REVIEW

The bulky and the sweet: How neutralizing antibodies and glycan receptors compete for virus binding

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Abstract: Numerous viruses rely on glycan receptor binding as the initial step in host cell infection. Engagement of specific glycan receptors such as sialylated carbohydrates, glycosaminoglycans, or histo-blood group antigens can determine host range, tissue tropism, and pathogenicity. Glycan receptor-binding sites are typically located in exposed regions on viral surfaces—sites that are also generally prone to binding of neutralizing antibodies that directly interfere with virus-glycan receptor interactions. In this review, we examine the locations and architecture of the glycan- and antibody-binding sites in four different viruses with stalk-like attachment proteins (reovirus, influenza virus, norovirus, and coronavirus) and investigate the mechanisms by which antibodies block glycan recognition. Those viruses exemplify that direct molecular mimicking of glycan receptors by antibodies is rare and further demonstrate that antibodies often partly overlap or bind sufficiently close to the receptor-binding region to hinder access to this site, achieving neutralization partially because of the epitope location and partly due to their sheer size.

Keywords: glycan receptors; viruses; neutralizing antibodies; structural characterization of binding epitopes and modes

Introduction

The attachment of a virus to its cognate host cell receptor is the first step of viral infection and serves as a key determinant of host specificity, tissue tropism and pathogenicity. For some viruses, a single receptor is sufficient to promote infection, while others require additional attachment factors or co-receptors for cell entry. Cell-surface carbohydrates linked to proteins or lipids are often-used receptors, and they are recognized by numerous viruses to facilitate attachment and entry. The carbohydrates that are typically hijacked by viruses can be grouped into three classes: sialylated carbohydrates, glycosaminoglycans (GAGs), and histo-blood group antigens (HBGAs). The glycosylation of a protein can also help mediating receptor recognition.

Sialylated carbohydrates are ubiquitously expressed among vertebrates and engaged by numerous viruses including influenza viruses, orthoreoviruses, human coronaviruses (CoVs) and adenoviruses.

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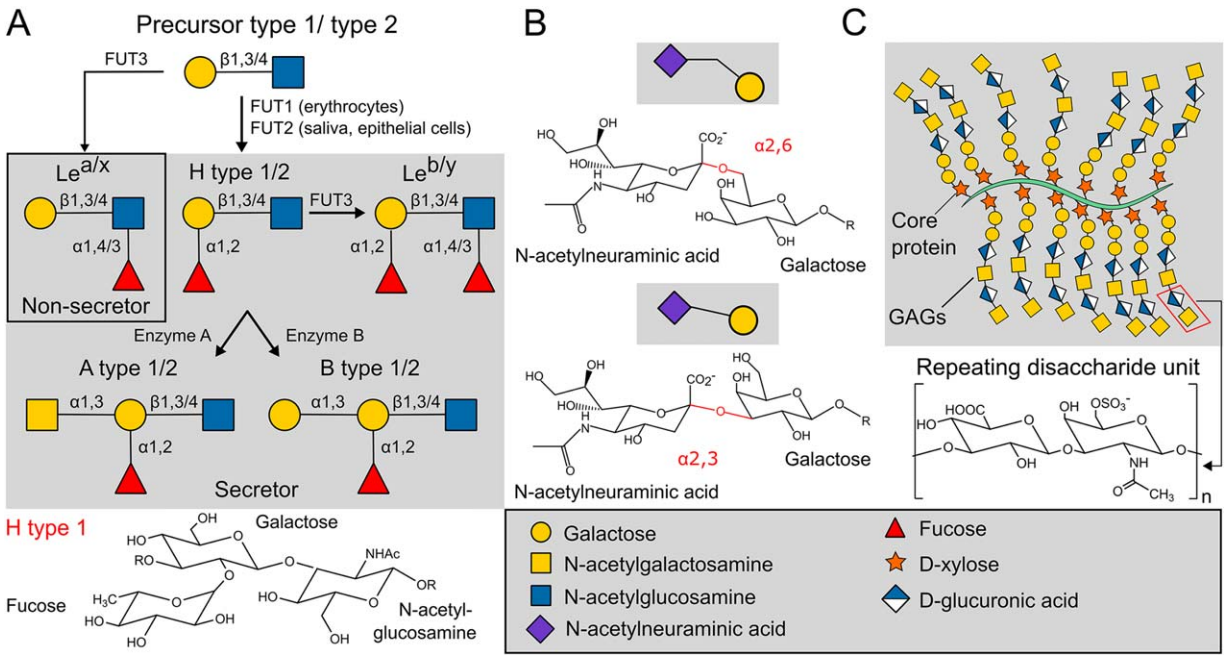

Figure 1. Glycan types that can function as viral receptors. (A) Biosynthesis of human ABH and Lewis HBGAs of Types 1 and 2. The types are defined by the glycosidic linkage of the precursor (Type 1 is β1,3 and Type 2 is β1,4 linked). Each step of the synthesis is catalyzed by a specific glycosyltransferase. FUT1 and FUT2 gene products control the same reaction. FUT1 is expressed in erythrocytes and FUT2 in secretory tissues giving rise to its glycosidic product in saliva and mucosal secretions. Sequential addition of monosaccharides to the precursor results in secretor-HBGAs in the presence and to non-secretor Lewis types in absence of FUT2 in secretions. FUT3 is primarily expressed in the epithelial cells of gastrointestinal tissue and adds a fucose to the precursor or H-type antigens. Enzyme A or enzyme B adds GalNAc or Gal via α1,3 linkages to H-type antigens, respectively, resulting in A and B type HBGAs. As an example H type 1 is shown in a structural representation. (B) Sialic acid variants. Sialic acids terminate N- and O-glycans as well as glycolipids. The two common types of linkages, the α2,6- and α2,3- linkage, are shown with the most prominent sialic acid in humans, N-acetylneuraminic acid, and Gal in a structural and schematic representation. The glycosidic linkage is highlighted in red. (C) In general GAGs are composed of repeating identical disaccharide units of N-acetylated or N-sulfated amino sugar linked to uronic acid or Gal. These units form long, unbranched GAG chains connected to a core protein. Depicted is chondroitin sulfate, a sulfated GAG consisting of repeating GalNAc and glucuronic acid units.

These glycans contain sialic acids, which are usually found at the termini of the branches of N-glycans, O-glycans, and glycosphingolipids, and they display a high level of diversity. This diversity arises from possible sialic acid modifications such as acetylation, methylation, hydroxylation, and sulfation in addition to different glycosidici linkage types that connect sialic acids to subsequent carbohydrate residues in the chain. Although α2,3 and α2,6 glycosidic linkages to galactose (Gal) or N-acetylgalactosamine (GalNAc) are the most common types found in these sialoglycan structures. To some degree, virus host range specificity can be determined by the glycosidic linkage type, as seen for example in influenza viruses.[1–3]

GAGs represent another class of virus glycan receptors or attachment factors and are recognized by, for example, herpesviruses and papillomaviruses. These linear polysaccharides are built from repeating units of β1,4-linked disaccharides, which contain an N-acetylated or N-sulfated amino sugar and an uronic acid or Gal unit.[4] Prominent examples for GAGs are chondroitin sulfate and heparan sulfate. Typically, several GAG chains are covalently attached via serine residues to a core protein, and together they form proteoglycans, which are produced by virtually all mammalian cells.[4] An important characteristic of GAGs is their overall negative charge, conferred by non-stoichiometric sulfation and the uronic acid carboxy groups.

HBGAs, on the other hand, are neutral terminal carbohydrate structures of lipid- or protein-linked glycan chains that can function as viral attachment factors for noroviruses and human rotaviruses, for example. These glycans are expressed on most epithelial cells and erythrocytes, and they are also secreted into saliva and other body fluids. Their biosynthesis is carried out through stepwise addition of monosaccharides by specific glycosyltransferases (Fig. 1).[5] Presence or absence of functional glycosyltransferase genes leads to different HBGA phenotypes among humans, leading to differences in susceptibility for certain virus strains.

Exposed virus surface areas that engage glycan and protein receptors may also be targeted by antibodies as part of the immune response. The binding of antibodies to free virus particles or infected cells

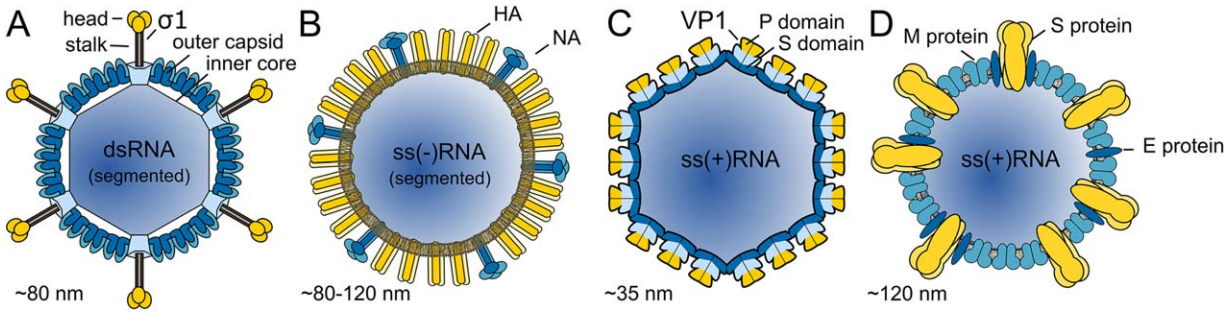

Figure 2. Morphology of viruses that contain spike-like viral attachment proteins and are discussed in this review. (A) Mammalian reovirus contains a segmented double-stranded (ds) RNA genome surrounded by two protein layers (inner core, outer capsid). The trimeric attachment protein σ1 is anchored into the capsid at the icosahedral vertices. Type 1 reoviruses engage sialylated carbohydrate receptors through the globular head domain (yellow) of σ1. (B) Influenza virus contains eight segments of single-stranded (ss) RNA. The external layer contains the envelope glycoproteins HA and NA in an approximate ratio of 4:1. The HA spike is a homotrimer, whereas NA forms a tetramer. Although HA is responsible for binding sialylated glycans, NA is a receptor-destroying enzyme that facilitates virus budding. (C) The norovirus particle is formed by 90 dimers of major capsid protein VP1 and encapsidates a (ss) RNA genome. The shell domain (S, in darker blue) of VP1 is involved in capsid formation, while the protruding domain (P, with subdomains P1 and P2 shown in light blue and yellow, respectively) projects from the shell surface at the icosahedral two-fold axes. The P domain plays an important role in immune recognition and also binds to HBGAs. (D) CoVs contains a linear (ss) RNA genome. The viral membrane is comprised of membrane (M) and envelope small membrane (E) proteins (shown in light and dark blue, respectively). The trimeric spike proteins (S, highlighted in yellow) project from the surface and harbor RBSs. Due to the high sequence diversity among the S protein of CoV strains they bind to different receptors.

can inhibit virus release from the host cell, block viral cell-to-cell transmission, or activate effector systems such as complement-dependent cytotoxicity.[6] Some antibodies are termed neutralizing, which refers to their capability to inhibit virus infectivity by direct binding to the antigen *in vitro*. Such antibodies usually bind to exposed structures on the virus surface with high affinity, and they can also protect cells from infection by interfering with attachment or cell entry.

The Protein Data Bank (PDB) contains few examples of viruses (or viral proteins) for which structural data for both glycan receptor and neutralizing antibody binding are available. Due to technical improvements in the field of structural biology and the emerging interest in glycobiology research, the number of such structures added to the PDB has increased during the past years.

We investigated a subset of these structures to examine to what extent the binding of glycan receptors and antibodies is mediated by the same structural determinants of the virus surface.

Four viruses (reoviruses, influenza viruses, noroviruses and CoVs, Fig. 2) have been chosen, and while these examples belong to different virus classes, they all contain protruding, stalk-like and multimeric viral attachment proteins that mediate interactions with glycans and, at the same time, provide a major target for neutralizing antibodies. Whereas all three glycan types can be used as receptors for viruses, structural data are available primarily for virus-sialyloligosaccharide and virus-HBGA interactions. We have therefore focused on representatives for these two interactions, as well as another example, where an N-glycosylated protein serves as receptor. A detailed comparison of the binding modes of antibodies and glycans provides insights into the determinants of glycan and antibody recognition for each case, and informs the design of improved antiviral strategies.

Mammalian Orthoreovirus (Reovirus)

Reoviruses are members of the non-enveloped *Reoviridae* family and enclose ten segments of double-stranded (ds) RNA within two concentric protein shells. Although these viruses usually cause asymptomatic infections in humans, they have been recently associated with the development of celiac disease.[7]

In newborn mice, reovirus spreads from the intestine to major organs including liver, spleen, lungs, heart and the central nervous system (CNS). The three reovirus serotypes, represented by prototype strains Type 1 Lang (T1L), Type 2 Jones, and Type 3 Dearing (T3D), differ in their route of spread and cell tropism, and they are responsible for different disease patterns in the CNS. These serotype-dependent differences have been linked to the S1 gene segment, which encodes the non-structural protein σ1s and the outer capsid protein σ1.[8,9] The homotrimeric σ1 protein is anchored in the capsid at the icosahedral vertices from where it protrudes as a filamentous stalk with a globular C-terminal head domain.

Initial attachment of the virus to the host cell is mediated by low-affinity binding of σ1 to sialylated carbohydrate receptors that is followed by an

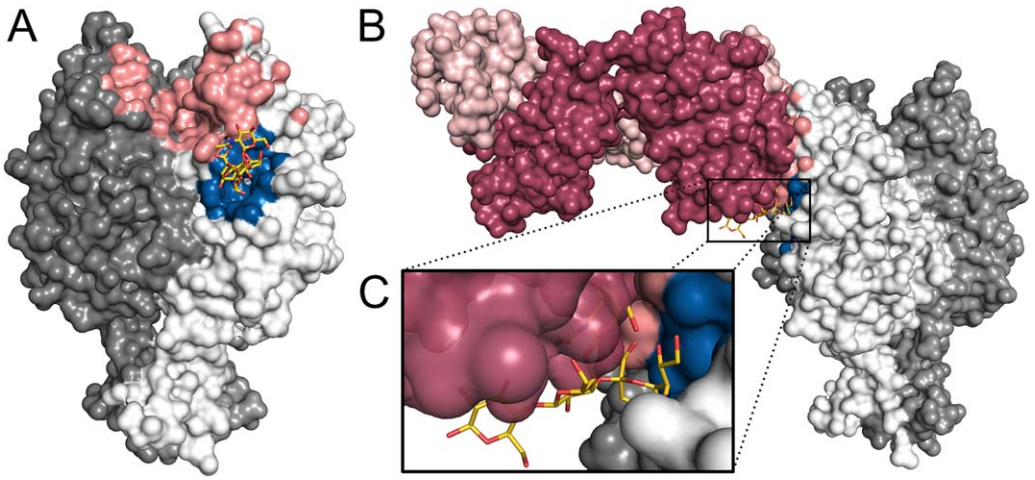

Figure 3. Binding of antibody 5C6 blocks glycan receptor engagement of the T1L reovirus protein σ1. Superposition of the T1L σ1 head domains of GM2 glycan (PDB ID: 4GU3) and Fab 5C6 (PDB ID: 5MHS) complex structures. (A) Surface representation of the trimeric σ1 head with monomers colored white, light and dark gray. The footprints of Fab 5C6 (salmon) and GM2 glycan (blue) binding have been calculated using a 4.5 Å distance cutoff. The carbohydrate molecules are shown as yellow sticks. (B) Side view of the σ1 head with one 5C6 Fab (light and heavy chain colored in light and dark violet, respectively). In this superposition, the Fab would clash with glycan moieties. For clarity, only one Fab and GM2 glycan are shown. (C) The close-up view shows that there is not enough space for simultaneous binding of 5C6 and the glycan receptor.

adhesion-strengthening step of high-affinity binding to protein receptors.[10] Reoviruses T1L and T3D both bind to sialic acid receptors but use entirely different regions of the σ1 protein for the interaction.[11,12] Furthermore, the two strains display different hemagglutination profiles, indicating different glycan specificities. Glycan array screening identified the oligosaccharide portion of ganglioside GM2 as a receptor for T1L.[11] Structural analyses of the interaction of the GM2 glycan and T1L σ1 revealed that the glycan-binding site lies in a small groove in the σ1 head domain [Fig. 3(A)]. Both terminal carbohydrate moieties of the branched tetrasaccharide contribute to σ1-binding.[11] For T3D σ1, no specific carbohydrate receptors have been identified thus far, but it was shown by structure-function studies that this strain can bind a range of differently linked sialylated glycans within the stalk of σ1. As has been observed for T1L σ1, most contacts are formed with the sialic acid glycan "cap".[12] Although both strains mostly use σ1 backbone atoms for hydrogen bond formation with the glycans, the sialic acid functional groups are engaged in different ways, for example, the sialic acid carboxyl group forms a hydrogen bond with a Gln side chain in case of T1L σ1, and a salt bridge with an Arg in case of T3D σ1 binding.

Mutations of one (T3D) or two (T1L) residues involved in σ1-glycan binding are sufficient to abolish the interaction.[12,13] T1 and T3 reoviruses that are not capable to bind to glycans are still infectious, but sialic acid-binding strains spread more rapidly from the intestine to sites of secondary replication and are substantially more neurovirulent.[13–15] Both strains also engage proteinaceous reovirus receptors, namely junctional adhesion molecule A (JAM-A) and the Nogo receptor (NgR1).[16,17] Although JAM-A binds to a conserved region in both the T1L and T3D σ1 head,[16,18] the interactions of reovirus with NgR1 are not known at the structural level. The currently available data support a model in which the interaction with the two protein receptors follows a common mechanism and is essential for T1 and T3 reovirus infection, while serotype-specific glycan interactions promote reovirus spread and influence viral cell tropism and disease, accounting for the observed differences in T1 and T3 pathogenicity.

Reovirus infection leads to the development of neutralizing and non-neutralizing antibodies. Neutralizing antibodies have been identified against three outer capsid components: σ3, λ2, and σ1.[19,20] The antibody response against σ3 and λ2 is usually group-specific, while σ1 typically elicits serotype-specific antibodies. Several regions of the σ1 protein are immunogenic and different T3 σ1-specific antibodies have been used to identify distinct functional domains of the protein.[21]

In vivo, protection from reovirus-induced disease occurs in neonatal mice with neutralizing and non-neutralizing antibodies.[20,22] The non-neutralizing antibodies that target σ3 were able to inhibit reovirus replication at a post-binding step, inhibiting either internalization or uncoating. The σ1-specific neutralizing antibodies act by blocking virus cell attachment and bind σ1 with high affinity.[23]

The neutralizing epitopes of T1- and T3-σ1 specific antibodies have been mapped to the σ1 head domain by viral escape mutant analysis.[24,25] Crystal structures of σ1 complexed with Fab fragments of

these antibodies revealed in both cases a quaternary epitope that spans two σ1 subunits[26] (Fig. 3). The binding site of receptor JAM-A is distal to these antigenic regions.

As JAM-A engagement by reovirus requires that the σ1 head closely approaches the cell membrane, the two antibodies probably block reovirus-JAM-A binding due to steric hindrance, blocking the cell membrane approach. These antibodies are also able to hinder reovirus binding to cell surface carbohydrates as indicated by hemagglutination inhibition assays. The T1-specific antibody, termed 5C6, binds next to the GM2 glycan-binding site with an interaction interface of around 700 Å^2. The heavy chain contributes about three times more to the epitope than the light chain, with its long CDR H3 loop forming most interactions. Two residues of σ1 that are involved in glycan binding are also involved in binding to 5C6. Several aromatic amino acids (mostly tyrosine residues) of the 5C6 complementarity determining regions (CDR) form hydrogen bonds and/or contribute to the binding via hydrophobic interactions with σ1. The heavy chain CDR H1 of this antibody partly occludes the glycan-binding site and thus directly blocks low-affinity glycan receptor engagement of T1L σ1.

Influenza viruses

Influenza viruses are responsible for respiratory and gastrointestinal diseases, posing a significant challenge to human health and resulting in a substantial economic burden.[27] Influenza viruses, which belong to the family of *Orthomyxoviridae*, are enveloped ss(-)RNA viruses. They are classified into three different subtypes, A–C, according to their serological cross-reactivity. The so called "flu" in humans is generally caused by Type A and B influenza viruses, with Type A being responsible for the more severe disease in humans.[28] Further classification is based on two glycoproteins projecting from the viral envelope, hemagglutinin (HA) and neuraminidase (NA), with H1N1 being the most prominent example in humans.[29,30] HA is the major surface glycoprotein and mediates cell attachment as well as fusion between the virus and host membranes.[30] The HA prefusion homotrimer undergoes a maturation process in the presence of host proteases, resulting in disulfide-linked HA1 and HA2 subunits. The HA generally consists of a head (HA1) and a stem (HA2) domain, where the head domain includes the receptor-binding pocket that interacts with sialic acid receptors on the host cell to mediate entry.[31] The head domain is therefore an important target of neutralizing antibodies.[32] It has been shown that human influenza A viruses bind preferentially to sialylated glycans with an α2,6 linkage between the terminal sialic acid and Gal. Avian viruses, on the other hand, have a preference for binding glycans with an α2,3 linkage at this position. The HA head is subject to constant antigenic drift, enabling the virus to evade the host immune response. Residues that make up the receptor-binding site (RBS) are more restricted in their mutational freedom because they are crucial for receptor recognition and binding. The analysis of sequences of pandemic virus strains allows to identify mutations in the RBS of HA that might be important for switching virus specificity. However, the Spanish Flu (H1N1) pandemic in 1918 was likely caused by only a pair of mutations in the RBS of an avian influenza virus, leading to a receptor switch from α2,3 to α2,6 linked glycans and therefore conferring human-to-human transmissibility.[33–35] Whereas α2,6 linked glycans are mainly found on human epithelial cells in the trachea and parts of the upper respiratory tract, avian viruses typically bind to α2,3 linked glycans, which can be found in the intestine of birds. Mutating only one of those sites in the H1N1 RBS already confers a dual binding to both α2,3 and α2,6 linked glycans.[34,36,37]

Over the last years, the number of available broadly neutralizing antibodies (bnAbs) against human influenza viruses has increased substantially. These bnAbs show different levels of cross-reactivity against divergent strains within and across subtypes. High resolution X-ray crystallography and cryo-electron microscopy aided in the discovery of so-called supersites of vulnerability on the HA that are recognized by bnAbs. Studies of bnAb-HA complexes revealed different modes of recognition according to which antibodies can be classified into two different groups: HA head-reactive antibodies and the more recently discovered HA stem-reactive antibodies. A subgroup of HA head-reactive antibodies are RBS-targeted antibodies, which further can be divided into antibodies either recognizing a single subtype or heterosubtypes, or antibodies employing receptor mimicry (see review[30]).

A small number of antibodies display a broader spectrum of reactivity and heterosubtypic neutralizing activity.[30,38,39] CH65 and CH67 exemplify receptor mimicry antibodies and have been shown to successfully neutralize H1 virus strains. Antibody 5J8 also employs receptor mimicry similar to that of CH65 and CH67, but additionally shows neutralization activity against a broader range of H1 strains, including the 1918 and 2009 pandemic strains. The HA RBS is contacted by a heavy chain CDR3 of 5J8 that is responsible for this binding mode: the side chain of residue Asp^{H100b} adopts an orientation similar to the one observed for the sialic acid carboxylate and additionally forms hydrogen bonds to conserved receptor-binding residues, which could also be observed for other broadly neutralizing H1 antibodies. The interaction of HA with host-cell sialyloglycans is therefore directly sterically blocked by the heavy chain CDR3 (Fig. 4).[39,40]

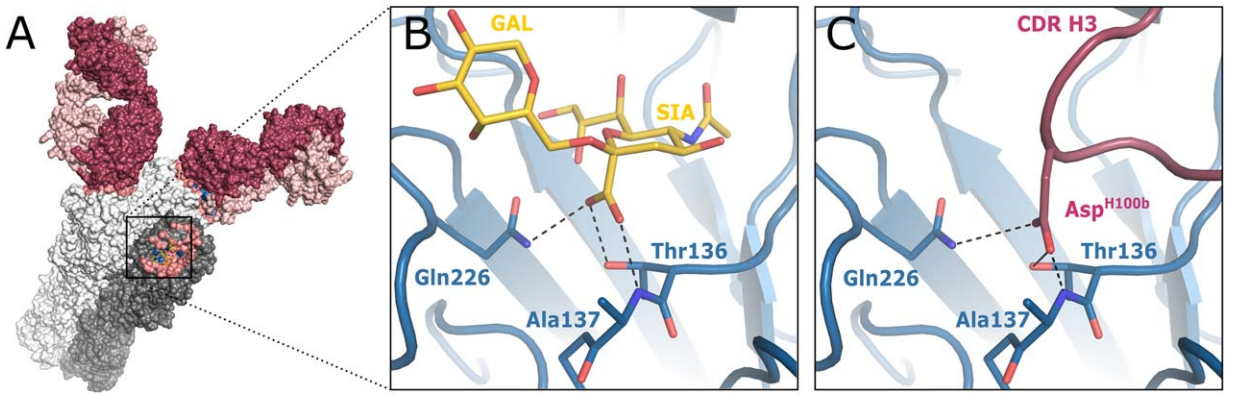

Figure 4. Structures of influenza virus HA and binding interface of glycan receptor (PDB ID: 3UBJ) and antibody 5J8 (PDB ID: 5UGY). (A) Surface representation of influenza virus Cali07/2009 HA (one monomer of the trimeric molecule is shown in darker gray) with the binding epitope of the 5J8 antibody shown in salmon and the overlapping α2,6-sialoglycan receptor-binding epitope in blue (cutoff of 4.5 Å). The sialoglycan is shown in stick representation with carbons in yellow. Two of the three bound Fabs are depicted in surface representation. (B) Detailed view of the binding interface of influenza virus Cali07/2009 HA with sialoglycan receptor. The sialoglycan receptor is shown in yellow as stick representation. Interactions of the sialic acid and the HA protein residues Gln226, Ala137, and Thr136 (also shown as sticks) are marked as black dashes. (C) Detailed view of the binding interface of influenza virus Cali07/2009 HA with the heavy chain CDR 3. The sialoglycan receptor mimicking residue Asp^{H100b} is depicted in stick representation. The residue engages the same residues as the sialoglycan receptor.

Nevertheless, there are features of the sialoglycan receptor that are not mimicked by antibody 5J8. The receptor mimicry is spatially constrained, since the RBS allows only the interaction with one antibody loop with the binding pocket. Only half of the buried surface area of the 5J8-HA complex involves the RBS. Regions adjacent to the RBS are contacted by CDR of both heavy and light chain and the total buried surface area on each HA subunit adds up to about 660 Å^2, involving several electrostatic and many van der Waals interactions stabilizing the interface.

From a conceptual point of view, bnAbs targeting the RBS are of special interest due to the limited mutational freedom of the residues forming the RBS. However, the footprint (i.e. surface area covered) of the sialoglycan receptor on the RBS is much smaller than that of the antibody. Hence, most antibodies that block the RBS also contact the adjacent regions of the head. These regions show high diversity in their sequence among different strains, which is why HA-head reactive antibodies are usually strain-specific, making a constant reformulation of vaccines on a yearly basis inevitable.

Norovirus

Noroviruses (NoVs), members of the genus *Caliciviridae*, are a major cause of acute viral gastroenteritis and form an antigenically diverse group of small non-enveloped ss(+)RNA viruses. These enteric pathogens are usually associated with short-term disease, but can be life-threatening to elderly and immunocompromised individuals.[41]

Susceptibility to most human NoVs is linked to the genetically determined expression of HBGAs, namely the ABH and Lewis family that serve as cell attachment factors for these viruses.[42] Interaction studies of virus-like particles with HBGAs revealed a diverse-binding pattern among different NoV strains. The relationship between NoV infection and histo-blood group-associated phenotypes was also shown in human volunteer studies.[43–46] Lack of specific glycosyltransferases that are involved in HBGA synthesis due to silent alleles leads to a polymorphic biosynthesis of these antigens among the human population. Presence of a functional fucosyltransferase 2 (FUT2) gene results in "secretor-positive" phenotypes, while absence leads to a "non-secretor" phenotype lacking ABH and Le^b antigens in saliva and mucosal secretions.[47]

NoVs engage HBGAs through the surface-exposed P2 subdomain of their major capsid protein VP1.[48] The structural basis of this interaction has been studied in detail by X-ray crystallography, revealing locally distinct HBGA-binding sites between the two genogroups GI and GII, which also differ structurally. These two genogroups include most human NoVs.[49–51] Genotype GI.1, also referred to as Norwalk virus, was first described and is the prototype strain of (GI) NoVs, while genotype GII.4 variants are most prevalent and account for most human NoV infections.

The majority of GI NoVs recognize H- and A-type as well as Le^b and Le^y HBGAs, while some NoVs are able to bind Le^a. In contrast, GII strains bind B-type HGBAs and exhibit a more diverse-binding pattern.[47] In GI NoVs, the glycan-binding site involves residues from a well-structured antiparallel β-sheet formed by one subunit, while the binding site of GII NoVs is located at the dimer interface and involves two exposed surface loops.

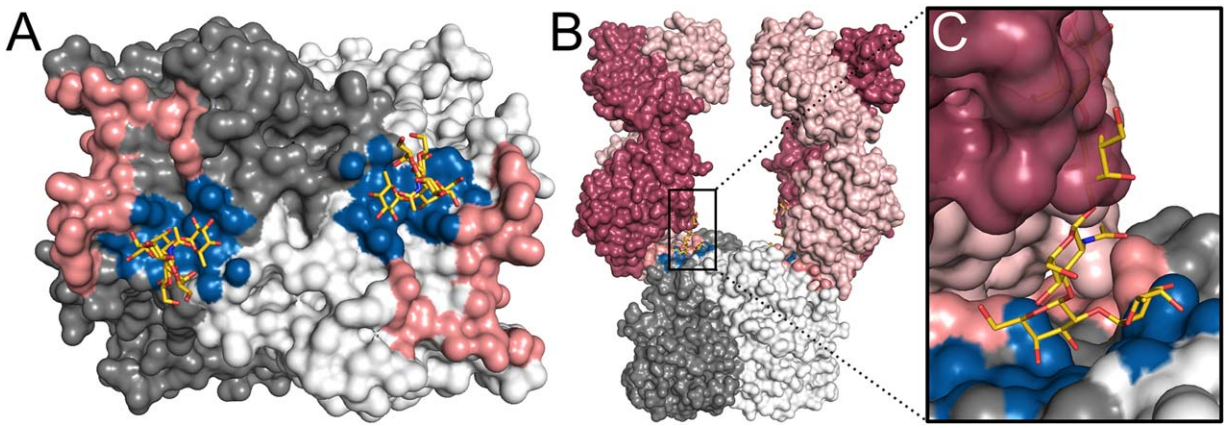

Figure 5. Binding of neutralizing antibody 5I2 to the Norovirus GI.1 P domain sterically hinders receptor HBGA binding. Superposition of the P domain complexed with HBGA (PDB ID: 2ZL6) and the P domain bound to Fab 5I2 (PDB ID: 5KW9). (A) Surface representation of the dimeric P domain with monomers colored white and gray. The epitope of Fab 5I2 (salmon) and the binding site of H-type HBGA (blue) are highlighted (distance cutoff 4.5 Å). The two binding sites are close to each other, but different P domain residues are involved in the interactions. The glycan moieties are shown as yellow sticks. Due to crystal contacts that prevent ligand binding, one H-type HBGA is modeled based on a superposition with the other subunit. (B) Side view of the P domain. Fabs 5I2 with light and heavy chain (colored in light and dark violet, respectively) binding their antigen- this engagement would partiallly mask and sterically block glycan binding. (C) The close up view shows a direct clash of glycan moieties and the heavy chain of 5I2.

The glycan-binding sites in both genogroups include a more conserved sub-site and a second (and sometimes third) site more prone to mutations, leading to strain-specific variation in HBGA binding. The conserved site of GI variants recognizes Gal (H-type, Lewis) or GalNAc moieties (A-type) whereas GII variants bind the α1,2-fucose of secretor antigens or α1,3-fucose of Le^x (and Le^y for some strains) within its conserved site. The variable site contributes less specific interactions but modulates HGBA specificity (reviewed in[52,53]).

Changes in the surface exposed P2-subdomain, which is the most variable region of NoVs, are believed to be driven by the selection pressure of the immune system. A NoV infection typically results in a homotypic adaptive immune response, eliciting antibodies that block HBGA binding. These antibodies have been shown to play a crucial role in NoV clearance.[47,54,55] Their epitopes have been mapped for different GI and GII strains, showing clustering in three regions of the P2-subdomain outside of the HBGA-binding site.[56–59]

The interaction of such a genotype-specific antibody (human IgA 5I2) with the P domain of GI.1 was recently investigated by X-ray crystallography (PDB ID 2ZL6).[60] The complex structure of the Fab-P domain revealed a conformational epitope that flanks the glycan-binding site (Fig. 5). No P domain residue of the HBGA-binding site contacts antibody 5I2 and antibody recognition does not alter any side-chain orientation in the glycan-binding site.

Nevertheless, modeling of simultaneous Fab- and glycan receptor binding to the P domain indicates that the antibody 5I2 directly blocks HBGA engagement of the P domain. The heavy chain occludes the glycan-binding site and clashes with carbohydrate moieties of the glycan. Thus, the likely mechanism by which antibody 5I2 neutralizes GI.1 is through direct steric hindrance and not by an allosterically disruption of the glycan-binding site or by blockade via binding to the same VP1 residues.

Coronaviruses

CoVs belong to the family of *Coronaviridae* and are enveloped, ss(+)RNA viruses that can cause respiratory, enteric, hepatic and neuronal infectious diseases in animals and humans. They are classified into the four genera: alpha-, beta-, gamma-, and delta-CoV.[61,62] CoVs possess a trimeric envelope spike glycoprotein (S), which is responsible for receptor binding, membrane fusion, and viral entry.[63] The S protein is organized into a membrane-distal N-terminal S1 domain, a membrane-proximal S2 domain, a transmembrane region, and an intracellular domain.[64] The receptor-binding domain (RBD) is part of the S1 domain, which determines cellular tropism, while the S2 domain mediates virus-cell fusion.[65]

CoVs differ substantially in their RBD with regard to sequence and structure, and they consequently recognize different entry receptors. The viruses show a complex receptor recognition pattern where even highly similar CoVs from the same genus recognize different receptors.[66]

A novel CoV, the Middle East respiratory syndrome CoV (MERS-CoV) was first identified in June 2012.[67] MERS-CoV infection causes symptoms such as severe pneumonia, septic shock, and multi-organ failure, which in many cases is fatal (http://www.

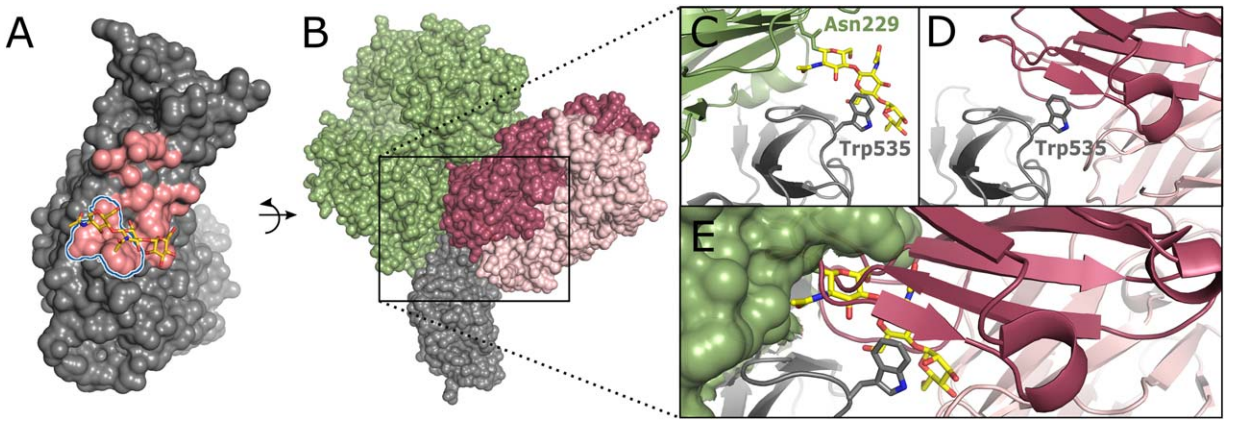

Figure 6. Structures of MERS-CoV RBD and binding surface of receptor DPP4 (PDB ID: 4KR0) and antibody MERS-27 (PDB ID: 4ZS6). (A) Surface representation of MERS-CoV RBD with the binding epitope of Fab MERS-27 shown in salmon and the overlapping DPP4 carbohydrate-binding epitope in blue (distance cutoff of 4.5 Å). The carbohydrate moiety is shown in stick representation, with carbons colored yellow. (B) Surface representation of MERS-CoV RBD with DPP4 shown in green and Fab MERS-27 with heavy and light chain (dark and light purple, respectively). Detailed view of the MERS-CoV RBD with focus on residue Trp535. (C) DPP4 receptor binding on RBD (shown in green and gray cartoon representation, respectively) with the Asn229-linked carbohydrate moiety in DPP4 (shown in yellow stick representation) interacting with residue Trp535. (D) Complex of RBD with MERS-27 Fab. The heavy and light chain are colored in dark and light purple, respectively, and the central Trp535 residue is depicted in stick representation. (E) Representation of steric clashes upon simultaneous receptor and antibody binding. Since the binding site of the carbohydrate moiety completely overlaps with the Fab-binding site, simultaneous binding is sterically not feasible.

who.int/emergencies/mers-cov/en/). So far, there is no specific treatment or vaccine against CoVs available but due to the epidemic and pandemic potential of emerging CoVs such as MERS-CoV or SARS-CoV, there is an urgent need of medical countermeasures.[68]

MERS-CoV utilizes human dipeptidyl peptidase 4 (DPP4), also known as CD26, as cellular receptor. DPP4 exhibits neither structural nor any significant sequence similarities to previously identified CoV receptors, such as the carcinoembryonic antigen-cell adhesion molecule 1, a receptor for mouse hepatitis virus, or aminopeptidase N, a receptor for several CoVs.[69,70] However, the importance of N-linked glycosylation for receptor recognition and specificity is common to these receptors. A number of CoVs from different genera (infecting mice, pigs, or birds) use sialic acids as attachment factors.[71,72]

DPP4 is a multifunctional, Type-II-membrane glycoprotein and is highly conserved among mammals, where it plays important roles in T-cell activation, glucose metabolism and apoptosis.[73,74] The structure of DPP4 contains an N-terminal β-propeller domain[75] consisting of eight blades, and structural analyses of complexes with the S protein show that DPP4 contacts the RBD of MERS-CoV with Blades 4 and 5.[76,77] The binding interface reveals a series of residues that are critical for the virus-receptor engagement.[78] Interactions involve some hydrophilic residues, which form a polar network, as well as three hydrophobic residues.[65,77] As for all CoVs, N-linked glycosylation of the receptor is important for binding to MERS-CoV. In the crystal structure of the MERS-CoV RBD-DPP4 complex (PDB code 4KR0), a trisaccharide attached to N229 contacting the RBD is well defined.[76,77] The first N-acetylglucosamine (NAG) residue of the glycan moiety forms a hydrogen bond with RBD Glu536, while the second NAG stacks onto the aromatic ring of Trp535. The third mannose residue contacts Trp535 via hydrogen bonding.[65,76,77] These interactions are crucial for binding to DPP4 and the viral entry of MERS-CoV.[79]

Since MERS-CoV uses DPP4 as single cellular receptor for viral entry, agents capable of blocking this interaction would be promising candidates for blocking the entry of the virus into the target cell.[80] It was shown that monoclonal and polyclonal antibodies directed against DPP4 inhibit MERS-CoV infection in different cell types.[74,81] As DPP4 plays important roles in multiple cellular processes, antibodies against DPP4 may however cause unwanted side reactions. Hence, antibodies targeting the RBD of MERS-CoV are preferred.[80] Additionally, it was shown in animal models that the entry of MERS-CoV could be inhibited by polyclonal antibodies against MERS-CoV RBD.[82–84] These studies fortify the hypothesis that antibodies directed against the RBD might efficiently block MERS-CoV entry. Recently, two neutralizing antibodies were identified[79] in a library screening approach against the RBD of MERS-CoV. These antibodies were also shown to inhibit *in vitro* infection of live MERS-CoV and pseudoviruses, making them potential candidates for combating MERS-CoV infection.[79,80] So far,

only the crystal structure for the complex of MERS-CoV bound to MERS-27 is available (PDB code 4ZS6).[79] In this complex, both the heavy and the light chain of MERS-27 interact with MERS-CoV RBD. The binding epitope of MERS-27 shows only a small overlap with the binding epitope of DPP4, yet the insertion of Trp535 and its carbohydrate moiety into the cavity between heavy and light chain completely disrupts protein-protein and protein-carbohydrate interactions between RBD and its receptor DPP4 (Fig. 6).[79] Hence, MERS-27 would prevent the engagement of DPP4 in part by clashing with the N-glycan attached to the receptor.

Conclusions

Viruses pose a constant threat to human health and are a substantial economic burden. Identifying and characterizing viral receptors and defining mechanisms of virus attachment and internalization improves our understanding of infection processes and viral replication cycles, and this mechanistic knowledge can ultimately inform strategies for antiviral therapy—for example, through targeted interference with attachment or release. Neutralizing antibodies play a central role for natural protection from viral infections. The characterization of antibodies from patients who survived life-threatening diseases, for example, ebola virus disease, demonstrates the vast therapeutic potential of antibodies[85,86]; however, our understanding of antibody-mediated neutralization has not yet reached a level that allows us to fully exploit this potential in antibody-based therapies or vaccination. The structural analysis of bnAbs and their binding epitopes on viral surfaces can help to further advance this understanding and, ultimately, the development of individual antiviral strategies.

In this review, we focused on four human viruses that all contain protruding, spike-like attachment proteins, and for which structural data for both glycan receptor and neutralizing antibody binding are available. The RBSs of these spikes are most distal to the virus particle, and, therefore, are often part of or in direct proximity to the epitopes recognized by neutralizing antibodies. It seems intuitive that both RBSs and epitopes for antibody recognition benefit from facile accessibility of the respective region on virus particle, and also that those antibodies that prevent simultaneous receptor binding via steric hindrance are particularly efficient immunological tools. Therefore, it is perhaps not surprising that the receptor and antibody-binding sites of the four viruses discussed in this article overlap in many cases. A comparison shows that while most often no direct glycan receptor mimicry is employed by the antibodies, partial (reovirus) or complete (CoV) blocking of the glycan-binding site is a common feature in virus-antibody complexes. Efficient neutralization can also be observed for antibodies that bind in the immediate vicinity but do not show any overlap with the glycan-binding site (norovirus). In these cases, all antibodies possess conformational epitopes, a common characteristic for neutralizing antibodies. A special case is quaternary epitopes that span multiple subunits, as is seen, for example, for the reovirus-targeting 5C6 antibody. Here, the antibody-binding site bridges two subunits in juxtaposition with residues responsible for glycan engagement found in a single monomer. There is no obvious requirement of such a subunit "bridging" for the ability of the antibody to block glycan binding, but the bridging might well prevent conformational changes of the viral attachment protein during later entry steps. This is well established for some enveloped viruses, for which antibodies have been found to stabilize a pre-fusion state of the virus, thereby preventing conformational changes that are required for receptor binding or membrane fusion. Examples are the neutralizing antibody 37.7H targeting lassa virus, or the monoclonal antibody 2D22 against dengue virus.[87,88]

From a biochemical point of view, there are profound differences between glycan and antibodies with respect to virus-engagement, first because of the relative size but also because of the physiological context in which these interactions occur. Viruses bind to individual glycan receptors with relatively low affinity (often in the mM K_d-range) using only a limited number of contacts. Because the local density of some glycoconjugates, for example gangliosides, on the plasma membrane can be very high, the low individual binding energy is typically enhanced by engaging multiple receptor copies at the same time, that is, low affinity is overcome by high avidity. Antibodies, on the other hand, need to have a high affinity (at least nM K_d-range) for virion surface antigens to mediate efficient neutralization. In part (and particular when compared with glycan binding) this is due to the limited number of the comparably large antibodies that can simultaneously engage a viral spike protein without clashing. Thus, while the location of glycan RBSs and antibody epitopes on viral capsids may often overlap at least in part, other aspects of glycan- and antibody-virus interactions are fundamentally different (Fig. 7).

The footprint of antibodies is in general significantly (roughly three times) larger than the footprint of the glycan, making up around 700 Å^2, with the contacts being formed by the heavy- and light-chain CDRs. Generally, a large fraction of the CDRs is comprised of aromatic residues such as tryptophane and tyrosine, whose antigen interactions can therefore be based on their hydrophobic, aromatic, and hydrophilic character. Glycans also rely on different types of non-covalent interactions to facilitate protein binding. Dependent on the orientation of the functional groups,

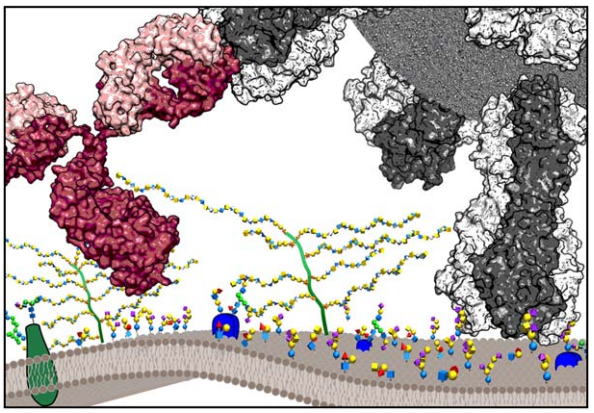

Figure 7. Schematic representation of the competition between antibodies and glycan receptors for virus binding. Exemplified is a virus with trimeric spike proteins shown in gray and an antibody with heavy and light chain in dark and light purple, respectively. Cell-surface carbohydrates (built with web-based glycan modeler tools of: Woods Group [2005–2017] GLYCAM Web. Complex Carbohydrate Research Center, University of Georgia, Athens, GA. [http://glycam.org]) are shown in a schematic representation and are roughly scaled to the size of the viral spikes and antibody.

the faces of glycan rings exhibit a different pattern between hydrophobic and hydrophilic properties.[89] They can participate in hydrogen bonds, van der Waals interactions, as well as in hydrophobic and CH-π interactions. Glycan receptor mimicry by antibodies is probably limited due to geometrical and spatial restraints of the CDRs. One rare example for glycan receptor mimicry is antibody 5J8, which targets the RBS on HA of human influenza A virus.

Although it would perhaps be easier for an antibody to mimic a proteinaceous virus receptor, our search of the literature did not yield many examples for such a strategy. The most prominent case is HIV, in which the receptor CD4 induces a profound structural change in the HIV envelope protein gp120.[90–92] Here, antibodies that mimic CD4-binding can elicit this change, thereby rendering gp120 vulnerable to the immune system. However, the situation for HIV is likely unique as the binding of glycan or protein receptors does not normally trigger such massive conformational changes in viral attachment proteins. In fact, in the cases reviewed here, the structural changes that occur upon glycan binding are minimal.

As antibodies are larger by far than most protein receptors, it may be that they have no need to exactly mimic receptor binding in order to achieve neutralization. It appears that just binding anywhere in the vicinity of the receptor is sufficient as this would readily prevent access of the virus to the receptor in most cases.

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