Trends in Microbiology

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Review

Effects of Sialic Acid Modifications on Virus Binding and Infection

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Sialic acids (Sias) are abundantly displayed on the surfaces of vertebrate cells, and particularly on all mucosal surfaces. Sias interact with microbes of many types, and are the targets of specific recognition by many different viruses. They may mediate virus binding and infection of cells, or alternatively can act as decoy receptors that bind virions and block virus infection. These nine-carbon backbone monosaccharides naturally occur in many different modified forms, and are attached to underlying glycans through varied linkages, creating significant diversity in the pathogen receptor forms. Here we review the current knowledge regarding the distribution of modified Sias in different vertebrate hosts, tissues, and cells, their effects on viral pathogens where those have been examined, and outline unresolved questions.

Interaction between Viruses and Sias

Sialic acids (see Glossary) generally occur as terminal monosaccharides on many different cell surfaces and secreted glycoconjugates and are therefore involved in key interactions between cells and many different viruses (as well as other pathogens) at various points in their infection and transmission cycles. Many viruses specifically bind to host Sias and use them as primary receptors for cell infection, or as components in a series of interactions that lead to infection. In vertebrates, Sias may be present as constituents of a variety of different complexes on the cell surface, including dense layers of glycoconjugates referred to as the glycocalyx. Mucosal surfaces are further protected by a viscous secretion, termed mucus, which varies in structure and composition depending on the location in the body [1]. However, mucus is formed in complex layers of cell-associated and secretory forms [2,3]. Sias in mucus and at cell surfaces can bind and trap viruses and prevent them from accessing their target tissues, and can also remove the bound virions in an active process mediated by mucocilliary transport [4]. Our understanding of virus–Sia interactions continues to be illuminated by new structural and biochemical analysis of viral proteins and bound glycans [5,6]. Currently we still do not clearly know which specific Sia structures are present on different cells or tissues of many viral hosts, including humans. It is clear that Sias exist in many diverse forms, and that their synthesis is tightly regulated yet highly variable on the cells and tissues of individual animals. The displayed glycan combinations vary under differing physiological conditions, stages of development, and also between different animal species. Many viruses also encode Sia-specific enzymes that alter the host Sia and therefore their specific interactions, including **sialidases** that remove the terminal Sia from glycans or **esterases** that remove ester-bonded acetyl modifications from the Sias where those are present.

Here we review examples of the various interactions between Sias and viruses in vertebrate animals, and examine how those can be modulated by variations in Sia structures, leading to

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Sialic acids (Sias) are components of cell-surface glycoproteins and glycolipids, as well as secreted glycoproteins and milk oligosaccharides. Sias play important roles in cell signaling, development, and host–pathogen interactions. Cellular enzymes can modify Sias, yet how modifications vary between tissues and hosts has not been fully elucidated.

Many viruses use Sias as receptors, with different modifications aiding or inhibiting virus infection. How modified Sias influence viral protein evolution and determine host/tissue tropism are poorly understood, and are important areas of research.

New advances in molecular glycobiology using pathogen proteins to detect varied forms allows for improved study of modified Sias that have otherwise proven difficult to isolate. This opens new avenues of inquiry for virology, as well as host interactions with bacterial and eukaryotic pathogens.

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differences in recognition and/or viral enzyme activity. We further summarize what is known about the diversity and distribution of modified Sias, the known and potential impacts of these specific glycan variants on different viruses, and define some questions that remain to be resolved.

Sias: Modifications and Variation

Sias are a highly diverse family of monosaccharides that serve as terminal residues of *N*- and *O*-linked glycoproteins and glycolipids, and they are also components of various polysaccharides. The core Sia structure contains nine carbons, and modifications are most commonly found at the 4, 5, 7, 8, and 9 positions. Modifications at the 5-carbon position determine the primary Sia forms: *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc), and 2-keto-3-deoxynononic acid (KDN) [7]. These primary Sia forms serve as core structures, and additional modifications can be generated enzymatically to form more than 30 variant types of Sia [8]. Those include acetyl, sulfate, lactoyl, and methyl group additions to various positions. Display of the different modified forms clearly varies between different organisms, tissues, and cell types, and as the modifications may be present in myriad combinations, there may be a wide diversity of glycan forms (Figure 1). In addition, the linkage of the Sia to the underlying glycan structure may also vary, with α2,3 or α2,6 linkages being most common, as well as α2,8 linkages in the formation of poly-Sia structures which are primarily found in the brain and on certain cells of the immune system [9].

Our understanding of the expression and biological roles of this wide diversity of variant Sias is rather limited for various practical and technical reasons. However, the modifications are widespread and clearly allow the Sia to serve a variety of physiologically important roles in normal cell and tissue development and in controlling cell–cell interactions. For example, the constitutive expression of influenza C virus 9-*O*-acetylesterase in transgenic mice resulted in complete developmental arrest of embryos as early as the two-cell stage [10]. Polysialic acids play an important role in neural development and are involved in neuron growth, axon guidance, and synapse formation, and their synthesis is tightly regulated [11]. Transgenic mice lacking expression of synthases and transferases necessary for oligosialic acid expression in the central nervous system showed atypical neuronal development and a 'sudden death' phenotype [12]. In addition, Sias are involved in immune cell maturation and activation. Regulated 9-*O*-acetylation of ganglioside GD3 (also called CD60) on T cells correlates with cell differentiation and blocking of pro-apoptotic pathways in proliferating T cells [13]. Similarly, regulated *O*-acetylation at the C9-position of Sia on B cells is required for the correct development and activation of those cells, as 9-*O*-acetylation blocks recognition by the Sia-binding immunoglobulin-type lectin CD22 (SIGLEC-2), which acts as an inhibitor of B cell receptor signaling and activation [14]. Given the regulatory role of Sias in so many different cell processes and pathways, it is not surprising that abnormal expression of modified Sias has been identified as a hallmark of many cancers, including some types of leukemia [15,16] and colorectal cancers [17].

Formation of specific glycans is regulated by the expression of a variety of enzymes, many of which are localized to the endoplasmic reticulum and Golgi apparatus, where they direct co- and post-translational modification of the glycans on glycoproteins or glycolipids. Enzymes specific for Sia addition and modification are expressed in the Golgi apparatus while others may be expressed in the cytoplasm and modify the Sia precursors. The varying expression of sialyltransferases, sialidases, esterases, and other Sia-modifying enzymes makes Sia synthesis and modification highly variable and dynamic, and as a result their display is influenced by both external and internal cellular stimuli. Several of the genes encoding Sia-modifying enzymes have been identified and analyzed in detail (Figure 1). Those include cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase (CMAH), the enzyme responsible for formation of Neu5Gc from the CMP-Neu5Ac precursor [18]. The CASD1 gene (capsule structure 1 domain

Glossary

Esterase (acetylesterase): in this context, an enzyme that removes *O*-acetyl groups from Sia sugars by cleaving the ester bond. Esterases have specificity for the position of the *O*-acetyl group in the Sia, typically 4-*O*-Ac or 9-*O*-Ac. Esterases are encoded by influenza C and nidovirus family viruses in the same glycoprotein (related-origin) encoding a hemagglutinin domain that has specificity for the same modified Sia.
Hemagglutinin (HA): a viral lectin that binds to Sia (sialolectin), historically characterized by the ability of a virus to agglutinate erythrocytes *in vitro*. Orthomyxo-, paramyxo-, and nidoviruses encode hemagglutinating glycoproteins either alone or fused to esterase or sialidase domains. Many surface capsid proteins of nonenveloped viruses also have *hemagglutination* sialolectin function.
Sialic acid (Sia): nine-carbon α-keto acidic sugar whose name is derived from its presence in saliva. It was discovered from human samples as primarily *N*-acetylneuraminic acid (Neu5Ac). It can be enzymatically modified at multiple positions. Sia is typically found at the termini of N- and O-glycans on glycoproteins, as well as components of glycolipids, where they perform important recognition reactions for other cellular factors and/or environmental agents such as pathogens.
Sialidase: an enzyme that removes the Sia sugar from glycocarbohydrate structures. Endo-sialidases cleave poly-Sia linkages. Sialidases of viruses have been traditionally defined as neuraminidases (NA) for their cleavage of Neu5Ac. Historically also identified as a virus-encoded receptor-destroying enzyme (RDE).
Tropism: the preferential viral infection of target hosts, tissues, or cells. Tropism is often related to permissiveness resulting from the presence of a specific receptor. Sia-binding virus tropisms may be greatly influenced by the relative distribution of Sia species on a given tissue. The degree of Sia diversity (including modified inhibitors or decoys) in alternative hosts or tissues may influence potential viral emergence.