Published in final edited form as:

Curr Opin Struct Biol. 2017 June ; 44: 211–218. doi:10.1016/j.sbi.2017.05.007.

Structural features of glycan recognition among viral pathogens

Sreejesh Shanker¹, Liya Hu¹, Sasirekha Ramani², Robert L Atmar^{2,3}, Mary K Estes^{2,3}, and BV Venkataram Prasad¹

¹Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, United States

²Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, United States

³Department of Medicine, Baylor College of Medicine, Houston, TX 77030, United States

Abstract

Recognition and binding to host glycans present on cellular surfaces is an initial and critical step in viral entry. Diverse families of host glycans such as histo-blood group antigens, sialoglycans and glycosaminoglycans are recognized by viruses. Glycan binding determines virus–host specificity, tissue tropism, pathogenesis and potential for interspecies transmission. Viruses including noroviruses, rotaviruses, enteroviruses, influenza, and papillomaviruses have evolved novel strategies to bind specific glycans often in a strain-specific manner. Structural studies have been instrumental in elucidating the molecular determinants of these virus–glycan interactions, aiding in developing vaccines and antivirals targeting this key interaction. Our review focuses on these key structural aspects of virus–glycan interactions, particularly highlighting the different strain-specific strategies employed by viruses to bind host glycans.

Introduction

Viruses recognize and bind host glycans as cell attachment factors, receptors or co-receptors, as a prerequisite for viral entry and subsequent infection [1]. Viruses exhibit a remarkable degree of selectivity and specificity while also evolving strain-specific strategies to engage host glycans. Glycan-binding can dictate virus–host specificity, tissue tropism, pathogenesis and interspecies transmission. Viruses predominantly recognize three distinct families of host glycans including: histo-blood group antigens (HBGAs) recognized by noroviruses (NoVs) [2] and human rotaviruses (HRVs) [3]; sialoglycans recognized by influenza viruses [4], orthoreoviruses [5] and specific picornaviruses [6,7]; and glycosaminoglycans (GAGs) recognized by papillomaviruses [8] and parvoviruses [9] among others. Understanding virus–glycan interactions is critical, as blocking this interaction using glycomimetics or antibodies can lead to reduced virus infectivity and targeting these interactions can lead to the development of antivirals and vaccines.

Corresponding authors: Shanker, Sreejesh (shanker@bcm.edu), Venkataram Prasad, BV (vprasad@bcm.edu).

Conflict of interest

MKE is named as an inventor on patents related to cloning of the Norwalk virus genome and has served as a consultant to Takeda Vaccines, Inc. RLA has received research grant funding from and is an unpaid consultant to Takeda Vaccines, Inc.

Recognition of HBGAs by gastroenteric viruses

HBGAs are genetically determined neutral glycans expressed on the surface of intestinal epithelial cells, and found in mucosal secretions of individuals. These polymorphic group of glycans are synthesized by the sequential addition of monosaccharide units onto a precursor disaccharide (Figure 1a). Two HBGA families, the ABH and Lewis family, are implicated in binding to viruses [10,11]. HBGA binding by the two leading causes of viral gastroenteritis, NoVs and HRVs is discussed below.

HBGA recognition by human NoVs

NoVs are non-enveloped positive-sense ssRNA viruses belonging to *Caliciviridae*. They are classified into 7 genogroups (GI–GVII) of which genogroups GI, GII, and GIV cause infections in humans [12]. Each genogroup is subdivided into multiple genotypes. The prototype Norwalk virus (NV) belongs to genogroup I, genotype 1 (GI.1). Genotype GII.4 is the globally dominant genotype [13], but recently GII.17 strains emerged as a predominant genotype in parts of Asia [14]. Differential strain-specific HBGA binding along with antigenic drift drives the evolution of NoVs.

NoVs recognize HBGAs as susceptibility [15] and cell attachment factors through the P-domain of their capsid protein VP1 [16–18]. Interestingly, HBGA bound P-domain structures from GI and GII NoVs show that the HBGA binding sites are distinct in both location and structural characteristics between these two genogroups (Figure 2a) [17,19]. The mode of HBGA binding is also distinct, with the GI strains primarily binding to the galactose (Gal) of the disaccharide precursor (Figure 2b), and the GII strains binding to the fucose (Fuc) moiety of the HBGA (Figure 2c). Closer examination of the HBGA binding site reveals that there are two subsites involved in HBGA binding. A conserved primary site (site 1), binding either the Gal residue in GI or the secretor-Fuc in GII, and a secondary evolving site (site 2) that is susceptible to sequence and structural alterations, allowing strain-specific HBGA binding [18,20,21] (Figure 2b, c). Site 2 in GII.4 variants identified post-2002 have evolved the ability to bind non-secretor Lewis HBGAs, and that might explain the prevalence of these variants [18].

Serum antibodies that bind NoVs and block HBGA binding are correlates of protection against NoV gastro-enteritis [22]. Recently the structure of one such human antibody in complex with the NV P-domain was determined and it revealed steric hindrance as the mechanism of HBGA blockade [23]. Other structural studies have shown that human milk oligosaccharides, 2'-fucosyllactose (2'FL) and 3'-fucosyllactose (3'FL) and molecules like citrate mimic HBGA binding, thus serving as decoy receptors and potential glycomimetics [24,25].

HBGA recognition by human RVs

While HBGA binding in human NoVs is well characterized, the HBGA binding among HRVs is a recent emerging paradigm shift that diverges from the sialoglycan binding observed in animal rotaviruses (ARVs) [26]. Recent epidemiological studies show that

secretor-positive individuals are significantly more susceptible to HRV infections suggesting that HBGAs are susceptibility factors for HRV infection similar to human NoVs [27].

RVs are non-enveloped multi-segmented dsRNA viruses belonging to *Reoviridae*. To date, they are classified into 32 G genotypes and 47 P genotypes based on their two antigenic outer capsid proteins, glycoprotein VP7 and protease-sensitive VP4, respectively [28,29]. VP4 is cleaved into VP8* and VP5*, with VP8* involved in glycan recognition. The structure of VP8* comprised of two twisted β -sheets separated by a shallow cleft resembles the galectin-like fold and is conserved among RVs (Figure 2d).

HRVs were recently shown to bind HBGAs. A P[14] HRV strain, with origins in even-toed ungulates, specifically binds A-type HBGA within a narrow cleft between the two β sheets (Figure 2d) [3]. The A-type HBGA binds to P[14] VP8* in a similar location as the sialic acid (SA) in the ARV VP8* (Figure 2d). The determinant of this receptor switch is an insertion of a residue serine 187 in P[14] VP8* that reorients the side chain of a neighboring residue tyrosine 188 such that it sterically blocks access to SA while engaging in hydrophobic interactions with A-type HBGA (Figure 2e). Further, the presence of A-type HBGA in both even-toed ungulates and humans provides the basis for potential interspecies transmission of this genotype. More recently, structural studies on VP8* of a neonatal bovine-human reassortant P[11] strain with a wider cleft show that they specifically bind precursor glycans, which are core elements of both HBGA and human/bovine milk glycans [30]. The glycan binding site is distinct from that observed in P[14] VP8* [30]. In P[11] VP8*, the glycan binding site is more expansive spanning the cleft region. Interestingly, a milk glycan array comparing glycan binding properties of P[11] VP8* of human neonatal strain and its bovine counterpart B223 showed that while the human strain recognized both type-1 and type-2 precursor glycans, the bovine strain is restricted to binding the type-2 precursor glycans. Comparisons of bovine and human P[11] VP8* show how subtle sequence and structural changes in the glycan binding site confers this specificity that is consistent with the glycan propensity in the respective gut milieus thus providing a structural basis for interspecies transmission [30]. Glycan bound VP8* structures from the dominant P [4], P[8] and P[6] HRVs are currently not available but biochemical studies suggest these bind the H-type HBGAs [31]. Thus these studies demonstrate that HRVs similar to human NoVs exhibit enormous strain-dependent diversity in recognizing polymorphic HBGAs.

Recognition of sialoglycans by human viruses

While recognition of sialoglycans is predominantly confined to ARV strains, several other human viruses including orthoreovirus, picornavirus, influenza virus, and mumps virus bind specifically to sialoglycans for cell attachment. These viruses, as discussed below engage sialoglycans using different strategies. Sialoglycans are negatively-charged and ubiquitously expressed among vertebrates and include the sialyllactosamine containing gangliosides, GM1 and GM2 (Figure 1b). *N*-acetylneuraminic acid (Neu5Ac) is the most commonly found sialic acid (SA) in humans. It is often found terminally attached to a Gal or GalNAc moiety using either α 2, 3 or α 2, 6 glycosidic linkages, with the SA functional groups held in optimal orientations that allow engagement with viral attachment proteins [32,33].

Sialic acid recognition by orthoreovirus

Orthoreoviruses (*Reoviridae*) are non-enveloped dsRNA viruses similar to RVs. These viruses were recently implicated in the pathogenesis of celiac disease in humans [34]. The two major orthoreovirus serotypes, type-1 Lang (T1L) and type-3 Dearing (T3D), bind differentially to SA receptors and exhibit clear differences in viral spread and tissue tropism [35]. These differences are attributed to variations in their outer capsid protein $\sigma 1$ that as a trimer protrudes out from the viral surface and is implicated in glycan binding. Each $\sigma 1$ subunit is composed of 3 distinct domains: a head domain comprised of eight antiparallel β -strands, a body comprised of β -spiral repeats, and a tail domain predicted to be α coiled-coil (Figure 3a) [36]. The glycan binding sites in T1L and T3D $\sigma 1$ are distinct in location and structural characteristics [5,37]. In T1L $\sigma 1$, the glycan binding site is located in the head domain, whereas, in T3D $\sigma 1$, the glycan binding site is located in the body domain (Figure 3a). Glycan interactions in both T1L (Figure 3b) and T3D (Figure 3c) primarily involve hydrogen bonding interactions with the Neu5Ac moiety. In both strains, the methyl group of Neu5Ac is inserted into a hydrophobic pocket, a feature common to Neu5Ac virus interactions. Another striking feature is that most of the hydrogen bonding interactions with Neu5Ac involve the main chain atoms of $\sigma 1$ rather than the side chains, as seen in most viral glycan interactions [38,39].

Sialic acid recognition by Picornaviruses

SA binding in picornaviruses represents a novel theme as these viruses commonly bind immunoglobulin-like (Ig-like) receptors. Enteroviruses (EVs) (*Picornaviridae*) are non-enveloped positive-sense ssRNA viruses. The icosahedral capsid of EV consists of 60 copies of the viral proteins VP1, VP2, and VP3 that form a pseudo T = 3 capsid symmetry. VP1 lies close to the icosahedral fivefold axis, VP2 – near the twofold, and VP3 – near the threefold axis. EV members bind Ig-like receptors at a depression on the viral surface called the “canyon” that encircles each fivefold axis. In most EV structures, a hydrophobic pocket in VP1 is located underneath the canyon which holds a branched elongated fatty acid-like ‘pocket factor’ that stabilizes the virion. Recent structural studies on EV members including EV-D68 that causes childhood respiratory diseases [6] and coxsackievirus A24 variant (CVA24v) that causes acute hemorrhagic conjunctivitis (AHC) [7] show that they utilize SA receptors with a preference for α 2, 6-linked Neu5Ac glycans. Interestingly, the SA-binding sites in EV-D68 and CVA24v capsid structures are distinct and lie 40 Å apart with respect to each other. In EV-D68, the SA binding site is located in the canyon similar to the Ig-like receptor binding site, however, this canyon is shallow and narrow when compared to the canyon in EVs that bind Ig-like receptors. EV-D68 recognizes and binds the terminal Neu5Ac in the sialoglycan using residues from both VP1 and VP3 (Figure 3e). These residues are conserved within EV-D68 isolates but not in CVA24v and other EVs that bind Ig-like receptors. Interestingly, in EV-D68, glycan binding induces significant structural changes in the loop regions that connects the glycan binding site to the VP1 hydrophobic pocket. These conformational changes disrupt the hydrophobic pocket resulting in the release of the ‘pocket factor’ to potentially facilitate destabilization and uncoating of the virion. In contrast to EV-D68, the SA binding site in CVA24v lies at a solvent-exposed protruding region on VP1 close to the fivefold axis [7] (Figure 3d). The residues binding the

Neu5Ac of the sialoglycan are contributed by loop regions from a VPI monomer and a clockwise (cw) rotated VP1 protomer (Figure 3f). The location of this site is similar to the LDL receptor binding site on human rhinovirus 2 (HRV2).

Sialic acid recognition by influenza viruses

In contrast to both orthoreo- and enteroviruses, influenza viruses, the cause of common flu, have a conserved SA binding site, and illustrate a strategy involving differential recognition of α 2, 3- and α 2, 6-glycosidic linkages by avian and human strains, respectively. Influenza viruses (*Orthomyxoviridae*) are enveloped viruses containing a segmented negative-sense ssRNA genome. Their two antigenic and serotype-defining viral glycoproteins, hemagglutinin (HA or H) and neuraminidase (NA or N), also determine their subtypes. Structural studies show that the HA trimer recognizes Neu5Ac-linked glycans to initiate cell entry. The glycan binding site is a conserved shallow groove at the distal region of the HA monomer (Figure 4a). Neu5Ac inserts deeply into HA with its functional groups making multiple interactions with HA (Figure 4b). The orientation of Neu5Ac and its interactions with HA are conserved among influenza subtypes [40,41]. The glycosidic linkage between the Neu5Ac and the Gal moiety is the determinant of host specificity, with avian members recognizing α 2, 3 linkage and human members recognizing α 2, 6 linkage [42,43]. Some avian influenza strains, such as the 1918 H1N1 (pandemic flu), overcame this interspecies barrier with catastrophic effects to humans. Glycan array analysis of the 1918 H1N1 strains [44] identified two key residues at positions 190 and 225 in the HA consensus sequence as the molecular determinants of strain specificity. Currently, there are two subtypes circulating in humans: the novel pandemic H1N1 (2009) and the H3N2. These strains maintain α 2, 6 linkage specificity, but preferentially bind branched glycans with extended poly-N-acetyl-lactosamine (poly-LacNAc) chains conferring potential for increased avidity by simultaneously binding two subunits of the HA trimer [45].

Sialic acid recognition by Mumps virus

Although influenza virus-SA interactions are structurally well characterized, the structural details of how the mumps virus (MUV), the causative agent of mumps, binds SA have been unclear until recently. The mumps virus (*Paramyxoviridae*) is an enveloped non-segmented negative-strand ssRNA virus. Its surface hemagglutinin-neuraminidase (HN) protein binds SA, and the structural details of this interaction were recently elucidated [46]. MUV-HN is a transmembrane protein composed of an N-terminal cytoplasmic tail, a transmembrane domain, a stalk, and a C-terminal head domain. The structure showed that the head domain of HN exhibits a six-bladed β propeller fold and forms a homodimer, with each subunit, tilted 90° towards each other. Similar to other members of paramyxoviruses, the homodimers form a tetramer [47]. The SA binding site lies atop the head domain (Figure 4c). It recognizes a terminal Neu5Ac, and preferentially binds α 2, 3 linked SA glycans through residues that are conserved across MUV genotypes (Figure 4e).

Recognition of GAGs by viruses

Another distinct class of glycans recognized by several viruses is glycosaminoglycans (GAGs). GAGs are ubiquitously expressed in most eukaryotic tissue and cell surfaces. GAGs are long unbranched polysaccharides composed of repeating disaccharide units of sulfated uronic acid and glucosamine residues (Figure 4c), and includes heparan sulfate (HS). Specific recognition of GAGs for cell attachment have been implicated for a number of non-enveloped icosahedral viruses such as human papillomavirus (HPV), belonging to *Papovaviridae* [48]; human parvovirus and adeno-associated viruses (AAV), belonging to *Parvoviridae* [9,49]; and enveloped icosahedral viruses such as Zika virus, Dengue virus and West Nile virus, belonging to *Flaviviridae* [50]. Although structural studies have provided general features of how these viruses interact with the negatively-charged HS, detailed understanding of the rules of glycan engagement is limited due to lack of atomic-level characterization of glycan interactions. Understanding glycan interactions in human flaviviruses, and parvoviruses such as AAV serotypes, which are being developed as gene-therapy vectors, may be of significant clinical importance. Albeit at low resolutions, the GAG-bound structure of HPV is discussed below as an example.

GAG recognition by Papillomaviruses

HPV strains HPV16 and HPV18 can cause oral and cervical cancers. Their major capsid protein L1 as 72 pentameric capsomeres form the icosahedral capsid of HPV. L1 is implicated in the specific recognition of HS for cell entry [51,52]. Crystallographic studies of heparin-bound pentameric L1 from HPV 16 and HPV 18 [8], together with mutational studies suggest that there are at least three (sites 1–3) conformational HS binding sites on L1. Though heparin is suggested to interact with all the three sites using a combination of positively charged lysine and polar residues, the electron densities for heparin is not well resolved. A recent cryo-EM structure of the heparin bound capsid of HPV16 shows that heparin binds only at the fivefold vertices [52]. The heparin binding site comprises of six residues contributed by two L1 chains. The side chains of these residues interact with the sulfated polysaccharide moieties of heparin. Two of these residues correspond to the residues in site 3 that were identified in the crystallographic studies, but the cryo-EM structure did not show the involvement of sites 1 and 2 in the glycan binding and suggest that heparin binding at site 3 will sterically hinder heparin binding at site 2. The cryo-EM study further suggests that though heparin binding does not induce conformational changes on L1, it might initiate conformational changes on the minor structural protein L2 by exposing L2 residues that may then bind heparin.

Conclusions

Attachment to cellular glycans is a crucial aspect for viral entry into cells leading to infection with multiple viruses targeting specific host glycans including HBGAs, sialoglycans, and GAGs. The gastroenteric pathogens NV and RV are unique among viruses in exploiting the polymorphic nature of HBGAs in a strain-specific manner. It is remarkable how the two major genogroups in NoVs have evolved to recognize distinct HBGA binding sites, with GI recognizing the galactose moiety and GII recognizing the fucose moiety to

bind HBGAs. Among HRVs, the major questions are: how the dominant strains including P [4], P[8], and P[6] exploit the polymorphic nature of HBGAs, will they target novel sites on VP8* to bind HBGAs similar to NV, and will HBGA blocking antibodies be a correlate of protection among RVs. Among SA binding viruses, some such as influenza and mumps virus employ a conserved SA binding site whereas others such as orthoreovirus and picornaviruses employ distinct SA binding sites. In general, they all primarily recognize the terminal Neu5Ac with the species specific linkages and other glycans adding to specificity and stability. One conserved feature is the insertion of the methyl group of the N-acetyl arm into a hydrophobic pocket on the viral protein. For viruses binding GAGs, high-resolution GAG bound viral protein structures are needed before we can elucidate the rules governing GAG binding and its role in cell entry. Another aspect of virus–glycan binding not discussed here is the role of viral glycosylation in virus virulence and immune evasion (reviewed in Ref. [53]). Overall this review summarizes our current knowledge of virus–glycan interactions specifically focusing on the different strategies employed by viruses to engage glycans.

Acknowledgments

We acknowledge support from National Institutes of Health [R37 AI36040 (BVVP), RO1 AI080656, RO1 AI105101 (MKE), PO1 AI057788 (MKE, BVVP, RLA)], and Robert Welch foundation [Q1279 (BVVP)].

References

1. Olofsson S, Bergstrom T. Glycoconjugate glycans as viral receptors. *Ann Med*. 2005; 37:154–172. [PubMed: 16019714]
2. Marionneau S, Ruvoen N, Le Moullac-Vaidye B, Clement M, Cailleau-Thomas A, Ruiz-Palacois G, Huang P, Jiang X, Le Pendu J. Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology*. 2002; 122:1967–1977. [PubMed: 12055602]
3. Hu L, Crawford SE, Czako R, Cortes-Penfield NW, Smith DF, Le Pendu J, Estes MK, Prasad BV. Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. *Nature*. 2012; 485:256–259. [PubMed: 22504179]
4. Connor RJ, Kawaoka Y, Webster RG, Paulson JC. Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. *Virology*. 1994; 205:17–23. [PubMed: 7975212]
5. Reiter DM, Frierson JM, Halvorson EE, Kobayashi T, Dermody TS, Stehle T. Crystal structure of reovirus attachment protein sigma1 in complex with sialylated oligosaccharides. *PLoS Pathog*. 2011; 7:e1002166. [PubMed: 21829363]
6. Liu Y, Sheng J, Baggen J, Meng G, Xiao C, Thibaut HJ, van Kuppeveld FJ, Rossmann MG. Sialic acid-dependent cell entry of human enterovirus D68. *Nat Commun*. 2015; 6:8865. [PubMed: 26563423]
7. Zocher G, Mistry N, Frank M, Hahnlein-Schick I, Ekstrom JO, Arnberg N, Stehle T. A sialic acid binding site in a human picornavirus. *PLoS Pathog*. 2014; 10:e1004401. [PubMed: 25329320]
8. Dasgupta J, Bienkowska-Haba M, Ortega ME, Patel HD, Bodevin S, Spillmann D, Bishop B, Sapp M, Chen XS. Structural basis of oligosaccharide receptor recognition by human papillomavirus. *J Biol Chem*. 2011; 286:2617–2624. [PubMed: 21115492]
9. Huang LY, Halder S, Agbandje-McKenna M. Parvovirus glycan interactions. *Curr Opin Virol*. 2014; 7:108–118. [PubMed: 25047752]
10. Marionneau S, Cailleau-Thomas A, Rocher J, Le Moullac-Vaidye B, Ruvoen N, Clement M, Le Pendu J. ABH and Lewis histo-blood group antigens, a model for the meaning of oligosaccharide diversity in the face of a changing world. *Biochimie*. 2001; 83:565–573. [PubMed: 11522384]

11. Le Pendu J, Nystrom K, Ruvoen-Clouet N. Host-pathogen co-evolution and glycan interactions. *Curr Opin Virol.* 2014; 7:88–94. [PubMed: 25000207]
12. Vinje J. Advances in laboratory methods for detection and typing of norovirus. *J Clin Microbiol.* 2015; 53:373–381. [PubMed: 24989606]
13. Kroneman A, Vega E, Vennema H, Vinje J, White PA, Hansman G, Green K, Martella V, Katayama K, Koopmans M. Proposal for a unified norovirus nomenclature and genotyping. *Arch Virol.* 2013; 158:2059–2068. [PubMed: 23615870]
14. de Graaf M, van Beek J, Vennema H, Podkolzin AT, Hewitt J, Bucardo F, Templeton K, Mans J, Nordgren J, Reuter G, et al. Emergence of a novel GII.17 norovirus—end of the GII.4 era? *Euro Surveill.* 2015;20.
15. Hutson AM, Airaud F, LePendou J, Estes MK, Atmar RL. Norwalk virus infection associates with secretor status genotyped from sera. *J Med Virol.* 2005; 77:116–120. [PubMed: 16032732]
16. Huang P, Farkas T, Zhong W, Tan M, Thornton S, Morrow AL, Jiang X. Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. *J Virol.* 2005; 79:6714–6722. [PubMed: 15890909]
17. Choi JM, Hutson AM, Estes MK, Prasad BV. Atomic resolution structural characterization of recognition of histo-blood group antigens by Norwalk virus. *Proc Natl Acad Sci U S A.* 2008; 105:9175–9180. [PubMed: 18599458]
18. Shanker S, Choi JM, Sankaran B, Atmar RL, Estes MK, Prasad BV. Structural analysis of histo-blood group antigen binding specificity in a norovirus GII.4 epidemic variant: implications for epochal evolution. *J Virol.* 2011; 85:8635–8645. [PubMed: 21715503]
19. Cao S, Lou Z, Tan M, Chen Y, Liu Y, Zhang Z, Zhang XC, Jiang X, Li X, Rao Z. Structural basis for the recognition of blood group trisaccharides by norovirus. *J Virol.* 2007; 81:5949–5957. [PubMed: 17392366]
20. Shanker S, Czako R, Sankaran B, Atmar RL, Estes MK, Prasad BV. Structural analysis of determinants of histo-blood group antigen binding specificity in genogroup I noroviruses. *J Virol.* 2014; 88:6168–6180. [PubMed: 24648450]
21. Prasad BV, Shanker S, Muhaxhiri Z, Deng L, Choi JM, Estes MK, Song Y, Palzkill T, Atmar RL. Antiviral targets of human noroviruses. *Curr Opin Virol.* 2016; 18:117–125. [PubMed: 27318434]
22. Ramani S, Estes MK, Atmar RL. Correlates of protection against norovirus infection and disease—where are we now, where do we go? *PLoS Pathog.* 2016; 12:e1005334. [PubMed: 27115358]
23. Shanker S, Czako R, Sapparapu G, Alvarado G, Viskovska M, Sankaran B, Atmar RL, Crowe JE Jr, Estes MK, Prasad BV. Structural basis for norovirus neutralization by an HBGA blocking human IgA antibody. *Proc Natl Acad Sci U S A.* 2016; 113:E5830–E5837. [PubMed: 27647885]
24. Weichert S, Koromyslova A, Singh BK, Hansman S, Jennewein S, Schrotten H, Hansman GS. Structural basis for norovirus inhibition by human milk oligosaccharides. *J Virol.* 2016; 90:4843–4848. [PubMed: 26889023]
25. Hansman GS, Shahzad-Ul-Hussan S, McLellan JS, Chuang GY, Georgiev I, Shimoike T, Katayama K, Bewley CA, Kwong PD. Structural basis for norovirus inhibition and fucose mimicry by citrate. *J Virol.* 2012; 86:284–292. [PubMed: 22031945]
26. Ramani S, Hu L, Venkataram Prasad BV, Estes MK. Diversity in rotavirus-host glycan interactions: a sweet spectrum. *Cell Mol Gastroenterol Hepatol.* 2016; 2:263–273. [PubMed: 28090561]
27. Payne DC, Currier RL, Staat MA, Sahni LC, Selvarangan R, Halasa NB, Englund JA, Weinberg GA, Boom JA, Szilagyi PG, et al. Epidemiologic association between FUT2 secretor status and severe rotavirus gastroenteritis in children in the United States. *JAMA Pediatr.* 2015; 169:1040–1045. [PubMed: 26389824]
28. Patton JT. Rotavirus diversity and evolution in the post-vaccine world. *Discov Med.* 2012; 13:85–97. [PubMed: 22284787]
29. Yinda CK, Zeller M, Conceicao-Neto N, Maes P, Deboutte W, Beller L, Heylen E, Ghogomu SM, Van Ranst M, Matthijnsens J. Novel highly divergent reassortant bat rotaviruses in Cameroon without evidence of zoonosis. *Sci Rep.* 2016; 6:34209. [PubMed: 27666390]

30. Hu L, Ramani S, Czako R, Sankaran B, Yu Y, Smith DF, Cummings RD, Estes MK, Venkataram Prasad BV. Structural basis of glycan specificity in neonate-specific bovine-human reassortant rotavirus. *Nat Commun.* 2015; 6:8346. [PubMed: 26420502]
31. Huang P, Xia M, Tan M, Zhong W, Wei C, Wang L, Morrow A, Jiang X. Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. *J Virol.* 2012; 86:4833–4843. [PubMed: 22345472]
32. Uusi-Kerttula H, Tamminen K, Malm M, Vesikari T, Blazevic V. Comparison of human saliva and synthetic histo-blood group antigens usage as ligands in norovirus-like particle binding and blocking assays. *Microbes Infect.* 2014; 16:472–480. [PubMed: 24631874]
33. Neu U, Bauer J, Stehle T. Viruses and sialic acids: rules of engagement. *Curr Opin Struct Biol.* 2011; 21:610–618. [PubMed: 21917445]
34. Bouziat R, Hinterleitner R, Brown JJ, Stencel-Baerenwald JE, Ikizler M, Mayassi T, Meisel M, Kim SM, Discepolo V, Pruijssers AJ, et al. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. *Science.* 2017; 356:44–50. [PubMed: 28386004]
35. Tyler KL, McPhee DA, Fields BN. Distinct pathways of viral spread in the host determined by reovirus S1 gene segment. *Science.* 1986; 233:770–774. [PubMed: 3016895]
36. Nibert ML, Dermody TS, Fields BN. Structure of the reovirus cell-attachment protein: a model for the domain organization of sigma 1. *J Virol.* 1990; 64:2976–2989. [PubMed: 2335823]
37. Reiss K, Stencel JE, Liu Y, Blaum BS, Reiter DM, Feizi T, Dermody TS, Stehle T. The GM2 glycan serves as a functional coreceptor for serotype 1 reovirus. *PLoS Pathog.* 2012; 8:e1003078. [PubMed: 23236285]
38. Stroh LJ, Stehle T. Glycan engagement by viruses: receptor switches and specificity. *Annu Rev Virol.* 2014; 1:285–306. [PubMed: 26958723]
39. Stencel-Baerenwald JE, Reiss K, Reiter DM, Stehle T, Dermody TS. The sweet spot: defining virus-sialic acid interactions. *Nat Rev Microbiol.* 2014; 12:739–749. [PubMed: 25263223]
40. Weis W, Brown JH, Cusack S, Paulson JC, Skehel JJ, Wiley DC. Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. *Nature.* 1988; 333:426–431. [PubMed: 3374584]
41. Watowich SJ, Skehel JJ, Wiley DC. Crystal structures of influenza virus hemagglutinin in complex with high-affinity receptor analogs. *Structure.* 1994; 2:719–731. [PubMed: 7994572]
42. Rogers GN, Paulson JC, Daniels RS, Skehel JJ, Wilson IA, Wiley DC. Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. *Nature.* 1983; 304:76–78. [PubMed: 6191220]
43. Rogers GN, Paulson JC. Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. *Virology.* 1983; 127:361–373. [PubMed: 6868370]
44. Childs RA, Palma AS, Wharton S, Matrosovich T, Liu Y, Chai W, Campanero-Rhodes MA, Zhang Y, Eickmann M, Kiso M, et al. Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray. *Nat Biotechnol.* 2009; 27:797–799. [PubMed: 19741625]
45. Peng W, de Vries RP, Grant OC, Thompson AJ, McBride R, Tsogtbaatar B, Lee PS, Razi N, Wilson IA, Woods RJ, et al. Recent H3N2 viruses have evolved specificity for extended, branched human-type receptors, conferring potential for increased avidity. *Cell Host Microbe.* 2017; 21:23–34. [PubMed: 28017661]
46. Kubota M, Takeuchi K, Watanabe S, Ohno S, Matsuoka R, Kohda D, Nakakita SI, Hiramatsu H, Suzuki Y, Nakayama T, et al. Trisaccharide containing alpha2,3-linked sialic acid is a receptor for mumps virus. *Proc Natl Acad Sci U S A.* 2016; 113:11579–11584. [PubMed: 27671656]
47. Crennell S, Takimoto T, Portner A, Taylor G. Crystal structure of the multifunctional paramyxovirus hemagglutinin-neuraminidase. *Nat Struct Biol.* 2000; 7:1068–1074. [PubMed: 11062565]
48. Richards KF, Bienkowska-Haba M, Dasgupta J, Chen XS, Sapp M. Multiple heparan sulfate binding site engagements are required for the infectious entry of human papillomavirus type 16. *J Virol.* 2013; 87:11426–11437. [PubMed: 23966387]

49. Huang LY, Patel A, Ng R, Miller EB, Halder S, McKenna R, Asokan A, Agbandje-McKenna M. Characterization of the adeno-associated virus 1 and 6 sialic acid binding site. *J Virol*. 2016; 90:5219–5230. [PubMed: 26962225]
50. Gould EA, Solomon T. Pathogenic flaviviruses. *Lancet*. 2008; 371:500–509. [PubMed: 18262042]
51. Buck CB, Day PM, Trus BL. The papillomavirus major capsid protein L1. *Virology*. 2013; 445:169–174. [PubMed: 23800545]
52. Guan J, Bywaters SM, Brendle SA, Ashley RE, Makhov AM, Conway JF, Christensen ND, Hafenstein S. Cryoelectron microscopy maps of human papillomavirus 16 reveal L2 densities and heparin binding site. *Structure*. 2017; 25:253–263. [PubMed: 28065506]
53. Vigerust DJ, Shepherd VL. Virus glycosylation: role in virulence and immune interactions. *Trends Microbiol*. 2007; 15:211–218. [PubMed: 17398101]

Figure 1.

Schematic representation of the diverse cellular glycans recognized by viruses: **(a)** Polymorphic HBGA: HBGA synthesis starts with a common disaccharide precursor. The glycosidic linkage and composition of this precursor disaccharide determines the HBGA types (type 1–4). Shown here are the type 1 (with $\beta 1,3$ linkage in the precursor) HBGA. Similarly, type 2 HBGA have $\beta 1,4$ linkage. Sequential addition of monosaccharides to this disaccharide precursor by various fucosyl (FUT2, FUT3) and glycosyltransferases (Enzyme A and B) results in FUT2-dependent secretor ABH and Lewis HBGA, and in the absence of functional FUT2, results in nonsecretor Lewis types of HBGA. **(b)** Representative sialoglycans: The commonly expressed sialoglycans recognized by viruses are shown and though sialoglycans with internal SA moieties are also recognized, only sialoglycans with terminal SA moieties are depicted. **(c)** Representative glycosaminoglycans: GAGs are long unbranched polysaccharides with repeating disaccharide units of sulfated glycans including iduronic and glucuronic acid. The disaccharide repeats of the GAGs that are commonly recognized by viruses are depicted.

Figure 2.

HBGA recognition in NoVs and HRVs: (a) NoV genogroups GI and GII exhibit distinct HBGA binding sites: Superposition of the P2 sub-domain of VP1 from GI (cyan) (PDB ID. 2ZL6) and GII (magenta) (PDB ID. 3SLN) genogroup NoVs bound to H-type HBGA (stick model; green GI/yellow GII). (b) HBGA binding in GI NoVs: Close-up of GI.7 HBGA binding site bound to secretor Le^y HBGA (yellow stick) (PDB ID. 4P25), highlighting the galactose (β Gal) dominant HBGA binding at the conserved primary site (Site 1) and strain dependent Lewis fucose (LeFuc) binding at the variation susceptible site (Site 2). The individual subunits of the P dimer are colored cyan and blue respectively with the residues from the opposite subunit labeled with a prime ('). Hydrogen bonding interactions are shown here and subsequently as black dashed lines. (c) HBGA binding in GII NoVs: Close-up of GII.4 HBGA binding site bound to secretor Le^b HBGA (yellow) (PDB ID. 3SEJ) highlighting their fucose (SeFuc) dominant HBGA binding at the conserved primary site (Site 1) and strain dependent Lewis fucose (LeFuc) binding at the variation susceptible site (Site 2). The individual subunits of the P dimer are colored brown and green respectively with the residues from the opposite subunit labeled with a prime ('). (d) Differential glycan binding among RVs: Superposition of VP8* from RV strains, HRV Hal1166 P[14] (purple) bound to A-type HBGA (yellow) (PDB ID. 4DRV), the neonatal HRV N155 P[11] (blue) bound to HBGA precursor glycan LNT (green) (PDB ID. 4YFZ) and ARV RRV P[3] (grey) bound to SA (black) (PDB ID. 1KQR) highlighting the switch from SA to HBGA between HRV and RRV strains and the distinct HBGA binding sites among the P[14] HRV and the P[11] neonatal HRV. The VP8*cleft is marked by a red arrow. (e) HRV strain P[14] HAL1166 binds A-type HBGA: Close-up of the A-type HBGA (yellow sticks) binding site in HRV strain P[14] HAL1166 (PDB ID. 4DRV) (purple) highlighting the residues S187 and

Y188 among others. These two residues play an important role in the observed receptor switch between the SA binding ARV strain (RRV P[3]) and HBGA binding human P[14] strains. **(f)** Neonatal HRV strain P[11] N155 binds HBGA precursors: Close-up of LNT bound structure of the neonatal HRV strain P[11] N155 (PDB ID. 4YFZ) showing the distinctive residue composition at this novel glycan binding site compared to the P[14] HRV strain.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

Sialic acid recognition among orthoreoviruses and picornaoviruses. **(a)** Orthoreoviruses exhibit strain-specific distinct sialic acid binding sites. Superposition of the SA-bound trimeric $\sigma 1$ protein from the two distinct orthoreovirus serotypes T1L and T3D with individual subunits shown in blue, cyan and green and SA glycans shown in red. In T1L $\sigma 1$ (PDB ID. 4GU3), the SA binding sites on individual subunits are localized on the head domain circled in red (top) in contrast in T3D $\sigma 1$ (PDB ID. 3S6X) wherein the individual SA binding sites are localized on the lower half of the body domain circled in red (bottom). The insets show zoomed up view of the respective red circled areas. **(b)** SA binding in serotype specific T1 Lang strain of orthoreoviruses: Close-up of the SA binding site in T1L $\sigma 1$ showing the hydrogen bonding interactions with SA. **(c)** P[14] Close-up of the SA binding site in T3D $\sigma 1$ showing the hydrogen bonding interactions with SA. **(d)** SA binding among enteroviruses: Superposition of capsid proteins VP1 (green), VP2 (cyan) and VP3 (magenta) of EV strains D68 (PDB ID. 5BNN) and CVA24v (gray) (PDB ID. 4Q4X), highlighting the distinct mode of SA engagement in these two closely related strains. The bound SA glycan on EV-D68 and CVA24v are shown in red and yellow, respectively, and the sites are circled in red. **(e)** SA binding in enterovirus EV-D68: Close-up of the SA binding site in EV D68 showing that the SA binding site lies on the east end of the “canyon” with residues from both VP1 (green) and VP2 (magenta) contributing to hydrogen bonding interactions with Neu5Ac. **(f)** SA binding in coxsackievirus CVA24v: Close-up of the SA binding site in CVA24v showing that the SA binding site lies at a solvent-exposed protruding region on VP1 close to the fivefold axis. The residues binding Neu5Ac are contributed by loop regions from a VPI monomer and a clockwise rotated VP1 protomer.

Residues contributed by the clockwise rotated protomer are labeled cw and marked with an asterix.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4.

Sialic acid binding among Influenza and Mumps virus. **(a)** Influenza virus exhibits a conserved sialic acid binding site: Surface representation of the trimeric HA protein (top view) from influenza showing the conserved SA binding site on each individual subunit (black circle) bound to SA containing glycan (yellow) (PDB ID. 1HGG). The subunits are colored in green, orange, and magenta, respectively. **(b)** Close-up of one of the HA subunit conserved SA binding site bound to SA. **(c)** Mumps virus binds SA through the HN protein: Surface representation of a monomeric HN protein (top view) (purple) showing a deep SA binding pocket on top of HN (circled black) bound to SA (yellow) (PDB ID. 5B2D) **(d)** Close-up of the HN SA binding site bound to sialoglycan (yellow). Interestingly, in addition to Neu5Ac, Glc also contributes to stabilizing interactions.