneuraminic acid, the terminal sialic acid residue is linked by the hydroxy group of the glycolic acid unit, synthesized from Neu5Ac catalyzed by CMP-N-acetylneuraminic acid (CMP-Neu5Ac) hydroxylase (CMAH) in animals including lower animals [19–22]. Neu5Gc is absent in humans as they lack CMAH gene. Oxygen

and reduced pyridine nucleotide play vital roles in enzyme activity together with an effective cofactor NADH and the substrate CMPNeu5Ac, and are activated by cytochrome *b5*. Neu5Gc is expressed in extraneural tissues but very low, absent, or reveal suppressed expression in the vertebrate brain [21].

2 Occurrence and function

Glycoconjugates are constituents of outer surface of animal cells, and their carbohydrate structures change dramatically during development. They are expressed characteristically in different stages of differentiation and are recognized by specific antibodies. In the mature organism, the expression of distinct carbohydrates is eventually restricted to specific cell types. Aberrant expression of cell surface carbohydrates in human is very often associated with malignant transformation.

Sialic acids are found in glycoconjugates of some bacteria, virus, protozoa, and fungi and in animals of the deuterostome lineage [3] constituting glycoproteins and glycolipid-like gangliosides and glycosaminoglycan in mammals and lower vertebrates [3, 5, 23], acting as ligands or receptors for cell-cell communication or host-parasite interaction (1–6, Fig. 1–3). Sialic acid has been reported to occur in *Drosophila melanogaster* (*D. melanogaster*) and other insects [24, 25]. *N*-acetylneuraminic acid is reported to occur in cicada of *Philaenus spumarius* (*P. spumarius*) [26]. Sialic acid has been reported to occur in mollusca *Arion lusitanicus* (*A. lusitanicus*) and *Arion rufus* (*A. rufus*) [27]. Sialoglycoconjugates deuterostome lineage of the echinoderms including starfish and sea urchin is an indicative of an origin of >500 million years ago compared to higher mammals [28]. Sialic acid content in insects and molluscan gastropods is low [28–30] and lack in plants [29].

In mammalian cells, the most common sialic acids are Neu5Ac and Neu5Gc but Neu5Gc is completely absent in normal human cells [30]. Gangliosides are sialylated glycolipids that act as receptors for pathogenic bacterial infection on the gut epithelial cells [3].

Acetylated and sulfated sialic acids act as receptors for viral infections while methylated sialic acids are not found to act as receptors.

2.1 Functions of sialic acid

Sialic acids affect the structure and function of glycoconjugates and act as ligands for lectins, antibodies, and enzymes. They mediate cell-cell recognition, communication, aggregation, development, carbohydrate-protein interactions, controlling the lifetimes of glycoconjugates in organisms,

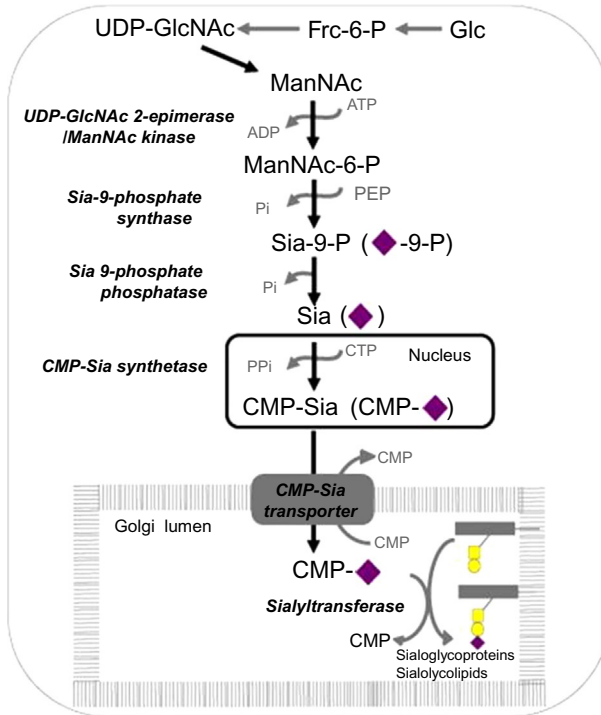


Fig. 2 Synthesis of sialoglycoconjugate. **Sialic acid** (Sia, purple diamond) is synthesized from Glc through UDP-GlcNAc as a key intermediate for sialic acid **metabolism**. UDP-GlcNAc is changed to ManNAc 6-phosphate (ManNAc-6-P) by UDP-GlcNAc 2-epimerase/ManNAc kinase. Sia-9-phosphate synthetase then condenses ManNAc-6-P with phosphoenolpyruvate (PEP) to give Sia-9-P. After **dephosphorylation** by Sia-9-phosphate **phosphatase**, Sia is activated to CMP-Sia by CMP-Sia synthetase (CMAS) in the nucleus. CMP-Sia is transported into the **Golgi apparatus** and many **sialyltransferases** (STs), such as ST3, ST6, and ST8, transfer Sia residues onto the **glycoproteins** and **glycolipids** forming sialoglycoproteins and sialoglycolipids (gangliosides). (Adapted with permission from Sato C, Hane M, Kitajima K. Relationship between ST8SIA2, polysialic acid and its binding molecules, and psychiatric disorders. A. Biosynthetic pathways of sialoglycoconjugates, *Biochim Biophys Acta* 2016,1860:1739-1752.)

mediating bacterial and viral infections, tumor growth and metastasis, with role in immunology, microbiome, cell signaling, reproduction, and biology of nervous system (Fig. 3).

They play a vital role in RBC stabilization and in preventing blood component aggregation by their negative charge and hydrophilicity. Sialic acids are known to affect the stability and function of hormones and enzymes. They play a significant role in reproduction, development, and sialylation of follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG), and contribute to their stability and function [1, 33–35].

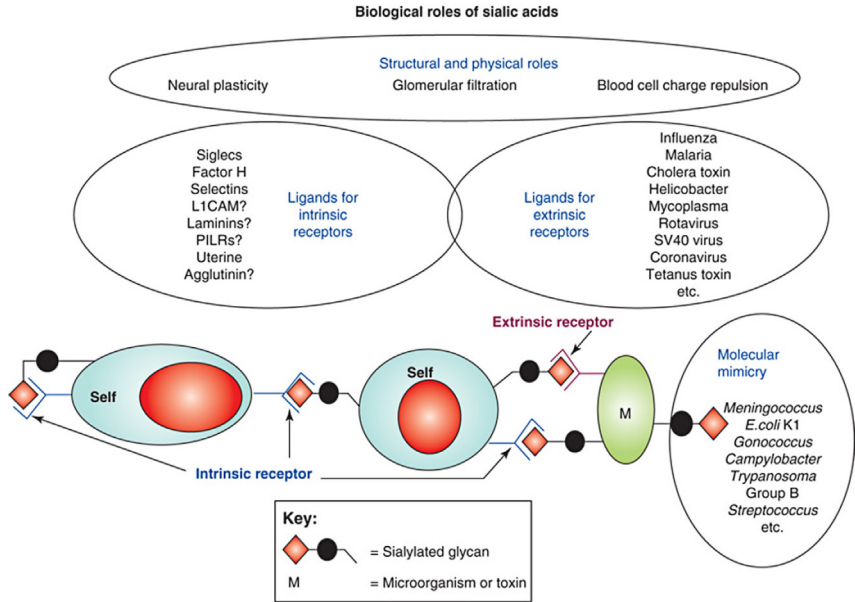


Fig. 3 Biological and pathological roles of sialic acids. The negative charge and hydrophilicity enable sialic acids to confer neural plasticity, glomerular filtration, or blood cell charge repulsion. They act as binding sites for pathogens and toxins, wherein a pathogen-binding protein or extrinsic receptor recognizes sialic acid forms in specific linkages to a defined underlying sugar chain. They also act as ligands for intrinsic receptors such as Siglecs and factor H. Sialic acids enable ‘molecular mimicry,’ by which microbial pathogens incorporate host sialic acids, thereby escaping the host immune responses. Abbreviations: L1CAM, L1 cell adhesion molecule; PILR, paired immunoglobulin-like receptor. (Adapted with permission from Ajit Varki *Sialic acids in human health and disease Trends Mol Med.* 2008; 14(8): 351–360.)

They function as ligands for glycan-binding proteins, including animal lectins like selectins and siglecs, viral lectins like hemagglutinins (HAs), and bacterial lectins like adhesins and toxins. Sialosides interaction with lectins regulates immune response, immune cell functions, and cell growth and survival. Human influenza A viral HA preferentially binds to α 2,6-linked sialic acid and avian viral HA preferentially binds to α 2,3-linked sialic acid on cell surface glycoproteins thus enabling viral attachment and entry. Thus sialic acid-binding specificity of HA determines viral tropism and host specificity [34].

Bacterial lectins, from *Helicobacter pylori* (*H. pylori*) and *Mycobacterium tuberculosis* (*M. tuberculosis*) adhesins or Cholera and Tetanus toxins, can interact with host cells by their sialic acid-containing ligands during infection [35].

Sialic acids are known to play a significant role in the development of the central and peripheral nervous system by controlling neuronal cells

function [23, 36]. Sialylated glycoconjugates like Lewis antigens interact with selectins, affect cell adhesion, lymphocyte homing, leukocyte migration to inflamed sites, observed in inflammation, angiogenesis, metastasis, thrombosis, and cancer [37–39]. Host cell's sialic acids are used by microbial pathogens *Trypanosoma cruzi* (*T. cruzi*) to mask their antigenic sites and to prevent recognition and elimination by host immune cells [40, 41].

3 Structure diversity of sialic acids

Modifications of sialic acids include diverse forms differing in position 5 of an amino group of neuraminic acid derivatives or an hydroxyl group of 3-deoxy-D-glycero-D-galactononulosonic acid (Kdn), different acylations of the NH_2 at position 5 (glycolyl, acetyl), and various substituent of the different hydroxyl groups including phosphate, sulfate, methyl, acetyl, etc. [3]. In the still growing family of sialic acids, >50 different derivatives [3, 5, 42] (Table 1) have been reported of which the two most commonly expressed members of sialic acid family are Neu5Ac and Neu5Gc followed by KDN (2-keto-3-deoxy-nononic acid) and Neu (neuraminic acid) [43].

Modifications to core structures of sialic acid by O-acetylation, O-methylation, or introduction of O-lactyl groups, sulfate, or phosphate esters at positions 4, 7, 8, and/or 9, generated by enzymes, have been reported. O-acetyltransferases catalyze the synthesis of O-acetylated sialic acid derivatives.

Sialic acids can be O-acetylated at positions C-4, C-7, C-8, and C-9 of the hydroxyl groups (Table 1). The monosaccharide and its linkage to other sugars in three main configurations such as α -2,3, α -2,6, and α -2,8 contribute to the diversity. Although mono-O-acetylated forms are predominant, combinations of acetyl groups at two or more positions generate oligo-O-acetylated derivatives. O-acetylation at positions C-7, C-8, and C-9 forming N-acetyl-7, 8, and 9-O-acetyl sialic acid (O-AcSA) are most common. 9-O-acetyl sialic acid (9-O-AcSA) and 4-O-acetylated sialic acid (4 O-AcSA) function as ligands for the agglutinin of *influenza virus C* [78] and murine hepatitis S virus [81], respectively.

3.1 O-acetylated sialic acid

Eukaryotic sialic acid anabolism is a complex enzymatic process in the cytosol leading to the activation of free Neu5Ac by CTP in the nucleus. The resulting CMP-Neu5Ac and CMP-Neu5Gc (synthesized by a cytosolic CMAH) serve as donor substrates in various acceptor-substrate-specific ST reactions in the Golgi. Finally, sialate-O-acetylation occurs at the α -glycosidically linked sialic acid (Fig. 4) [82].

Table 1 Naturally occurring sialic acid family members [5, 44–80, 83–88]

Name	Abbreviation
5- <i>N</i> -Acetyl-4,9-di- <i>O</i> -acetyl-neuraminic acid 1,7-lactone	Neu4,5,9Ac ₃ 1,7lactone
1-Tauryl 5- <i>N</i> -acetyl-neuraminic amide	Neu5Ac1Tau
5- <i>N</i> -Glycolyl-neuraminic acid	Neu5Gc
4- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu4Ac5Gc
7- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu7Ac5Gc
8- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu8Ac5Gc
9- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu9Ac5Gc
4,7-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu4,7Ac ₂ 5Gc
4,9-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu4,9Ac ₂ 5Gc
7,9-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-neuraminic acid ^k	Neu7,9Ac ₂ 5Gc
8,9-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu8,9Ac ₂ 5Gc
4,7,9-Tri- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu4,7,9Ac ₃ 5Gc
7,8,9-Tri- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu7,8,9Ac ₃ 5Gc
4,7,8,9-Tetra- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-neuraminic acid	Neu4,7,8,9Ac ₄ 5Gc
5- <i>N</i> -Glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu5Gc9Lt
4- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu4Ac5Gc9Lt
7- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu7Ac5Gc9Lt
8- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu8Ac5Gc9Lt
4,7-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu4,7Ac ₂ 5Gc9Lt
7,8-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu7,8Ac ₂ 5Gc9Lt
5- <i>N</i> -Glycolyl-8- <i>O</i> -methyl-neuraminic acid ^l	Neu5Gc8Me
4- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu4Ac5Gc8Me
7- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu7Ac5Gc8Me
9- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu9Ac5Gc8Me
4,7-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu4,7Ac ₂ 5Gc8Me
7,9-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu7,9Ac ₂ 5Gc8Me
5- <i>N</i> -Glycolyl-9- <i>O</i> -methyl-neuraminic acid	Neu5Gc9Me
5- <i>N</i> -Glycolyl-8- <i>O</i> -sulfo-neuraminic acid	Neu5Gc8S
5- <i>N</i> -Glycolyl-9- <i>O</i> -sulfo-neuraminic acid	Neu5Gc9S
5- <i>N</i> -(<i>O</i> -Acetyl)glycolyl-neuraminic acid	Neu5GcAc
5- <i>N</i> -(<i>O</i> -Methyl)glycolyl-neuraminic acid	Neu5GcMe
2-Deoxy-2,3-didehydro-5- <i>N</i> -glycolyl-neuraminic acid ^g	Neu2en5Gc
8- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu8Ac5Gc9Lt
4,7-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu4,7Ac ₂ 5Gc9Lt
7,8-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid	Neu7,8Ac ₂ 5Gc9Lt

Continued

Table 1 Naturally occurring sialic acid family members [5, 46–82, 85–90]—cont'd

Name	Abbreviation
5- <i>N</i> -Glycolyl-8- <i>O</i> -methyl-neuraminic acid ^l	Neu5Gc8Me
4- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu4Ac5Gc8Me
7- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu7Ac5Gc8Me
9- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu9Ac5Gc8Me
4,7-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid	Neu 4,7Ac ₂ 5Gc8Me
7,9-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl neuraminic acid	Neu7,9Ac ₂ 5Gc8Me
5- <i>N</i> -Glycolyl-9- <i>O</i> -methyl- neuraminic acid	Neu5Gc9Me
5- <i>N</i> -Glycolyl-8- <i>O</i> -sulfo-neuraminic acid	Neu5Gc8S
5- <i>N</i> -Glycolyl-9- <i>O</i> -sulfo-neuraminic acid	Neu5Gc9S
5- <i>N</i> -(<i>O</i> -Acetyl)glycolyl-neuraminic acid	Neu5GcAc
5- <i>N</i> -(<i>O</i> -Methyl)glycolyl-neurammic acid	Neu5GcMe
2-Deoxy-2,3-didehydro-5- <i>N</i> -glycolyl-neuraminic acid ^g	Neu2en5Gc
9- <i>O</i> -Acetyl-2-deoxy-2,3-didehydro-5- <i>N</i> -glycolyl-neuraminic acid ^g	Neu2en9Ac5Gc
2-Deoxy-2,3-didehydro-5- <i>N</i> -glycolyl-9- <i>O</i> -lactyl-neuraminic acid ^g	Neu2en5Gc9Lt
2-Deoxy-2,3-didehydro-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl neuraminic acid ^g	Neu2en5Gc8Me
2,7-Anhydro-5- <i>N</i> -glycolyl-neuraminic acid ^g	Neu2,7an5Gc
2,7-Anhydro-5- <i>N</i> -glycolyl-8- <i>O</i> -methyl-neuraminic acid ^g	Neu2,7an5Gc8Me
4,8-Anhydro-5- <i>N</i> -glycolyl-neuraminic acid ^l	Neu4,8an5Gc
5- <i>N</i> -Glycolyl-neuraminic acid 1,7-lactone	Neu5Gc1,7lactone
9- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-neuraminic acid 1,7-lactone	Neu9Ac5Gc1,7lactone
7-Acetamido-9- <i>O</i> -acetyl-7-deoxy-5- <i>N</i> -glycolyl-neuraminic acid	Neu9Ac5Gc7NAc
7-Acetamido-8,9-di- <i>O</i> -acetyl-7-deoxy-5- <i>N</i> -glycolyl-neuraminic acid ^m	Neu8,9Ac ₂ 5Gc7NAc
2-Keto-3-deoxy-nononic acid	Kdn
5- <i>O</i> -Acetyl-2-keto-3-deoxy-nononic acid	Kdn5Ac
7- <i>O</i> -Acetyl-2-keto-3-deoxy-nononic acid	Kdn7Ac
8- <i>O</i> -Acetyl-2-keto-3-deoxy-nononic acid	Kdn8Ac
9- <i>O</i> -Acetyl-2-keto-3-deoxy-nononic acid	Kdn9Ac
4,5-Di- <i>O</i> -acetyl-2-keto-3-deoxy-nononic acid	Kdn4,5Ac ₂
4,7-Di- <i>O</i> -acetyl-2-keto-3-deoxy-nononic acid	Kdn4,7Ac ₂
5,9-Di- <i>O</i> -acetyl-2-keto-3-deoxy-nononic acid	Kdn5,9Ac ₂
7,9-Di- <i>O</i> -acetyl-2-keto-3-deoxy-nononic acid	Kdn7,9Ac ₂
8,9-Di- <i>O</i> -acetyl-2-keto-3-deoxy-nononic acid	Kdn8,9Ac ₂
2-Keto-3-deoxy-5- <i>O</i> -methyl-nononic acid	Kdn5Me
2-Keto-3-deoxy-9- <i>O</i> -phospho-nononic acid ^{g,n}	Kdn9P

Adapted with permission from Schauer R, Kamerling JP. Exploration of sialic acid world Chapter 1, Advances in carbohydrate chemistry and biochemistry, vol. 75, 2018, Elsevier.

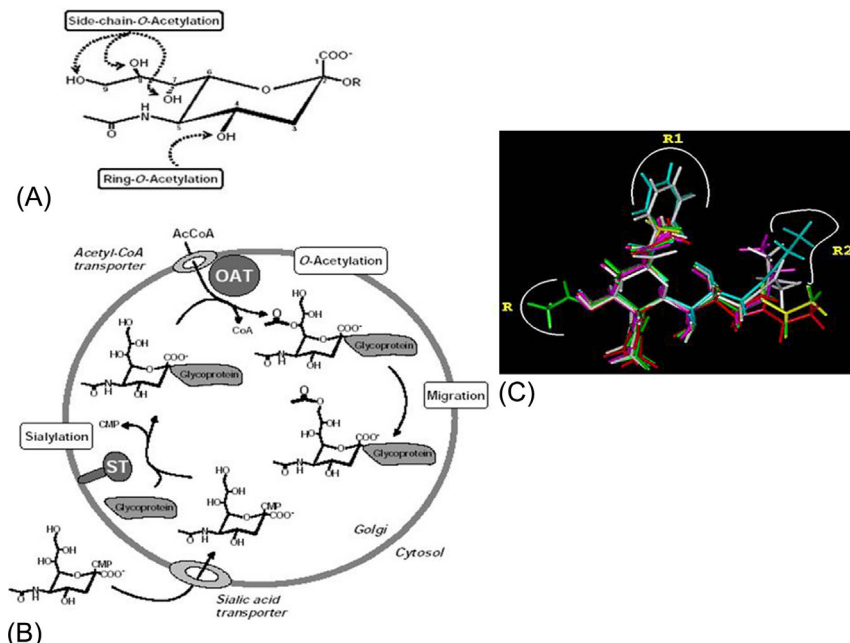


Fig. 4 (A) Naturally occurring O-acetylated sialic acids. Sialic acid O-acetylation can take place at the positions C-4, C-7, C-8, and C-9. They can be mono-O-acetylated, oligo-O-acetylated, combined acetylation at C-7 and C-9 leading to di-O-acetylated Neu5,9Ac3 form. (B) Model of side-chain O-acetylation of sialic acids. Sialic acids are transported in their CMP-activated form into the Golgi serving as substrates for different sialyltransferase (ST). Acetyl-CoA (AcCoA) enters the Golgi by an AcCoA-transporter and provide it for the O-acetyltransferase (OAT), which transfers the acetyl moiety to the glycosidically bound sialic acid probably at position C-7. After a migration to position C-9, which could be enzymatically catalyzed, an additional O-acetylation reaction can take place at position C-7. (C) Disease-specific glycotope Neu5,9Ac2-GPs, expressed on PBMCALL [PBM of childhood acute lymphoblastic leukemia (ALL)]. Superimposed three-dimensional (3D) structures of all the sialic acid derivatives which were used as inhibitors of the binding of Neu5,9Ac2-GPsALL to Achatinin-H. (Source: (B) Reproduced from Schauer R, Schmid H, Pommerenke J, Iwersen M, Kohla G. Metabolism and role of O-acetylated sialic acids. *Adv Exp Med Biol* 2001;491:325-42 with permission. (C) Adapted with permission from Ghosh S, Bandyopadhyay S, Mukherjee K, Mallick A, Pal S, Mandal C, Bhattacharya DK, O-acetylation of sialic acids is required for the survival of lymphoblasts in childhood acute lymphoblastic leukemia (ALL). *Glycoconj J* 2007, 24:17-24.)

3.1.1 Enzymes in O-acetylated sialic acid metabolism

The metabolism of O-AcSA can be divided into two parts.

- (i) It is initiated by the formation of acetyl esters at different hydroxyl groups of sialic acids by the transfer of activated acetyl groups from acetyl coenzyme A (AcCoA) by O-acetyltransferases (Fig. 4).

Differing in their regioselectivity, two groups of sialate-*O* acetyltransferases exist: (a) acetyl-CoA:sialate 7 [9]-*O*-acetyltransferase responsible for the frequently found *O*-acetylation side chain and (b) acetyl-CoA:sialate 4-*O*-acetyltransferase which leads to the pyranose ring-*O*-acetylation at C4.

- (ii) Several enzymes degrade free or glycosidically bound *O*-AcSA acids such as esterases for Neu5,9Ac2 and Neu4,5Ac2, respectively, which are found in influenza C virus, lysosomes of mammalian cells, mouse hepatitis virus, and in horse liver.

3.1.2 Functions of *O*-acetylated sialic acids

In the past three decades, *O*-acetylated sialic acids (*O*-AcSA) have been shown as important cell membrane components that play fundamental roles in the development, immune regulation, cancer processes, and many other biological and pathophysiological events [17, 82, 84]. The biological effect depends on the position relative to the 9-carbon scaffold in Neu5Ac or Neu5Gc. The *O*-acetyl group being a more hydrophobic moiety when introduced into the sialic acid molecule the parameters like size, net charge, hydrogen bonding and conformation of glycoconjugate alters and the terminal location enables their involvement in different functions such as cell-cell adhesion, signaling, differentiation and metastasis [85–88]. The extended nature of oligosaccharide chains, and possibly their negative charges, plays an important role in cell-cell and cell-matrix interactions [86]. *O*-acetylated sialic acids reported to occur in bacteria and parasites are known to act as receptor determinants for some viruses, well-known cancer markers in childhood acute lymphoblastic leukemia (ALL), and also regulate ganglioside-mediated apoptosis. Sialic acid-specific *O*-acetyltransferases and *O*-acetylsterases regulate their synthesis [89].

Modifications of sialic acids exhibit tissue-specific and developmentally regulated expression. *O*-AcSA occurrence is predominant in growing and developing tissues of neuroectodermal origin and in B and T lymphocytes [90], 9-*O*-Ac-GD3 distribution on the normal tissues is largely restricted to brain [91] while 9-*O*-acetylsialylated GT3 ganglioside [92] expression is developmentally regulated in rat embryonic cerebral cortex. Sialic acids acetylation is selectively developmentally regulated in the central and peripheral nervous system, the retina, and the medulla of kidney [93] in rat tissue.

O-acetylated GD3 on human lymphocytes [94] is reported as a marker for human CD8+ T helper cells [95]. Sialyl-Le x and Sialyl-Le a ligands for selectins are known to play a major role in the early steps of leukocyte rolling on

endothelial cells [96]. 9-*O*-acetylations on immune cells have been reported to regulate CD22 β adhesion negatively through masking of the carbohydrate epitope. 9-*O*-acetyl sialoglycoconjugates (9-*O*-Ac-SGs) on erythrocytes in visceral leishmaniasis (VL) serve as diagnostic and prognostic markers [97, 98] and are known to activate the alternate complement pathway enhancing hemolysis accounting for anemia [99]. Sialic acids on *Leishmania donovani* (*L. donovani*) amastigotes and two 9-*O*-AcSGs with Mw 158,000 and 150,000 have been identified as components of amastigote cell surface [99–101]. *O*-acetyl GD3 is not expressed on human normal tissues [102]. 9-*O*-Ac-GD3 is considered as an oncofetal marker in animal and human tumors like neuronal tumors, melanoma, basaloma or breast cancer (BC), and in psoriatic lesions [88].

9-*O*-acetylated sialic acid and glycoconjugates have been reported as diagnostic and prognostic markers in childhood ALL [83, 103–107]. 9-*O*-acetylations have been reported to be upregulated in basal cell carcinoma tissues than in the surrounding skin [108].

O-acetylation of disialoganglioside GD3 by human melanoma cells has been reported to be a unique antigenic determinant [109]. BC cells have recently been reported to express b-series gangliosides GD3 and GD2, and *O*-acetylated GD2 (*O*-AcGD2) in which 9-*O*-acetyl-*N*-acetylneuraminic acid (Neu5,9Ac₂) is predominant *O*-acetylated sialic acid species of GD2 [110].

Two disease-specific 9-*O*-Ac-SGs, of molecular weight 90 and 120 kDa, have been reported on PBMC of patients from childhood acute lymphoblastic leukemia (PBMCALL) [103, 111] with diagnostic and prognostic importance [103, 111–114] and biological function [83, 103–107], which have been demonstrated by Achatinin-H, a lectin isolated from *Achatina fulica* (*A. fulica*) snail, with 9-*O*-AcSA α 2–6GalNAc₂ as its lectinogenic epitope. 9-*O*-AcSA-specific IgM and IgG antibodies have been reported in ALL patients and finds importance in the detection and monitoring of patients [115, 116].

3.2 *N*-acetylation of sialic acids

The *N*-acetyl group of Neu5Ac at fifth position is known to originate from AcCoA [117–120] (Fig. 5) during conversion of GlcNH₂-6-P to GlcNAc-6-P, the precursor of UDP-GlcNAc. This then converts to ManNAc by epimerization reaction, and finally to CMP-Neu5Ac [117–119, 121]. Neu5Ac is transferred to macromolecules from the nucleotide sugar, which can later be released into the lysosomes, and exported into the cytosol [122, 123]. The *N'*-acetyl group can be converted to *N'*-glycolyl group by a specific hydroxylase [124].

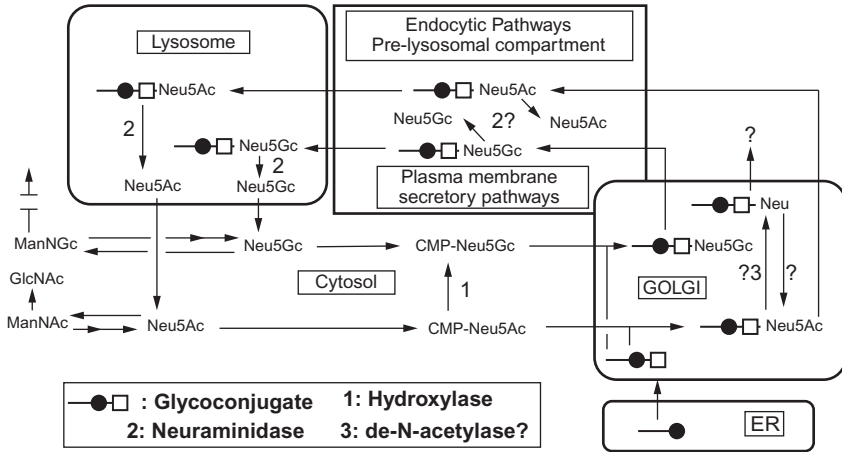


Fig. 5 Steps in *N*-acetylation of sialic acid. (Adapted from Varki A. *Diversity in the sialic acids*. *Glycobiology*, 1992, 2:25–40 with permission.)

3.3 Other modifications of sialic acids

Unsaturated sialic acids occur in nature or biological fluids generated by enzymes or chemical processes such as 2,7-anhydro sialic acids released by sialidases [125–129], 2,3-didehydro 2,6-anhydro from mild CMP-sialic acids [130], and 4,8-anhydro compounds from release or deacetylation of 4–0-acetylated compounds [131, 132]. The phosphate group of Neu5Ac9P emerges from ManNAc–6-P.

- (i) Deaminated neuraminic acid (KDN) although reported to occur in vertebrates and bacteria, is predominantly expressed in lower vertebrates. KDN is linked to most glycan structures in place of Neu5Ac and is found to occur as glycoconjugates, including glycolipids, glycoproteins, and capsular polysaccharides. They exhibit linkage types such as α 2,3, α 2,4, α 2,6, and α 2,8 bearing similarity to Neu5Ac. KDN de novo biosynthesis involves mannose as a precursor, activated to CMP-KDN and transferred to acceptor sugar residues. Predominant KDN expression has been reported to occur in fetal human red blood cells and ovarian tumor tissues as compared to adult RBC and normal individuals. KDNase, which cleaves KDN linkages, is reported to occur in bacteria [133]. KDN could arise from sequential deacetylation and deamination of Neu5Ac.

C8 acidic sugar 3-deoxy-d-manno-2-octulosonic acid (Kdo) is reported to occur in Gram-negative bacteria lipopolysaccharide (LPS) and plant cell pectic rhamnogalacturonan II. In the light of recent discoveries although hitherto unknown, sialic acid expression in algae Kdo has been

reported. *de novo* biosynthesis of the deaminated sialic acid, 3-deoxy-d-glycero-d-galacto-2-nonulosonic acid (Kdn), has been reported to occur in *Prymnesium parvum* (*P. parvum*) with probable indications of role in host pathogen [134].

- (ii) Other types of substitutions of the hydroxyl groups arise from use of the appropriate donors such as S-adenosylmethionine for methylated sialic acids and 3'-phosphoadenosine 5'-phosphosulphate for sulfated molecules. However, not many studies have been done on other modifications like O-lactyl groups.

Sialic acid family has now been designated as subclass of the superfamily of naturally occurring non-2-ulosonic acids (NulOs), the parent molecule of the family being neuraminic acid (Neu), which is not found in free form in nature due to its immediate cyclization to form an internal Schiff base, and is a nine-carbon-containing monosaccharide, comprising 2-keto-carboxylic acid, a deoxysugar, and an aminosugar. Other members of the NulO superfamily include 5,7-diamino-3,5,7,9-tetraoxynon-2-ulosonic acids, with the mother molecules pseudaminic acid (Pse), legionaminic acid (Leg), 4-epi-legionaminic acid (4eLeg), 8-epilegionaminic acid (8eLeg), acinetaminic acid (Aci), and 8-epi-acinetaminic acid (8eAci), found in bacterial polysaccharides and glycoproteins [5] (Fig. 6).

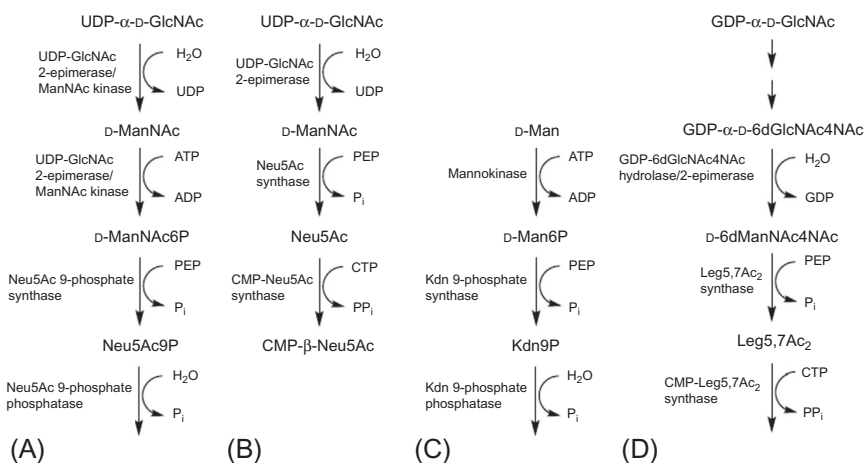


Fig. 6 Metabolism of non-ulosonic acids: (A) Neu5Ac in vertebrates, (B) Neu5Ac in bacteria, (C) Kdn in vertebrates, and (D) Leg 5,7Ac₂ in bacteria. (Adapted with permission from Schauer R and Kamerling JP Exploration of sialic acid world Chapter 1, Advances in carbohydrate chemistry and biochemistry, vol. 75, 2018, Elsevier.)

4 Sialic acid and the living world

4.1 Sialic acid and bacteria

Glycoconjugates occur on both prokaryotic and eukaryotic cell surfaces with important biological functions like cell-cell and small molecule-cell recognition and communication. Sialic acids are predominantly found on eukaryotic cell surfaces. Pathogens (Table 2) reveal property of acquiring host sialic acid on cell surfaces, thereby mimicking the host and escaping the host immune responses.

Neu5Ac and its structural variants such as substitutions at carbon 5, or covalent modifications of the hydroxyl groups in sugars find importance in this context. Bacteria can either express sialic acid by de novo biosynthesis or acquire sialic acid from their environment and transport in the cell surface using transporters, thereby leading to the formation of sialic acid-acquired surfaces that can affect the host-parasite interaction. Bacteria can process them by common pathways for sialic acid metabolism and use sialic acid in different roles such as colonization owing to its disease causing property.

Table 2 Pathogens expressing sialic acids on their surfaces

Pathogen	Major disease
Sialic acid synthesized by pathogen	
<i>Neisseria meningitidis B</i>	Meningitis
<i>Escherichia coli K1</i>	Neonatal meningitis
Group B <i>Streptococcus</i>	Neonate and infant infections
<i>Campylobacter jejuni</i>	Enteritis, Guillian-Barré syndrome
Host sialic acid taken up by pathogen	
<i>Hemophilus influenza</i>	Respirator infections
<i>Hemophilus ducreyi</i>	Chancroid
Host sialic acid transferred by trans-sialidase	
<i>Trypanosoma cruzi</i>	Chagas disease
<i>Corynebacterium diphtheria</i>	Diphtheria
Host CMP-sialic acid used by sialyltransferase	
<i>Neisseria gonorrhoea</i>	Gonorrhoea
<i>Neisseria meningitides group A</i>	Meningitis
Source of sialic acid un known	
<i>Sporotrichium schenckii</i>	Skin infection
<i>Aspergillus fumigates</i>	Opportunistic infections

Adapted with permission from Ajit Varki Sialic acids in human health and disease Trends Mol Med. 2008; 14(8): 351–360.

De novo synthesis of sialic acid is reported in *Escherichia coli* (*E. coli*) K1, *Neisseria meningitidis* (*N. meningitidis*), and *Campylobacter jejuni* (*C. jejuni*) [8] wherein UDP-GlcNAc acts as sialic acid biosynthesis precursor and finds importance in cell wall biosynthesis, and NeuC and NeuB proteins enable Neu5Ac by ManNAc conversion (Figs. 7 and 8).

Some bacterial pathogens acquiring sialic acid from host secrete sialidase that releases sialic acid from host [135]; but *Haemophilus influenzae* (*H. influenzae*), lacking sialidase genes [136], acquires free host sialic acid by other sialidase-expressing bacteria in the host [137], or by the action of host sialidases [138, 139], activated during disease/inflammation. Bacteria use specific transporters to take in this free sialic acid including NanT sialic acid transporter from *E. coli* K-12 [8] that transports Neu5Ac uptake [8]. *H. influenzae* and *Pasteurella multocida* (*P. multocida*) use high affinity tripartite ATP-independent periplasmic (TRAP) transporter, SiaPQM [140–142] and extracytoplasmic solute receptor (ESR) protein for transport; *Haemophilus ducreyi* (*H. ducreyi*, SatABCD), causing chancroid, uses a high affinity ABC transporter [143] for the transport of sialic acid.

Neisseria gonorrhoeae (*N. gonorrhoeae*) uptakes activated form of sialic acid CMP-Neu5Ac by secreting the enzymes that sialylates its LPS contributing to virulence factor.

Neu5Ac-inducible porin NanC (YjhA) from *E. coli* K-12 has recently been studied for playing a role in growth on Neu5Ac even though lacks both OmpC and OmpF porins [144]. *H. influenzae* and *E. coli* can also utilize the transported sialic acid as a carbon and nitrogen source [8, 156], the *N*-acetylneuraminase aldolase NanA cleaves Neu5Ac to ManNAc and pyruvate (Fig. 7). ManNAc is later converted to fructose 6-phosphate and ammonia by NanK, NanE, NagB, and NagA proteins for further metabolism [8].

After either de novo synthesis or acquiring from host, sialic acid is converted to activated form CMP-Neu5Ac by CMP-sialic acid synthetases, while linkage-specific sialyltransferases enable their addition to appropriate acceptors. *N. gonorrhoeae* uses outer membrane-associated sialyltransferase to scavenge CMP-Neu5Ac directly from host [145].

In *E. coli*, NeuA activates Neu5Ac before incorporation into the K1 and K92 capsules while the *N. meningitidis* ortholog enables both capsule and LPS synthesis. In *E. coli*, NeuS acts as polysialyltransferase adding Neu5Ac to oligosialic acid receptors to form the polysialic acid (PSA) polysaccharide capsule, exported through the Kps system [8]. After synthesis sialic acid in the PSA capsule of both *N. meningitidis* and *E. coli* can be modified by O-acetylation [146–148] like O-acetyltransferases NeuO and NeuD in

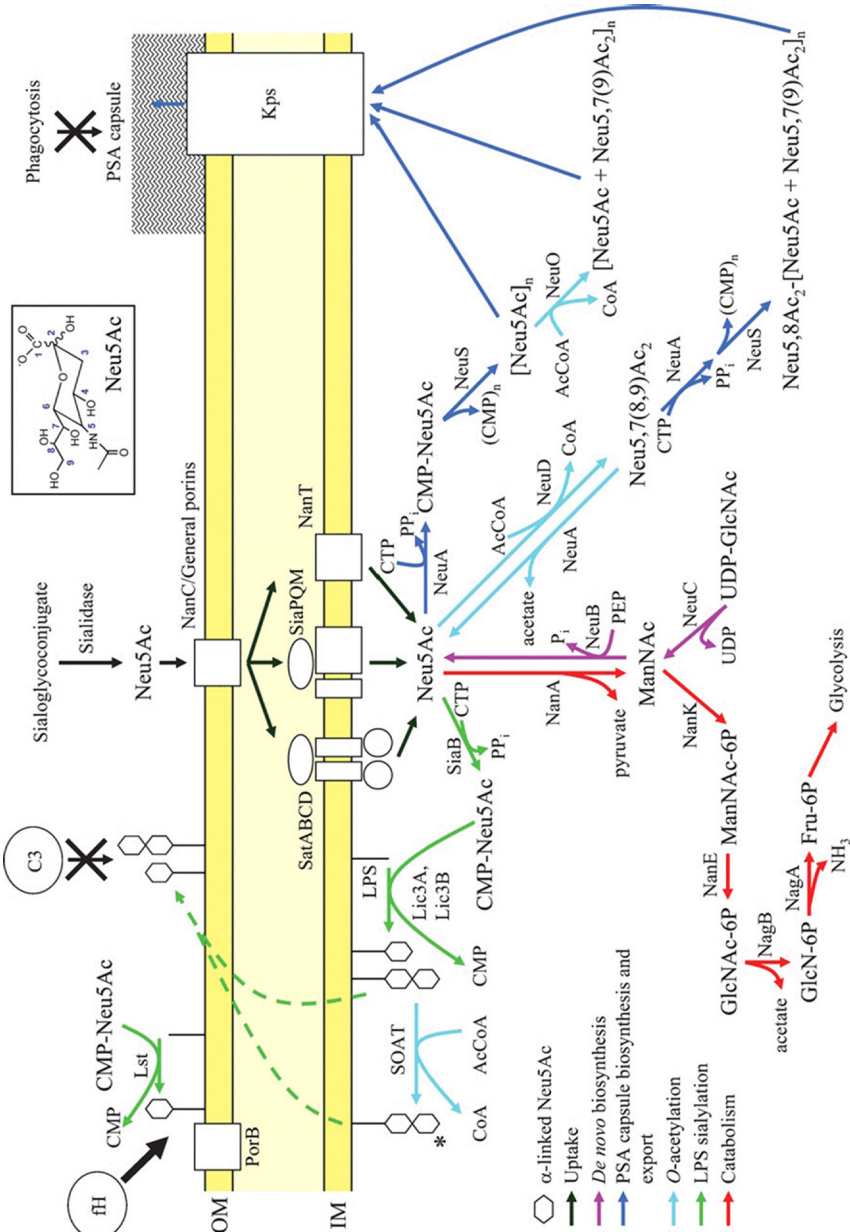


Fig. 7 See figure legend on opposite page.

Fig. 7, Cont'd Overview of the major pathways for sialic acid utilization in bacterial pathogens. Sialic acid/Neu5Ac. Black arrow: The site of interaction of factor H (fH) on the gonococcal cell surface. Monomeric *O*-acetylated Neu5Ac produced by *E. coli* NeuD is believed to enter the normal PSA biosynthetic pathway via NeuA/NeuS [164]; light-green discontinuous arrow: LPS is exposed on the outer membrane. Asterisk: *O*-acetylation of the disialylated LPS of *C. jejuni*; catalyzed by the product of the gene *orf11*, or sialic acid *O*-acetyltransferase (SOAT). IM, inner membrane; OM, outer membrane; Neu5Ac, *N*-acetylneuraminic or sialic acid; ManNAc, *N*-acetylmannosamine; GlcNAc, *N*-acetylglucosamine; GlcN, glucosamine; Fru, fructose; PSA, polysialic acid; PEP, phosphoenolpyruvate; Lst, *Neisseria* LPS sialyltransferase; NanC, *E. coli* Neu5Ac-specific porin; Kps, *E. coli* PSA capsule export system; SatABCD, *H. ducreyi* Neu5Ac ABC (ATP-binding cassette) transporter; SiaPQM, *H. influenzae/P. multocida* Neu5Ac TRAP (tripartite ATP-independent periplasmic) transporter; NanT, *E. coli* Neu5Ac MFS (major facilitator superfamily) transporter; SiaB and NeuA, respectively *H. influenzae* and *E. coli* CMP-Neu5Ac synthetases; Lic3A, Lic3B: *H. influenzae* sialyltransferases; SOAT, *C. jejuni* Neu5Ac *O*-acetyltransferase; NeuC, *E. coli* UDP-GlcNAc 2-epimerase; NeuB, *E. coli* Neu5Ac synthase; NeuS, *E. coli* polysialyltransferase; NeuO, *E. coli* PSA *O*-acetyltransferase; NeuD, *E. coli* Neu5Ac *O*-acetyltransferase; NanA, Neu5Ac aldolase; NanK, ManNAc kinase; NanE, ManNAc-6P epimerase; NagB, GlcNAc-6P deacetylase; and NagA, GlcN-6P deaminase (the catabolic pathway is present in several bacteria: 8). (Image Reproduced with permission from Severi E et al. Sialic acid utilization by bacterial pathogens. *Microbiology*. 2007;153(Pt. 9):2817-22.)

E. coli can modify PSA and monomeric Neu5Ac, respectively, the latter can be deacetylated by NeuA acting as a bifunctional enzyme [148–150].

Streptococcus agalactiae (*S. agalactiae*) is the only Gram-positive bacteria causing serious infections in newborns, and can produce sialic acid-containing capsule by using sialyltransferase (CpsK) adding terminal α -2,3-linked Neu5Ac to galactose within the capsule's oligosaccharide repeat [151]. Neu5Ac can be modified by *O*-acetylation [152].

Sialylation of the LPS is mediated by linkage-specific sialyltransferases. Both *N. meningitidis* and *N. gonorrhoeae* sialylate their LPS by α -2,3 sialyltransferase. LPS sialylation is reported to occur in *Pasteurellaceae*, including *H. ducreyi*, *H. influenzae*, *Haemophilus somnus* (*H. somnus*), and *P. multocida* [153]. Lic3A, sialyltransferase adding α -2,3-Neu5Ac [154], is associated with bacterial survival [155]. Lic3B can add mono- or disialic acid to the LPS acceptor [153]. *C. jejuni* possesses mono- or bifunctional LPS sialyltransferases transferring either α -2,3-Neu5Ac or disialic acid [156]. Terminal sialic acid residue in the disialylated LPS can also be modified by an *O*-acetyltransferase (Figs. 7) [157] as identified in *C. jejuni*.

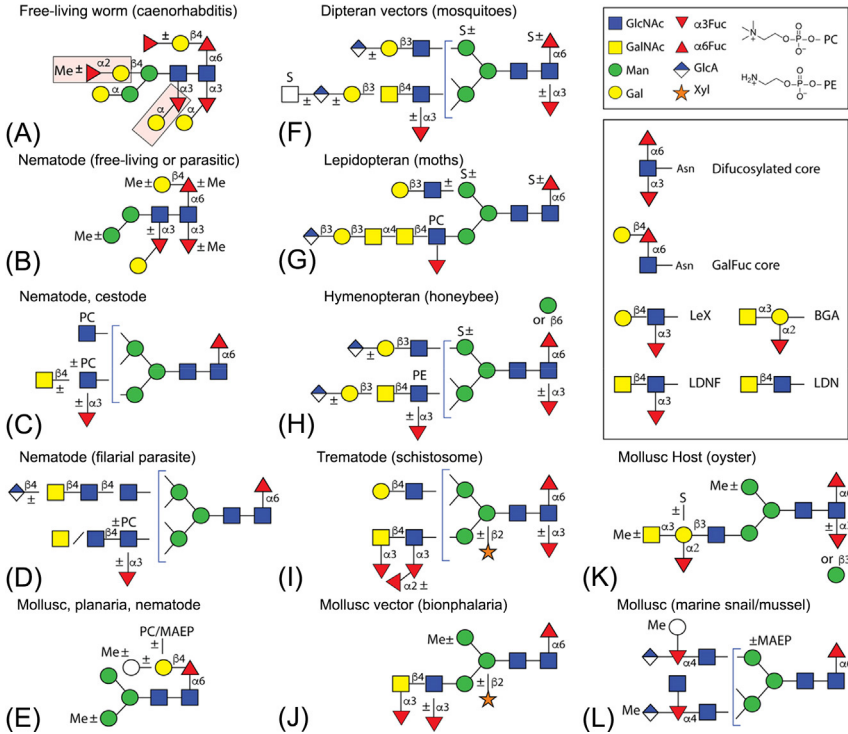


Fig. 8 Example of N-glycans from invertebrates including parasitic or free-living organisms, hosts, or vectors for parasites. A non-exhaustive selection of core and antennal epitopes is shown in the inset: core difucosylation, core ‘GalFuc’, Lewis X (LeX), fucosylated and non-fucosylated LacdiNAc (LDN), and blood group A. (Image adapted with permission from Paschinger K, Wilson IBH. Comparisons of N-glycans across invertebrate phyla. *Parasitology* 2019, 3:1-10.)

Sialylated LPS and PSA capsules confer protection to the bacteria to escape host immune responses by ‘molecular mimicry’ [158] as was observed in studies on neisseria and hemophilus bacteria.

Neisseria spp., *H. influenza* and *C. jejuni*, reveal reversible ON to OFF switching leading to variable expression and also O-acetylation of the PSA capsule of *E. coli* K1 [148, 159].

4.1.1 Bacterial sialylation and host immune system

The PSA capsule of *N. meningitides* serogroup B and *E. coli* K1 is poorly immunogenic and PSA reveal structural similarities to mammalian neuronal cell adhesion molecule, NCAM [160]. The sialylated capsule of *S. agalactiae* inhibits phagocytosis, impairs C3 deposition on the cell surface, and prevents complement alternative pathway [161] activation.

LPS sialylation inhibits the complement alternative pathway in both *N. gonorrhoeae* and non-typable *H. influenza* (NTHi) [162, 163]. Gonococcal sialylated LPS increases the binding of bacteria to factor H (fH), a complement alternative pathway inhibitor [163], thus conferring protection from C3 attack [163]. In NTHi, LPS sialylation inhibits deposition of C3 without fH binding [162].

4.2 Archaea

N-glycosylation is a posttranslational modification that occurs in all three domains. In Archaea, N-linked glycans reveal diversity which is not observed in either Eukarya or Bacteria with the lack of expression of nonulosonic acids (NulOs), sialic acids, pseudaminic acids, and legionaminic acids, in contrast to Eukarya and Bacteria. In haloarchaea *Halorubrum* sp. PV6 includes an N-formylated legionaminic acid and a biosynthetic pathway [9].

4.3 Virus

Viral sialic acid-recognizing lectins or HAs can agglutinate RBC. Viruses use sialic acids linked to glycoproteins and gangliosides to attach to host cells, followed by their entry, for example, corona virus, DNA tumor viruses, hepatitis virus, influenza viruses (A, B, and C), mouse polyoma virus, mumps, Newcastle disease virus (NDV), norovirus, parainfluenza viruses, rotavirus, and Sendai virus. HAs from influenza A, C, NDV, and polyoma viruses have been crystallized. Sialic acid-recognizing lectins from adenoviruses and picornaviruses have not been identified.

Some of these viruses carry neuraminidase or sialyl-O-acetyl-esterase that destroys the receptor, promotes virus release from infected cells, and removes sialic acid on host cell affecting cell surface binding of the virus. Influenza A virus enters the host by using host surface sialic acids. Influenza C virus HA-esterase specific for 9-O-acetylated sialic acids can break down 9-O-acetyl ester. HA-esterase from mouse hepatitis virus is specific to sialic acids substituted by O-acetyl group at the C-4 position (Neu4,5Ac2). HA-neuraminidase of NDV84 and parainfluenza viruses perform vital functions in infection biology [6, 165].

4.4 Fungi

Sialic acids have been reported to occur in some pathogenic fungal cells such as *Candida Cryptococcus neoformans* (*C. neoformans*), *Aspergillus fumigatus* (*A. fumigatus*), and *Sporothrix schenckii* (*S. schenckii*). Neu5Ac, Neu5Gc, and Neu5,9Ac2 were reported to have been expressed. It is hypothesized that probably fungi may have unique ways to synthesize sialic acid however they may acquire it from the environment [166–169].

A. fumigatus pathogenic variety reveals greater sialic acid density as compared to that of non-pathogenic *Aspergillus* species [170]. *Scedosporium apiospermum* (*S. apiospermum*), *Scedosporium aurantiacum* (*S. aurantiacum*), *Scedosporium minutisporum* (*S. minutisporum*), and *Lomentospora prolificans* (*L. prolificans*) reveal lack of sialic acid [171, 172].

A. fumigatus has been known to express a sialidase termed as KDNase that prefers sialic acid substrate, 2-keto-3-deoxy-D-glycero-D-galactononic acid (KDNase) and plays an important role in maintaining cell wall integrity and virulence [173]. Core 1 O-linked glycan-specific lectin, *Herichium erinaceus* lectin (HeL), has been isolated from the fruiting body of the mushroom *Herichium erinaceus* (*H. erinaceus*), which acts as a natural source for a sialic acid-binding lectin (SABL) [174].

A lectin has been reported to occur in Australian indigenous mushroom *Psathyrella asperospora* (*P. asperospora*) termed as PAL with cytotoxic properties on human colon cancer HT29 and monkey kidney VERO revealing binding preference toward N-acetylglucosamine (GlcNAc) and sialic acid (Neu5Ac) [175]. Most *Fusarium* lectins exhibit binding affinity to D-ribose, l-fucose, D-glucose, l-arabinose, D-mannitol, d-galactosamine hydrochloride, d-galacturonic acid, N-acetyl-d-galactosamine, N-acetylneuraminic acid, 2-deoxy-D-ribose, fetuin, asialofetuin, and bovine submaxillary mucin (BSM) [176].

4.5 Plants

Plants lack sialic acid but studies on recombinant plant glycoproteins are being conducted. Neu5Ac however has been reported to occur in buckwheat using mass spectrometry. R-keto acids in plants include 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP) and Kdo in cell wall polysaccharides. *Arabidopsis thaliana* (*A. thaliana*) genome revealed lack of genes for biosynthesis, activation, or transfer of sialic acid [177–179].

4.6 Invertebrates

N-glycosylations have been reported to occur in invertebrates (Fig. 8).

Sialic acid is synthesized and expressed by different invertebrates. We discuss in brief the expression of sialic acid in invertebrate animals and its functions.

4.6.1 Protozoa

Parasitic protozoa are known to reveal sialoglycoconjugates which play an important role in their biological function (Table 3) and *T. cruzi* (Fig. 9), the causative agent of life-threatening Chagas disease in South America, is

Table 3 Biological role of sialoglycoconjugates in parasitic protozoa [99–101, 186–210]

Parasite	Sialoglycoconjugates	Biological relevance
<i>Trypanosoma cruzi</i>	Acquisition of sialic acids by mucins	(a) Invasive determinant (b) Modulation of host immune response (c) Protection against cytolytic agents.
<i>Trypanosoma brucei</i>	Acquisition of sialic acids by procyclic repetitive proteins (PARPs).	(a) Invasion of host cells (b) Survival within insect vector.
<i>Entamoeba histolytica</i>	Appearance of sialic acid during encystation and gangliosides in trophozoites.	(a) Decreases parasite adherence to target cells thereby reducing its cytolytic activity.
<i>Plasmodium falciparum</i>	Absence of Neu5Ac, instead sialic acid-binding protein EBA-175 is present.	Ligands for EBA-175 are essential for erythrocyte invasion.
<i>Trichomonas vaginalis</i> and <i>Trichomonas foetus</i>	Sialic acid-specific lectin identified in parasite culture supernatant	Enhances parasite adhesion to mucosal surfaces
<i>Toxoplasma gondii</i>	Uptake of fetuin from the culture medium.	Not known
<i>Leishmania donovani</i>	Polyanionic adsorption	(a) Determinant of virulence (b) Complement mediated cell lysis

Adapted from Chava AK, Bandyopadhyay S, Chatterjee M, Mandal C. Sialoglycans in protozoal diseases: their detection, modes of acquisition and emerging biological roles. *Glycoconj J* 2004a. 20:199–206 with permission.

known to express sialic acids transferred from the host glycoconjugates to the terminal β -galactopyranosyl residues of mucin-like molecules on parasite surface by using the enzyme trans-sialidase [180]. These sialic acids might play a role in conferring protection from the recognition and response by the host immune system. Sialic acid such as Neu5Ac, Neu5Gc, Neu5,7Ac2, and Neu5,9Ac2 have been reported to occur in *Dictyostelium discoideum*, a trypanosome *Crithidia fasciculata* (*C. fasciculata*), piroplasmid *Theileria sergenti* (*T. sergenti*), and amoebae *Entamoeba invadens* (*E. invadens*) and *Entamoeba histolytica* (*E. histolytica*) [6, 181–185].

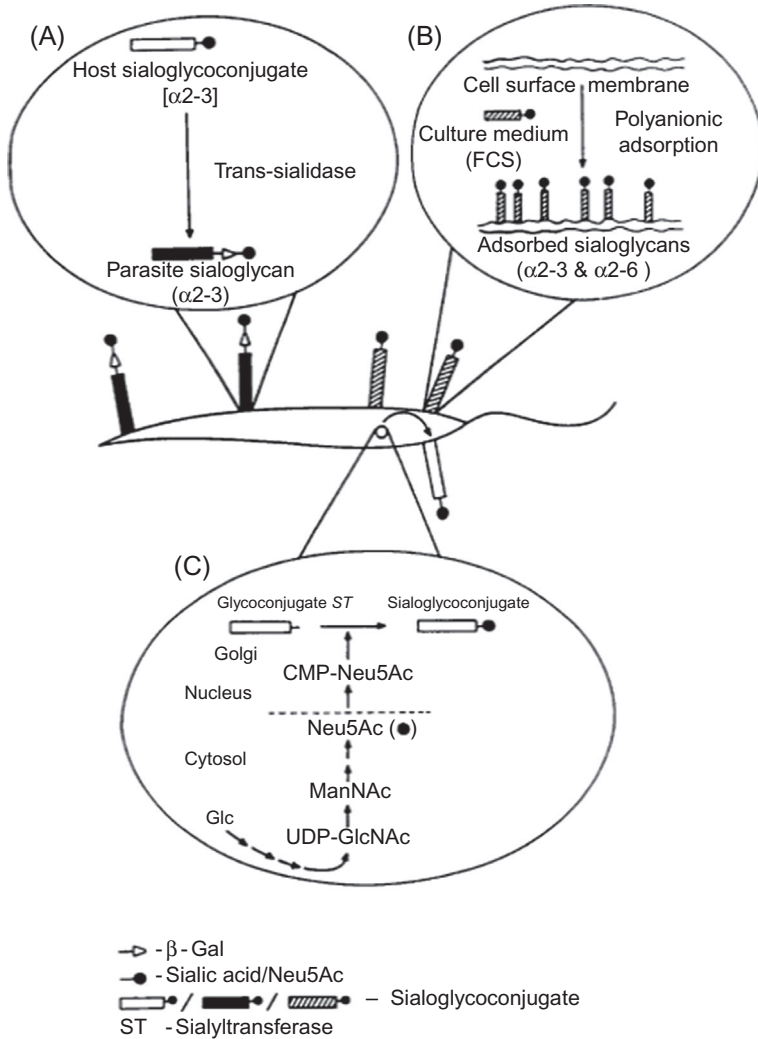


Fig. 9 Representative profile of sialic acid acquisition by protozoa: (A) with the help of trans-sialidase in *T. cruzi* and *Trypanosoma brucei* (*T. brucei*), (B) polyanionic adsorption of serum sialoglycans by *L. donovani* promastigotes, and (C) de novo synthesis of sialic acid in *E. histolytica*. (Adapted from Chava AK, Bandyopadhyay S, Chatterjee M, Mandal C. Sialoglycans in protozoal diseases: their detection, modes of acquisition and emerging biological roles. *Glycoconj J* 2004;20:199–206.)

4.6.2 Cnidarians

Mechanoreceptors in sea-anemone tentacles are activated on binding to acetylated sugars and proline from prey [211] while tentacles of certain sea anemones bind *N*-acetylneuraminic acid (NANA) predisposing

contact-sensitive mechanoreceptors (CSMs) [212–215] to trigger discharge upon physical contact of prey and cyclic AMP (cAMP) has been reported to be involved in NANA-sensitized nematocyst discharge [212, 216]. NnL lectin from a jellyfish *Nemopilema nomurai* (*N. nomurai*) revealed hemagglutinating activity that was inhibited by *N*-acetyl-D-galactosamine NANA and Neu5Gc [217].

4.6.3 Helminths

Lymphatic filariasis reveals altered IgG glycosylation, and while decreased galactosylation bears relation with inflammation, increased sialylation is associated with anti-inflammatory responses [218]. Mucins of *Haemonchus contortus* (*H. contortus*) or *Teladorsagia circumcincta* (*T. circumcincta*) parasites in sheep revealed fucose, glucosamine, galactose, and galactosamine and minute amounts of sialic acids [219]. *Schistosoma bovis* (*S. bovis*) a parasite of wild and domestic ruminants lack sialic acid expression but expressed complex-type *N*-glycans and immunogenic GalNAc β 1–4GlcNAc (LDN) terminate antennae on excretory-secretory (ES) glycoproteins [220]. Dogs infected with adult tapeworms of *Echinococcus granulosus* (*E. granulosus*) release fecal antigens (coproantigens) constituting α -D-mannose and/or α -D-glucose, β -galactose and *N*-acetyl- β -glucosamine, *N*-acetyl- β -glucosamine, and sialic acid residues [221] and antigenic properties occur in cyst-derived glycolipids [222]. Sialic acids were identified in the acidic fraction of glycolipids of *E. granulosus* metacestode tissue [221]. The altered glycosylation of intestinal mucins of mouse infected with *Nippostrongylus brasiliensis* (*N. brasiliensis*) revealed a transferase adding a terminal GalNAc to sialic acid-containing epitope in rat [223]. The sialylation of mucins during a 13-day infectious cycle in Sprague-Dawley rats infected with *N. brasiliensis* parasite revealed a relative decrease in Neu5Gc compared with Neu5Ac by decreased expression of a CMAH hydroxylase [224]. The removal of adult worms of parasite *N. brasiliensis* from the small intestinal goblet cell mucins of mice is hypothesized to be possibly associated with terminal GalNAc and sialic acid residues of the small intestinal goblet cell mucins prior to infection [225].

Recently *Schistosoma mansoni* (*S. mansoni*), causing human schistosomiasis, has been reported to be rich in fucose, containing terminal beta-GalNAc residues but lack sialic acid [226, 227]. The expressed fucosylated glycans containing Lewis x (Le(x)) antigen common to human leukocytes and other tissues produce autoantibodies, thereby probably playing a role in affecting lymphocyte functions. Triantennary- and biantennary-like complex-type asparagine-linked glycoproteins with the expression of mannose, fucose,

N-acetylglucosamine, and *N*-acetylgalactosamine in *S. mansoni* have been associated with a role in the host immune response to infection [228]. A cysticercus membrane glycoprotein antigen has been identified with hexoses and sialic acids [229].

4.6.4 *Annedida*

Very few studies have reported the observation of sialic acid in earthworm. Glycolipid fraction of earthworm *Lumbricus terrestris* (*L. terrestris*) has been reported to include cerebrosides and sulfatides containing glucose and galactose, and gangliosides containing glucosamine and sialic acid [230].

4.6.5 *Arthropoda*

Sialylation pathway in *Drosophila* reveals similarities in the initial steps to the mammalian sialylation pathways indicating a probable common evolutionary origin [231, 232]. *N*-glycan processing in insects reveal similarity at early steps with differences in subsequent steps to mammalian *N*-glycan synthesis with the insect cell lines not processed to terminally sialylated complex-type structures but modified to paucimannosidic or oligomannose structures thus differing from the mammalian cells due to the lack of enzymes including glycosyltransferases involved in generating complex-type structures and appropriate sugar nucleotides [233]. The baculovirus-insect cell expression system has been used to produce recombinant therapeutic glycoproteins [234]. Neu5Ac has been reported to occur in larvae of the cicada *Philaenus spumarius* (*P. spumarius*) [26].

4.6.6 *Mollusca*

Sialic acid-binding lectins (SABLs) SgSABL-1 and SgSABL-2 of *Solen grandis* (*S. grandis*) have been revealed to have functions like pattern-recognition receptor (PRRs) and hypothesized to be involved in the innate immune response of *S. grandis* [235, 236]. Siglec gene or CgSiglec-1 has been characterized from the Pacific oyster, *Crassostrea gigas* (*C. gigas*) [237].

Gastropod *Haliotis tuberculata* (*H. tuberculata*) foot epithelium reveal *N*-glycoproteins rich in fucose and mannose while secretory cells reveal expressions of acidic sulfated glycoconjugates such as glycosaminoglycans and mucins, enriched with galactose, *N*-acetylgalactosamine, and *N*-acetylglucosamine but foot epithelium lack sialic acid [238]. Lectins were known to participate in immune recognition and host defense and Chsalectin, a novel sialic acid-binding lectin, was reported to occur in *Crassostrea hongkongensis* (*C. hongkongensis*) with a role in immune recognition and

host defense against bacterial infection caused by *C. hongkongensis* [239]. SABL from Manila clam *Venerupis philippinarum*, VpSABL [240], has been reported. A novel sialic acid-specific lectin (MCsialec) was detected from Manila clam hemocytes infected with *Perkinsus olseni* that plays a vital role during pathogenic infection [241]. Scalarin from the eggs of *Pomacea canaliculata* (*P. canaliculata*, Lamarck, 1822) and *Pomacea scalaris* (*P. scalaris*, d'Orbigny, 1835) revealed expression of terminal sialic acid residues possibly resistant to neuraminidase and O-linked residues derived from the T and Tn antigens [242]. N-linked oligosaccharides are expressed in the nacreous layer of Japanese pearl oyster *Pinctada fucata* (*P. fucata*) [243]. A novel α/β -galactoside α -2,3-sialyltransferase was expressed by luminous marine bacterium, *Photobacterium phosphoreum* JT-ISH-467, isolated from the Japanese common squid (*Todarodes pacificus*, *T. pacificus*) [244]. Garden snail *Cepaea hortensis* (*C. hortensis*) [245, 246] has been reported to express sialic acid-specific lectin. *C. hortensis* agglutinin-I (CHA-I) lectin binds to O-linked sialic acids [247]. Newly hatched Hawaiian squid *Euprymna scolopes* (*E. scolopes*) rapidly become colonized by the bioluminescent marine bacterium *Vibrio fischeri* which exhibited the unusual ability to migrate to nucleosides, nucleotides, and sialic acid, a component of squid mucus [248]. Neu5Ac and Neu5Gc have been reported to occur in slug *Arion lusitanicus* (Gastropoda) revealing specificity toward MAA [249]. Eye lenses of common squid (*T. pacificus*) and Pacific octopus (*Octopus vulgaris*, *O. vulgaris*) has been known to express gangliosides including gangliotetraose species and c-series gangliosides [250]. Acidic lipids from *T. pacificus* and *O. vulgaris* revealed expression of Neu5Ac. Lipid-bound sialic acid in cerebral ganglia were significantly lower as compared to the expression in hepatopancreatic tissues indicative of ganglioside expression in protostomia [167].

Achatinin, a 9-O-acetyl sialic acid (9-O-AcSA) binding lectin, has been isolated from *A. fulica* snails with sugar specificity toward 9-O-AcSA α 2 \rightarrow 6GalNAc [251]. Sialic acids have been reported to occur in two marine bivalves, the Pacific oyster *C. gigas* and the horse mussel *Modiolus modiolus* (*M. modiolus*) [252]. A heterogeneous SABL with affinity toward bacterial LPS was expressed in hemolymph of *M. modiolus* [253]. The *M. modiolus* SABL is reported to agglutinate erythrocytes and bacterial LPS and react with sialoconjugates [254]. A cDNA library of *Limax flavus* (*L. flavus*) was constructed and screened for sialic acid-specific lectin [255]. A total of 16 lectins have been reported to be expressed in the digestive gland of the bivalve mollusc *Mytilus galloprovincialis* (*M. galloprovincialis*) [256]. *Salmonella djakarta* (*S. djakarta*) and *Salmonella isaszeg* (*S. isaszeg*) LPS were investigated

for neuraminic acid expression [257]. Neu5Gc-specific lectin (AFL) has been isolated from the foot muscles of the marine clam *Anadara granosa* (*A. granosa*) [258]. A Neu5Gc-specific lectin (PAL) has been isolated from apple snail, *Pila globosa* (*P. globosa*) [259]. A sialic acid lectin is expressed in slug *Limax* [260]. Sialic acid-containing substrates as intracellular calcium receptors have been reported to be involved in transmitter release [261].

4.6.7 Echinoderms

In echinoderms including starfish and sea urchin, expression of Neu5Gc is predominant [50, 59, 60, 64]. Di-sialoglycoconjugates have additional Neu5Gc, O-methyl-Neu5Gc, N-acetyl-O-methylneuraminic acid, and N-glycoloyl-O-methyl neuraminic acid [262, 263]. Starfish *Asterias rubens* (*A. rubens*) reveals expression of 8-O-methyl-5-Neu5Gc (Neu5Gc8Me) [264] with Neu5Ac, Neu5Gc, and their O-acetylated derivatives, while starfish additionally possess 8-O-methylated sialic acids [264, 265]. Neu5Gc is formed by CMP-Neu5Ac hydroxylase found in gonads of starfish *A. rubens* revealing similarities to the mammals. However, the echinoderm hydroxylase reveals differences from the mammalian counterpart in membrane association and a requirement for high ionic strength for optimal activity [266, 267] which has been cloned [266].

4.6.8 Vertebrates

Intestinal glycoconjugates of the blunthead pufferfish *Sphoeroides pachygaster* (*S. pachygaster*) and gray triggerfish *Balistes capriscus* (*B. capriscus*) reveal expression of GalNAc and GlcNAc residuals with GalNAc residuals in *S. pachygaster* subterminal to sialic acid [268].

Zebra fish *Danio rerio* (*D. rerio*) has been studied extensively for glycosylation [269–271] and development of vertebrates and reported the expression of protein and lipid-associated alpha2–8-linked oligosialic acid motifs in the early development [272]. CMP-Sia synthetase (CMAS) has been reported to occur in *D. rerio* (dreCmas) [273]. PSA action has been reported during axon growth and pathfinding in the developing zebra fish CNS [274].

Sialic acid acetyltransferase (SIAE) removes acetyl moieties from the carbon 9 and 4 hydroxyl groups of sialic acid and adult fish reveal expression of siae mRNA in heart, eye, muscle, liver, brain, kidney, and ovary revealing their role in immune system function and the development of central nervous system [270].

The epidermis of sea lamprey *Petromyzon marinus* (*P. marinus*) reveals expression of glycoconjugates including sulfated glycosaminoglycans

(*N*-acetylglucosamine and *N*-acetylgalactosamine) and *N*-glycoproteins rich in mannose in the mucous. The skin cells, a unique cell of lampreys, reveal expression of l-fucose and sialic acid residues that is lost with metamorphosis [275].

Sialidase removes sialic acids from glycoconjugates and medaka sialidase Neu1 has been reported to exhibit desialylation of α 2–3 sialic acid linkage [276]. Gangliosides has been reported to occur in fish containing only *N*-acetylneuraminic acid/sialic acid, while beef, chicken, and pork contained GD1a/b species that incorporated both Neu5Ac and Neu5Gc and hydroxylated fatty acids [277]. Mucin *O*-glycosylation of five freshwater acclimated Atlantic salmon have been reported to contain sialylated intestinal mucins, Neu5Ac and sialylated core 5 was the most dominant structure with a probable role in host–pathogen interactions [278]. Fish skin mucus reveals NANA/sialic acid, glucose, *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose, and fucose residues [278]. Trisialyllactosylceramide, GT3, containing an *O*-acetylated sialic acid has been reported to occur in cod fish brain [279]. In fish neu4 sialidase, neu4 gene has been reported and was cloned from medaka brain mRNA with a probable role in embryonic development [280]. Polysialic acid (PSA) linked to neural cell adhesion molecule NCAM1 forms PSA-NCAM1 by polysialyltransferases STX with functions during the development of vertebrate nervous systems including axon extension and fasciculation. NCAM1 and NCAM2 have been reported in tetrapods and fishes [281].

PSA-NCAM plays a vital role in neuronal differentiation, maintenance, plasticity, and regeneration. In zebra fish homologues of STX (St8sia2) and PST (St8sia4) have been studied and found that PSA-NCAM regulates motility for cerebellar neuronal progenitors [282] and NANS-mediated synthesis of sialic acid was essential for the development of brain and skeleton [283]. Sialyltransferase gene found in the tunicate *Ciona intestinalis* (*C. intestinalis*) reveals a possible ortholog of the common ancestor of galactose α 2,3-sialyltransferases and ST3Gal II gene from the bony fish *Takifugu rubripes* (*T. rubripes*) [284]. Polysialoglycoprotein (PSGP) in salmonid fish egg is a unique glycoprotein bearing α 2,8-linked PSA on its *O*-linked glycans and two α 2,8-polysialyltransferases (α 2,8-polySTs), PST (ST8Sia IV) and STX (ST8Sia II), have been reported for PSA biosynthesis on *N*-glycans of glycoproteins in mammal [285].

Unambiguous orthologs of mammalian siglec-4, exclusively expressed in the nervous system, has been identified from fugu and zebra fish. As in mammals, fish siglec-4 is expressed by nervous tissue. Fish Siglec-4 recombinant protein, fish siglec-4 has been reported to bind to sialic acids with a

specificity similar to the mammalian orthologs indicating that siglec occurs in the nervous system of all vertebrates [286]. Infectious salmon anemia virus (ISAV) causes infections in farmed Atlantic salmon. Purified ISAV hydrolyzed free 5-*N*-acetyl-4-*O*-acetyl neuraminic acid and the enzymatic activity of the HA-esterase of ISAV revealed similarities to sialate-4-*O*-esterases of murine coronaviruses and related group 2 coronaviruses [287]. PSA-NCAM facilitates axon growth. In lizard, retinal ganglion cell axons have been reported to be transiently PSA-NCAM positive, whereas in goldfish retinal ganglion cell (RGC) axons are PSA-NCAM negative. PSA-NCAM is negative both in normal animals and throughout regeneration with the exception of a PSA-NCAM-positive fascicle arising from newly generated RGCs [288].

Epidermal, branchial, and digestive mucous cells, and the gastric glands of larvae/postlarvae of three fish species (two teleostean and a chondrosteian) revealed negative Con A lectin staining, but oesophageal mucous cell of sturgeon revealed expression of mannose -Man- and/or glucose -Glc-, L-fucose -Fuc-, *N*-acetyl-*D*-galactosamine -GalNAc-, *N*-acetyl-*D*-glucosamine, -GlcNAc-, and/or sialic acid-NANA-residues [289]. Fish type III antifreeze protein is homologous to the C-terminal region of mammalian sialic acid synthase [290]. In all 18 gangliosides were isolated from dogfish *Squalus acanthias* (*S. acanthias*) brain, including GM2, GQ1c, GP1c, and GD2 [291]. CRPs in *Labeo rohita* (*L. rohita*) reveal microheterogeneity [292]. Cichlid fish and rat brains also contained GM1b-, GT1b-, and GQ1c-synthase and sialyltransferase activities [293].

α 2 \rightarrow 8-Linked PSA chains terminate *O*-linked oligosaccharide chains on Salmonidae fish egg polysialoglycoproteins (PSGPs) the expression of which are developmentally regulated [294]. Gangliosides expression is reported to occur in electric organ of *Torpedo marmorata*: synaptosomes, presynaptic membranes, postsynaptic membranes, and synaptic vesicle membranes [295]. A 9-*O*-acetylated GT2 has been reported to occur in cod fish brain [296]. A trisialyllactosylceramide GT3 was found in cod fish brain with a chemical structure of II3(9-*O*-Ac-NeuAc2-8NeuAc2-8NeuAc2-3)Lac Cer [297]. Trout liver has been reported to express sialate cytidylyltransferase activity. The sialic acid fraction of trout liver after hydrolysis is composed of *N*-acetylneuraminic acid, *N*-acetyl-9-*O*-acetylneuraminic acid, and *N*-acetyl-9-*O*-lactoylneuraminic acid [298].

The luminal surface of the saccular macula in the rainbow trout revealed expression of a glycoconjugate constituting glucose, galactose,

fucose, mannose, *N*-acetylglucosamine, *N*-acetylneuraminic acid, and *N*-acetylgalactosamine [299].

Carbohydrate-rich sialoglycopolyprotein was isolated from the fertilized eggs of the Medaka fish species, *Oryzias melastigma* (*O. melastigma*), as a member of the L-hyosporin family [300].

The mucin-like keratan sulfate glycopolymer has been reported to occur in ampullae of lorenzini [301]. Amphibians have been studied for molecules with anticancer properties. Onconase, amphinase, cSBL from *Rana catesbeiana* (*R. catesbeiana*) eggs, and jSBL from *Rana japonica* (*R. japonica*) eggs, which belong to the RNase A family, were purified from the oocyte cells and eggs of three amphibians, and they were found to induce cytotoxicity by degrading cellular RNA [302]. *Xenopus* embryos revealed expression of polysialoganglioside including asialo-GMI as the core structure of the ganglioside XI and palmitic and oleic acid as the fatty acids of the ceramide moiety [303].

N-glycan structures have been identified from snake venoms [304]. Four different sialidase forms are known in vertebrates: the lysosomal NEU1, the cytosolic NEU2 and the membrane-associated NEU3 and NEU4. Sialidase orthologs from 21 different organisms have been identified and shown distribution in the evolutionary tree including Metazoa relative, marine choanoflagellate *Monosiga brevicollis* (*M. brevicollis*), early Deuterostomia, precursor of Chordata, and Vertebrata (teleost fishes, amphibians, reptiles, avians and early and recent mammals [305]. Mucins in the alimentary tract of the grass snake, *Natrix natrix* (*N. natrix*), has revealed expression of mannosylated sialosulfomucins and fundic mucosa of stomach reveal expression of sialomucins with terminal sialic acid linked to galactose, with neck cells expressing sialomucins with mannose, *N*-acetylglucosamine, galactose, *N*-acetylgalactosamine, and fucose- α - (1,2)-linked residues [306]. A serine protease with thrombin-like activity (TLBan) expressed by *Bothrops andianus* (*B. andianus*) commonly called Andean Lancehead has been reported to contain both N-linked carbohydrates and sialic acid [307]. Asian pit viper venom reveals expression of partially *O*-acetylated NeuAc α 2-8NeuAc α 2-3Gal β 1-4GlcNAc β 1-terminal epitope [308]. Platypus revealed expression of Neu5Ac. Among nine reptiles and one turtle, Neu5Gc was expressed in the egg and an adult basilisk, belonging to the lizard family. BLAST analysis of platypus, chicken, and zebra finch genomes did not reveal similarity to CMAH structure. Monotremes including Platypus and Sauropsids including birds and reptiles lacked Neu5Gc synthesis machinery. Neu5Gc found in eggs may probably have been acquired from diet or by an alternative pathway [309]. Carbohydrate components of the ductus epididymis

epithelium of a lizard revealed location differences [310]. DM43, an opossum serum protein inhibitor of snake venom metalloproteinases, revealed constitution of *N*-acetylglucosamine, mannose, galactose, and sialic acid forming four biantennary *N*-linked chains [311]. The expression of PNA-binding glycoproteins has been reported to occur in lizard lymphocytes [312]. A heavily glycosylated protein fraction was isolated from cobra venom containing both *O*- and *N*-linked oligosaccharides; 1 *N*-linked chain for every 8–10 *O*-linked oligosaccharides and the *O*-linked chains revealed expression of fucose, galactose, and *N*-acetylglucosamine more than *N*-acetylgalactosamine; with minute level of sialic acid lacking sulfate esters [313]. N-CAM has been shown to undergo decrease in sialic acid content during embryonic to adult conversion with an increase in binding efficacy thus regulating morphogenesis [314].

Sialic acids are found to be constituents of milk oligosaccharides, components of glycoproteins in blood, sera, or plasma of mammals. Human sera reveal expression of Neu5Ac and Neu5Ac9Lt (Lt=lactoyl) and minute quantities of *O*-acetylated sialic acid derivatives, Neu5,9Ac2 [89]. Sialic acid derivatives in serum glycoproteins reveal differences in expression in mammalian species and level and position of *O*-acetylation and Neu5Gc expression [2]. Sialic acid and its acetylated derivatives have been reported to occur in epithelial and mucous glycoproteins revealing the presence of Neu5Ac, Neu5,9Ac2, and Neu5,7,9Ac3 [2]. Gangliosides have also been reported. Pathogens are known to bind to sialic acid on human cell surfaces (Table 4).

Table 4 Pathogens that bind to sialic acids on human cell surfaces

Pathogen	Binding protein	Known target sialylated sequence
Human Influenza A	Hemagglutinin	Sia α 2–6Gal(NAc)
Avian Influenza A	Hemagglutinin	Sia α 2–3Gal β 1–
Human Influenza C	Hemagglutinin- esterase	9-O-Ac-Sia α 2–
Vibrio cholera	Toxin	Gal β 1–3GalNAc β 1,4(Sia α 2–3) Lac-Cer
Plasmodium falciparum	EBA-175	Sia α 2–3Gal β 1–3(Sia α 2–6) GalNAc-O-
Clostridium botulinum	Toxin	Polysialogangliosides
Helicobacter pylori	SabA	Sia α 2–3Gal on gangliosides

Table adapted with permission from Ajit Varki Sialic acids in human health and disease Trends Mol Med. 2008; 14(8): 351–360.

Sialic acid-binding protein

Sialic acid components of oligosaccharide side chains in glycoconjugates occur in most higher animals and a few microorganisms act as ligands in glycobiological interactions on binding to a specific sialic acid-binding protein acting as receptors [315].

Siglecs

Siglecs are sialic acid-binding immunoglobulin (Ig)-type lectins which are the members of the immunoglobulin superfamily that act as transmembrane cell surface immune regulatory receptors predominantly found on hematopoietic cells containing an N terminal V-set Ig-like domain with sialic acid-binding sites that recognizes different sialylated glycoconjugates, leading to the activation or inhibition of the immune response. Siglecs include (a) CD33-related Siglecs and (b) Siglec-1 (Sialoadhesin), Siglec-2 (CD22), Siglec-4 (myelin-associated glycoprotein, MAG), and Siglec-15. Phylogenetic studies in higher vertebrates including fishes, amphibians, birds, reptiles and mammals have revealed that Siglecs are conserved in evolution [316]. A loss of Siglec genes in rodents have been reported.

The cytoplasmic domain of most Siglecs contain immune receptor tyrosine-based inhibitory motifs (ITIMs) that recruit tyrosine phosphatases SHP-1 and SHP-2 and function as inhibiting receptors, inhibiting signal transduction. Siglec-14, Siglec-15, and Siglec-16 associate with tyrosine-based activation motif (ITAM) adaptor DAP12 and act as activating receptors by recruiting SYK kinase. Siglecs are known to play a vital role in immune regulation in host-pathogen interaction in infectious diseases, inflammation, neurodegeneration, autoimmune diseases, and cancer [317]. The sialic acid-Siglec axis has been recently reported to be exploited by tumors and pathogens for the induction of immune tolerance [318].

Sialic acid-binding lectins

SABLs are lectins that specifically recognize sialic acid residues [319]. They have been reported to occur in plants and animal sources with diverse specificity and are being exploited for analytical properties (Table 5).

Selectins

Selectins are a diverse group of calcium-dependent, type I transmembrane molecules that bind to sialylated, fucosylated carbohydrates, function in vascular adhesion, and play a significant role in inflammation, immunity, hemostasis, and cancer progression. Selectin ligand reveal overexpression of

Table 5 Vertebrate sialic acid-specific lectins [18, 320–348]

Names (synonyms)	Expression (source) ^a	Binding specificity
E-selectin (ELAM-1, CD62E)	Activated endothelium	sialyl Le ^x , sialyl Le ^a
L-selectin (MEL 14 antigen, CD62L)	Leucocyte	6'-sulfo sialyl Le ^x , heparan sulfate
P-selectin (GMP 140, PADGEM, CD62P)	Activated endothelium, platelet	sialyl Le ^x , sialyl Le ^a , heparan sulfate (HS)
Siglecs		
Siglec-1 (sialoadhesin)	Macrophage	Neu5Ac α 2–3Gal > Neu5Ac α 2–6Gal
Siglec-2 (CD22)	B-cell	Sia α 2–6Gal
Siglec-3 (CD33)	Myeloid precursor, Mo ^{b,c}	Sia α 2–6Gal \geq 2 Sia α 2–3Gal
Siglec-4 (MAG)	Glial cells	Neu5Ac α 2–3Gal on complex gangliosides
Siglec-5	Mo, Gr	Sia α 2–6Gal \approx Sio α 2–3Gal, Neu5Ac α 2–8
Siglec-6 (OB-BP1)	Placenta, B-cell	Sia α 2–6Gal NAc
Siglec-7 (AIRM-1)	NK cells, Mo, Gr	Sia α 2–6Gal \approx Sia α 2–3Gal (3-Ig isoform), Sia α 2–6Gal (2-Ig isoform)
Siglec-8	Eosinophil, basophil	Sia α 2–3Gal \geq Sia α 2–6Gal
Siglec-9	Mo, Gr	Sia α 2–6Gal \approx Sia α 2–3Gal
Siglec-10	B-cell	Sia α 2–6Gal \approx Sia α 2–3Gal
Siglec-11	Fibroblasts	Neu5Ac α 2–8Neu5Ac
Others		
Complement factor H	Blood	Sia; C7–C9 side chain is a part of epitope
CD83	Dendritic cell	Sia
LI	Mouse neuron	Neu5Ac α 2–3 (on CD24)
Interleukin-1 α	Blood	Neu5Ac α 2–3Gal β 1–4GlcNAc, biantennary
Interleukin-1 β	Blood	Neu5Ac α 2–3Gal β 1–Cer (GM4)
Interleukin-2	Blood	GD1b
Interleukin-4	Blood	Neu5Ac1,7lactone
Interleukin-7	Blood	Neu5Ac α 2–6GalNAc
Laminin	Extracellular matrix	Neu5Ac α 2–3Gal β 1–4GlcNAc (α 2–3 > α 2–6)
Sialic acid-binding protein	Endometrium	Neu5Gc > Neu5Ac
Sarcolectin	Placenta	Neu5Ac, Neu5Gc

Table 5 Vertebrate sialic acid-specific lectins [18, 323–351]—cont'd

Names (synonyms)	Expression (source) ^a	Binding specificity
Calreticulin	Ovine placenta	Neu5Gc > Neu5Ac, prefer O-acetyl
Sialic acid-binding protein	Rat uterus	Sia
Calcyclin	Bovine heart	Neu5Gc
Sialic acid-binding protein	Frog egg	Sia
Ganglioside binding protein*	Rat brain myelin	Gangliosides (CT1b, CQ1b, GD1b)
Hemagglutinin*	Rat brain	Neu5Ac, Neu5Gc

^a Expression (source) is in human, unless otherwise stated.

^b Mo, monocyte; Gr, granulocyte.

^c Most of the proteins are cloned and/or purified to homogeneity, except for the entities marked with an asterisk (*).

Adapted with permission and updation from Angata T, Varki A. Chemical diversity in the sialic acids and related α -keto acids: an evolutionary perspective. *Chem Rev* 2002;102:439–469.

tumor cells leading to increased metastasis, poor prognosis, mediate tumor cell adhesion, extravasation during metastasis, and activate signaling cascade in tumor.

Selectins play a vital role in leukocyte homing. L-Selectin binding to ligands on leukocytes activates leukocytes. Selectins enable interactions with platelets and endothelial cells. The interaction between selectin ligand P-selectin glycoprotein ligand 1 PSGL-1 on leukocytes and P-selectin on platelet or E-selectin on endothelial cells triggers intracellular signaling in leukocytes.

Selectins and their ligands play an important role in the human implantation. L-selectin on interaction with its ligands plays a critical role in the adhesion of the blastocyst to the endometrium at the maternal-fetal interface. P-selectin and E-selectin play a vital role in human implantation [349]. PSGL-1 is now known as a major participant in inflammation, thrombus, along with cancer [350]. Due to their role in cancer immune system modulation they are being studied as possible targets for controlling tumor immunity [351].

4.6.9 Sialylation and disease

Although sialic acids are found to occupy the terminal position of glycans of all cell types, in disease states, like cancer or immunological disorders, the sialylation profile of cells in affected tissues manifest altered downregulation or overexpression—or neoexpression of certain glycan structures [352].

Aberrant sialylation of oligosaccharide branches of *N*-glycans (β 1,6-GlcNAc branching) or affected terminal glycan sequences, like premature sialylation of truncated saccharide units, are reported to occur due to affected or altered enzyme expression [352]. In rheumatoid arthritis, an incomplete IgG glycosylation with galactose and sialic acid is observed to lead to immune disorder [353].

But upregulated expression of sialyltransferases like ST3Gal-I is observed in human BC [354] and upregulated ST6Gal-I is reported in human BC and colon cancer. Ganglioside overexpression in cancer has been reported such as CNS-specific GD3s from tumor tissues [355]. The overexpression of 9-*O*-acetylated sialic acid in BC [356], childhood ALL [83, 103–107] are reported.

Altered cell sialylation in cancer affects tumor cell interactions with other cells affecting cell adhesion, migration, and metastasis [357–359]. Sialylation affects natural killer (NK) cell cytotoxicity [360]. Tumor cell hypersialylation has been observed to enable tumor cell to evade recognition by NK cells, thus escaping the immune responses. Hypersialylation of tumor cells enables it to escape the immune surveillance [358].

Sialic acid ligand-protein interaction is being exploited for design and development of therapeutics and anticancer therapies containing sialoside that targets siglecs [361]. Nanoparticle formulations are being tested to deliver potential therapeutic agents to the target cell [317].

4.6.10 Tumor-associated carbohydrate antigens

Upregulated sialyltransferases and fucosyltransferases lead to the overexpression of tumor-associated carbohydrate antigens (TACA), being mucin related to Thomsen nouvelle (Tn) antigen (Tn), the sialyl-Thomsen nouvelle (sTn) antigen, Thomsen-Friedenreich antigen (TF-Ag), the blood group-related Thomsen-Lewis antigens Lewis^Y, Sialyl Lewis^X and Sialyl Lewis^A, and Lewis^X (or specific embryonic antigen-1, SSEA-1), the glycosphingolipids Globo H, stage-specific embryonic antigen-3 (SSEA-3), sialic acid-containing glycosphingolipids, the gangliosides GD2, GD3, GM2, fucosyl GM1, and Neu5GcGM3, and PSA [362] that have been implicated in tumor, metastasis, and poor prognosis. Some TACAs are also expressed in fetal tissue, and termed as oncofetal antigens [362].

TF-Ags increased the expression in pancarcinoma and carcinomas of the breast, colon, bladder, prostate, liver, and stomach as compared to normal cells and its role in metastasis renders it as an important tumor target [362].

The overexpression of Lewis^Y in ovarian, breast, prostate, colon, and epithelial cell lung cancers than in the normal cells makes them potential targets. The overexpression of SLe^X in breast, ovarian, melanoma, colon, liver, lung, and prostate cancers and SLe^A in breast, colon, and pancreas cancers, and in melanomas due to the upregulated expression of ST3Gal-III and FucT-III enzymes, synthesizing sLea and ST3Gal-IV, ST3Gal-VI, and FucTVII, catalyzing the synthesis of sLex, or due to deficiency in the enzymes responsible for sulfation, and their overexpression acting as *E*-selectin ligands contribute to metastasis and poor prognosis makes them potential targets. The treatment of these antigens with antibodies inhibited metastasis in pancreatic tumor mouse models. PSA is overexpressed in small cell lung cancer (SCLC), rhabdomyosarcoma, Wilms tumor, and neuroblastoma and PSA-NCAM is involved in increased tumor growth and metastasis with decreased patient survival. Endoneuraminidase N, cleaving PSA could stop cell growth in rhabdomyosarcoma and neuroblastoma cells [362].

Downregulated sialyltransferases and fucosyltransferases lead to reduced cell surface sialylation and reduced TACA expression, decreasing adhesion and migration potency and metastatic activity [363].

sTn antigen generated by sialylation of Tn antigen [364] by ST6GalNAc I occurs rarely in healthy tissues but has been observed in epithelial cancer cells and breast tumors [365].

4.6.11 Sialic acids and therapeutics: Where we stand

Strategies like enzyme inhibitors for disease-associated carbohydrates are being studied, designed, and developed. Inhibitors for the carbohydrate-binding receptors are also being conceived as a strategy [366]. X-ray crystallographic structures of mammalian sialyltransferases (STs) complexed with their ligands are enabling us to better understand the substrate specificities and inhibitor design [367].

Inhibition of STs, glycosyltransferases, is a strategy to reduce cell surface sialylation particularly in cancer [368]. Lack of ST3Gal-IV and ST3Gal-VI reduces sialic acid containing ligands for selectin interactions, thus affecting leukocyte homing and leukocyte recruitment at inflammation sites [369]. *Streptococcus pneumonia* leads to septicemia, meningitis, and community-acquired pneumonia in human. Although prophylactic vaccines are the strategies available for disease targeting, *Streptococcus pneumonia* (*S. pneumonia*) sialidases, including NanA and NanB, implicated in the pathogenesis of *S. pneumonia* and design of novel NanB-selective inhibitors are being tested for drug targets [370].

Binding with selectin antagonists, like bimosiamose (TBC-1269) and GSC-150, sialoside-selectin interactions can be blocked; selectin antagonists also find application in the treatment of inflammatory disorders, autoimmune diseases and metastatic cancers [371]. 11a sLex-derived inhibitor of E-selectin, CGP69669A, with affinity higher than natural ligand sLex has been designed [372]. Modified sLex-containing glycopeptide analogues for E-selectin inhibition has been designed [373]. P-selectin antagonist, GMI-1070, is in phase II clinical trials for the treatment of sickle cell crisis, associated with sickle cell disease (SCD) [374]. Bimosiamose (TBC-1269), a P-selectin antagonist, has been reported to have successfully completed phase II clinical trials for psoriasis and chronic obstructive pulmonary disease (COPD) [375].

N-acetylglucosamine 2-epimerase and *N*-acetylneuraminic acid aldolase allow conversion of simple and sugars into different sialic acid-related compounds from whole cell extracts enabling large scale and economical synthesis of sialic acid and sialyloligosaccharides [376].

Low molecular weight antagonists, targeting sialoside interaction with the lectin MAG, have been designed as a therapeutic strategy to promote axon regeneration after neuronal injury [377]. Zanamivir (*Relenza*) and oseltamivir (*Tamiflu*) are designed as sialic acid-based inhibitors of the viral enzyme neuraminidase for targeting influenza infections [378].

But design of carbohydrate-derived drug molecules faces challenges such as pharmacokinetic profiles, poor bioavailability, requirement for active transport through membranes, short plasma half-life, poor metabolic stability, and are rapidly excreted [379, 380]. However, this area of biology is developing with studies or approaches to improve their pharmacokinetic profiles like systematic structural modifications such as the replacement of certain moieties, the introduction of hydrophobic moieties, and/or the incorporation of a prodrug strategy leading to compounds that mimic the biological activity of their carbohydrate precursors called as 'glycomimetics' like neuraminidase inhibitors zanamivir and oseltamivir [381, 382].

Drugs targeting glycan-lectin interactions need to be designed by synthetic introduction of moieties that increase the affinity. Inhibition of enzymes in the synthesis of tumor-associated glycans is another strategy [383].

Liposomal nanoparticle are being exploited in selective delivery of drugs and therapeutics like chemotherapeutic doxorubicin in B cell lymphoma, wherein high-affinity ligand, 9-*N*-biphenylcarboxyl (BPC)-Neu5Ac- α 2,6-Gal- β 1,4-GlcNAc, on the liposomal surface confers the selectivity being recognized by CD22 (Siglec-2), exclusively expressed on B cells and

trisaccharide derivative BPC-Neu5Ac- α 2,6-Gal- β 1,4-GlcNAc conjugated to pegylated lipid is used in vesicle formation with encapsulated doxorubicin thereby selectively targeting CD22-expressing cells, including B cell lymphoma [384–386].

Selective ligands targeting the lectins CD22 and CD33 are being designed [387–389]. 4-Cyclohexyl-1,2,3-triazole residues at position 5 of sialic acid in combination with meta-substituted moieties at position 9 have been reported to improve affinity and selectivity profile [390].

Therapeutic antibodies

Therapeutic antibodies find application in leukemia and lymphoma. CD33 in leukemic cells and CD22 in B-cell lymphoma are being exploited for targeting. Anti-siglec-monoclonal antibodies conjugated to toxins or chemotherapeutic agents are being designed like gemtuzumab (*Mylotarg*), an anti-CD33-antibody in acute myeloid leukemia (AML), approved by the FDA in 2000 but faces challenges of safety issues [390, 391] and epratuzumab, an anti-CD22- antibody, is being designed for targeting non-Hodgkin lymphoma (NHL) and the autoimmune disorder systemic lupus erythematosus (SLE) [392].

Carbohydrate-based vaccines

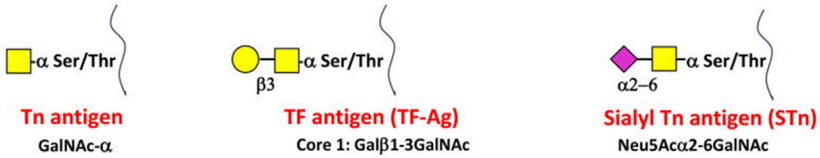
Carbohydrate-based vaccines designed based on altered cell surface glycans in disease as compared to normal cells are the latest strategy for targeting cancer, developed over the last two decades, and are used after conventional therapeutic modes of surgery, radiation, and chemotherapy [390, 392].

TACA are being exploited as vaccine targets for their elimination. However, they suffer challenges of being poorly immunogenic self-antigens and strategies to improve antigenicity like conjugating to a carrier protein, for example, keyhole limpet hemocyanin (KLH), and attachment sites for carbohydrate antigen conjugation [390, 393]. Vaccine constructs need to be designed to activate both cell mediated and humoral immune responses against specific carbohydrate epitopes of tumor cells [390, 394, 395]. *Theratope*, a sTn-KLH conjugate, is in clinical practice since 2002 for metastatic BC and colorectal cancer, however, is still in phase III clinical trials since 2003 [390, 396, 397].

Globo H, cancer vaccine against a carbohydrate epitope is unique to target prostate and ovarian cancer cells, such as TACA, including Globo H, sTn, Tn, TF, and Ley antigens attached to a MUC1 glycopeptide backbone conjugated to KLH [362, 390, 394, 398, 399] (Fig. 10). Mimicking of the

Tumor related carbohydrates

The mucins



The blood group related antigens: Lewis structures

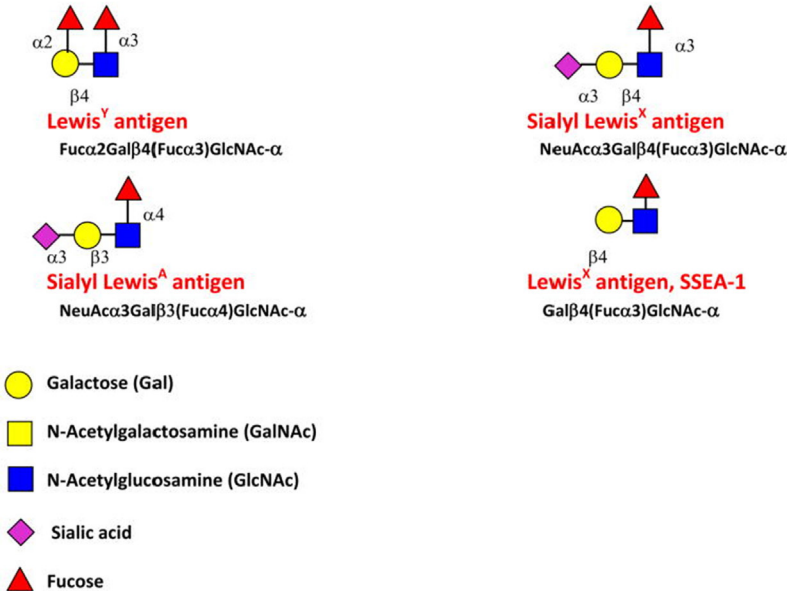


Fig. 10 Structures of the mucin carbohydrate antigens Tn, Sialyl Tn, and TF, and the Lewis blood group-related antigens. (A) Lewis^Y, Sialyl Lewis^X and Sialyl Lewis^A, and Lewis^X. (B) Structures of the glycosphingolipids, the globo series Globo H and SSEA-3, and the gangliosides GM2, GD2, GD3, and Fucosyl GM1. (C) Structures of the additional sialic acid-containing compounds. NeuGcGM3 and polysialic acid. (Adapted with permission from Heimbürg-Molinaro J, Lum M, Vijay G, Jain M, Almogren A, Rittenhouse-Olson K, Cancer vaccines and carbohydrate epitopes. *Vaccine* 2011;29:8802–26.)

tumor cell's natural carbohydrate epitope composition is hypothesized for a more effective immune response.

Sialic acid containing SSEA3 (Gb5) and SSEA4 detected on BC cells and other types of cancer [390, 400–402] finds application in anticancer vaccine development. Hypersialylation has been observed to increase their

serum half-lives with increased stability of luteinizing hormone (LH) and erythropoietin (EPO) [390, 403]. Ligand for Siglec-7 is being exploited in anticancer therapy.

Efficiency of Gp120 immunogens (MN- and A244-rgp120) used in the RV144 trial conferring protection against HIV were improved by enriching the sialic acid content and lacking N-linked glycosylation sites needed for binding by bN-mAbs [404].

Thus sialic acid is offering advantages in designing therapeutics and in targeting diseases like cancer and other immunological disorders.

5 Discussions

Sialic biology, with huge diversity in forms, structures, expression, metabolism, and functions in the diverse living world, is a fascinating science and our knowledge in this domain is growing day by day. While the pathogens primarily use sialic acid to mimic the host and escape immune responses, it has different functions in mediating cell-cell interaction, signaling, immune reactions, development and function in animals, and completely lacks in plants. Glycoengineering is enabling modification of plant glycosylation pathways toward the synthesis of mammalian glycoproteins which we have discussed in subsequent chapters. Insect sialylation revealing differences from the mammalian sialylation pathway has been discussed in the subsequent chapters.

In the cancer cells, overexpression of sialylation and enzymes involved is reported and forms an interesting area in cancer targeting. Glycomimetics is another such developing domain in the designing of sialic acid-related therapeutics. Nanobiotechnology and its application in sialic acid biology is an emerging domain and has been discussed in subsequent chapters.

At this point although a lot of studies has been done, the scope of applications of sialic acid in therapy is an emerging science which seems to be just the tip of the iceberg for the immensely important potential of sialic acid biology.

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