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# Expanding diversity of glycan receptor usage by rotaviruses

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Rotaviruses are major etiologic agents of severe gastroenteritis in human and animals, infecting the mature intestinal epithelium. Their attachment to host cell glycans is mediated through the virion spike protein. This is considered to be crucial for successful host cell invasion by rotaviruses. Recent studies have greatly expanded our understanding of the diversity of glycans commonly recognized by rotaviruses, to include the ganglioside GM1a and histo-blood group antigens. Here, these new findings are integrated with advances in knowledge of spike protein structure, rotavirus entry mechanisms and innate intestinal immunity to provide an overview of the variety of rotavirus glycan receptors and their roles in cell penetration, host tropism and pathogenesis.

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## Introduction

The species *Rotavirus A*, a member of the *Reoviridae* family of double-stranded RNA viruses, is a major agent of severe infantile gastroenteritis in humans and animals worldwide [1,2]. Infection is targeted to the differentiated epithelial cells of the small intestine. The infectious rotavirus particle is triple-layered, comprising a core containing the segmented genome, an inner protein shell and an outer capsid of virus protein (VP) 7 trimers penetrated by VP4 spikes. Double-layered rotavirus particles lacking the outer capsid, which are non-infectious but transcriptionally active, are delivered to the host cell cytoplasm after entry of infectious rotavirus [1]. Rotavirus infectivity is increased by exposure to intestinal proteases, particularly trypsin, which cleaves VP4 to promote conformational change related to cell penetration [3•]. On the basis of sequences of the serotype-determining *glycoprotein* VP7 and *protease*-sensitive VP4, rotaviruses are classified in a binary system of G (VP7) and P (VP4) types. VP4 protease cleavage yields N-terminal VP8* and C-terminal VP5* domains that remain virus-associated [3•]. VP8* is localized to the dimeric VP4 spike head and binds to cell surface glycans, suggesting that this essential interaction occurs at an early stage. VP5* forms most of the spike body and foot, and is proposed to undergo major conformational change to expose a hydrophobic domain important for host cell membrane penetration [3•,4•]. Several rotaviruses use VP5* to bind the α2β1 integrin and facilitate infection [5–7]. VP7 participates in rotavirus cell entry via interactions with the αxβ2 and αvβ3 integrins [5,6,8]. The α4β1/7 integrins are implicated in rotavirus entry into certain cell types [5,9]. VP8* is implicated in opening tight junctions between cells, which may facilitate virus access to receptors [10]. Perhaps through their co-localization with integrins and glycan receptors, membrane lipid microdomains and heat shock cognate protein 70 also appear to play a role in this intricate multifaceted pathway of rotavirus-cell entry [11•,12].

The ability of a virus to invade host cells is a crucial determinant of pathogenicity and host tropism. In addition to point mutation and gene rearrangement, the segmented rotavirus genome and large range of P and G types (>25 of each) facilitate extensive gene reassortment, resulting in very substantial genetic diversity. Notably, VP8* is the most variable domain in rotavirus structural proteins. Here, I review the significant progress made in the last two years in our appreciation of the breadth and flexibility of glycan receptor usage by rotaviruses through their VP8* domains, and in rotavirus-cell entry mechanisms. Possible implications for host range restriction and interspecies transmission are canvassed.

## Sialic acids and gangliosides as rotavirus receptors

It is long established that the infectivity of a number of animal rotaviruses is strongly reduced in cells treated with certain bacterial sialidases, due to the removal of the sialic acid (Sia) residues from the termini of the main chains of cell surface glycans [13,14]. It is now clear that the VP8* of these sialidase-sensitive rotaviruses mediates this terminal Sia binding (Table 1). *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc) are the most common Sia family members in nature with Neu5Gc differing from Neu5Ac by an additional hydroxyl group. In contrast to most mammals, humans have lost the biosynthetic machinery for Neu5Gc [15]. Most animal

**Table 1**

**Glycans established as receptors for rotaviruses**

| Virus strain | Origin | Type[a] | Sialidase sensitive[b] | Glycans bound by virus and/or VP8* that also inhibit infection by this virus/Ref |
|---|---|---|---|---|
| RRV | Simian | G3P[3] | + | Neu5Ac > Neu5Gc [18,20]<br>a-GM3 [25] |
| NCDV | Bovine | G6P[1] | + | Neu5Gc > Neu5Ac [18] |
| CRW-8 | Porcine | G3P[7] | + | Neu5Gc > Neu5Ac [18]<br>a-GD1a [28], a-GM3 [24,25] |
| TFR-41 | Porcine | G5P[7] | + | Neu5Ac, a-GM1a [11•] |
| UK | Bovine | G6P[5] | – | Neu5Gc, a-GM1a [11•] |
| DS-1 | Human | G2P[4] | – | A-type HBGA [33••]; *(a-GM1a ND)*[c] |
| RV-5 | Human | G2P[4] | – | a-GM1a [11•]; *(A-type HBGA ND)* |
| RV-3 | Human neonatal | G3P[6] | – | a-GM1a [11•]; A-type HBGA [33••] |
| ST-3 | Human neonatal | G4P[6] | – | A-type HBGA [33••]; *(a-GM1a ND)* |
| Wa | Human | G1P[8] | – | a-GM1a [11•,28,33••] |
| K8 | Human | G1P[9] | – | A-type HBGA [33••,34,35]; *(a-GM1a ND)* |
| HAL1166 | Human | G8P[14] | – | A-type HBGA [33••,34]; *(a-GM1a ND)* |
| N155 | Human neonatal | G10P[11] | – | H-type II core HBGA [29,36]; *(a-GM1a ND)* |

[a] G serotype and P genotype (square brackets) are given.
[b] Sialidase-sensitive rotaviruses (+) show reduced infectivity in permissive cells from which main chain (terminal) Sia have been removed by sialidase treatment. Sialidase-insensitive rotaviruses (–) show enhanced or unchanged infectivity in such sialidase-treated cells.
[c] Italics refer to data not yet obtained that is needed to fully understand the roles of a-GM1a and A-type HBGA. ND, not determined.

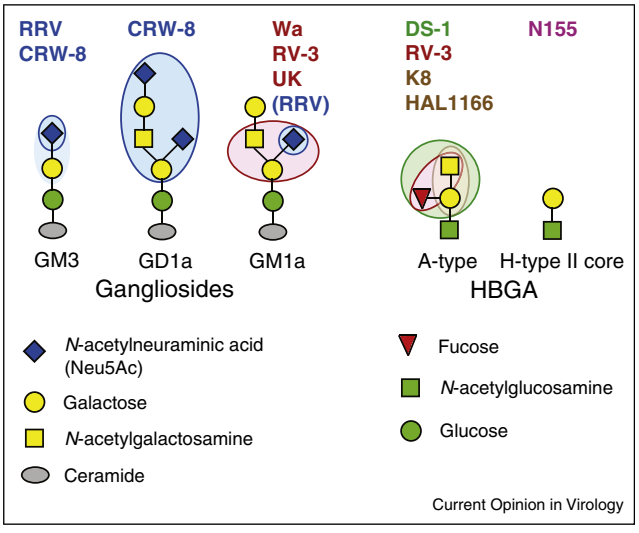
rotaviruses utilize both Neu5Ac and Neu5Gc, with simian strain RRV preferring Neu5Ac and bovine (NCDV, UK) and porcine (CRW-8) strains showing a higher affinity for Neu5Gc (Table 1). Crystallographic studies with RRV and CRW-8 VP8* have identified a common Sia-binding groove [16,17]. The latest of such studies provides evidence that Sia binding preference is influenced by the nature of the amino acid at VP8* position 187, and the residue at position 157 may affect Sia binding affinity [18].

In structural studies with disaccharides, the Neu5Ac moiety contributes most of the interaction with RRV VP8* [19,20]. However, the potential for RRV VP8* recognition of addition glycans in the oligosaccharide chain was shown [21]. The Neu5Gc usage by animal rotaviruses is consistent with Neu5Gc expression in animals. Whether any human rotaviruses use Neu5Gc is currently unknown, although this is unlikely given the low Neu5Gc levels in humans [15]. As only one human rotavirus strain (HCR3a) has been reported to be sialidase-sensitive [13], dependence on terminal Sia may be a factor in restricting spread amongst humans.

Sialidase-sensitive animal rotaviruses have been found to use gangliosides with terminal Sia as receptors, via VP8* binding [22–26]. Gangliosides form a class of cell membrane glycosphingolipids, with a functional glycan head group typically including main chain (terminal) and/or branched (internal) Sia and a ceramide tail that is inserted into the membrane ([27]; Figure 1). The infectivity of RRV and CRW-8 is inhibited by blockade of the acetamido form of ganglioside GM3 (a-GM3) [24,25]. Further analysis by crystallography and saturation transfer difference nuclear magnetic resonance (STD-NMR) indicates that the terminal Sia of a-GM3 is the main glycan moiety recognized by VP8* of these rotaviruses ([24,25]; Figure 1). Similar approaches showed CRW-8 also uses the GD1a ganglioside, with both its terminal and internal Sia moieties involved in VP8* binding ([28]; Figure 1).

The P[8] rotaviruses predominate globally in humans, with P[4] strains also frequently found. Certain less common VP4 genotypes, such as P[6] and P[11], are associated with asymptomatic infection of neonates in

**Figure 1**




Gangliosides and HBGA bound by rotaviruses. Binding epitopes on glycans are indicated for each listed rotavirus strain by an ellipse of matching colour.