Viral Interactions with Glycans

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Abstract

The cell surface of mammalian cells is covered with complex glycans or polysaccharides. Several viruses attach to cells via glycans present on the host cell surface. These cell surface glycans such as heparan sulfate proteoglycan enhance the infectivity of host cells. Cell surface glycans also serve as cellular receptors involved in the transmission of endocytosis-inducing signals or in the induction of fusion between viral envelope and cellular membrane for some other viruses. In contrast, viruses can use host cell functions to glycosylate viral proteins,

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which affects viral glycoprotein stability and function during host cell entry. Glycosylation of viral glycoproteins is also involved in viral antigenicity responsible for immune evasion by viruses. In this review, functions of glycan-mediated interactions between host cells and viruses are discussed.

Keywords
Glycoprotein • Viral entry • Viral attachment • Entry receptor • Attachment factor • Antigenicity • Immune evasion • Lectin

Introduction

The cell surface and extracellular matrix of mammalian cells contain many complex glycans or polysaccharides. The highly heterogeneous cell surface glycans are covalently attached to proteins or lipids. These glycans are classified as either linear or branched. Branched glycans exist as *N*- and *O*-linked glycans on proteins or in glycolipids. The majority of linear sugars are glycosaminoglycans, containing long polymers of sulfated and epimerized disaccharide repeats that are *O*-linked to a core protein. Proteoglycans can form aggregates such as heparan sulfate proteoglycan (HSPG). These complex properties of glycans affect various biological and pathological processes, such as cell growth, differentiation, adhesion, tumor invasion and metastasis, and microbial pathogenesis. Several viruses attach to cells via glycans present on the host cell surface. These cell surface glycans enhance the infectivity of host cells. In contrast, viruses can use host cell functions to modify viral proteins, ultimately impacting the roles of viral glycoproteins in stability, antigenicity, and host cell entry. In order to discuss the interactions between viruses and glycans, a comprehensive understanding of glycans on both the host cell and viral surfaces is important.

Host Cell Surface Glycans During Viral Entry

Viral particles comprise double- or single-stranded DNA or RNA molecules surrounded by a capsid. For some types of viruses, the capsids are further surrounded by a tegument (matrix) protein and lipid bilayer envelope; other viruses possess no tegument and envelope. Various types of molecules are expressed on the surfaces of viruses, including envelope proteins, glycoproteins, spikes, or fibers with knobs. Viral particles initially attach to the host cell surface to enter host cells by associating with the host cell surface molecules such as proteins, lipids, and glycans (Fig. 1). Following this attachment, viruses can enter host cells through two pathways (Connolly et al. 2011). One pathway is the direct fusion pathway, where the viral envelope directly fuses with the cell membrane through interactions between envelope proteins and cellular receptors. The other pathway is the endocytosis pathway. In this pathway, virions are initially endocytosed in a manner similar to that of clathrin-dependent endocytosis, caveolae-mediated

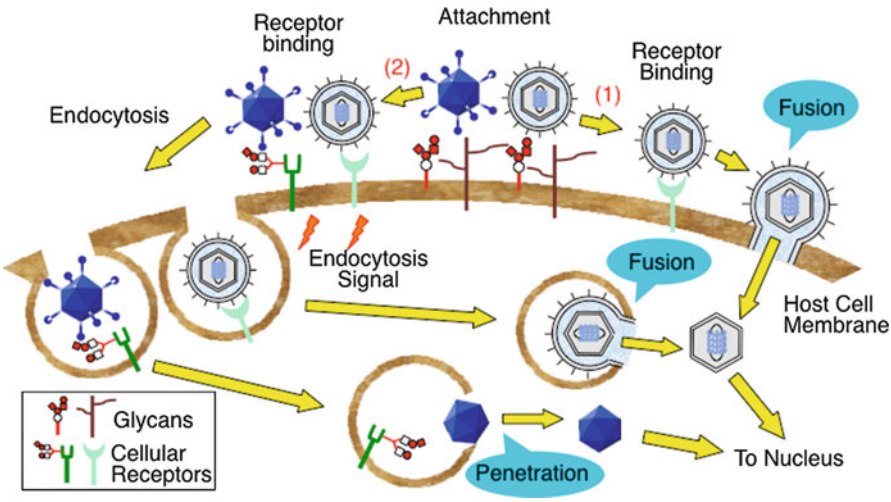

Fig. 1 Glycans on the host cell surface and viral entry routes. Following its attachment to host cell glycans, such as HSPG, gangliosides, or sialic acid-binding proteins, a virus can enter via two routes. (*1*) Direct fusion of the viral envelope with the host cell membrane, which is mediated by an interaction between the viral envelope protein and its cellular receptor. (*2*) Internalization of viral particles via endocytosis, which is enhanced by signal transduction consequent to the interaction between the viral protein and its cellular receptor. The viral envelope fuses with the endosomal membrane or the capsid penetrates through the endosome. After fusion or penetration, the capsid can enter the cytosol, where it is transported to the host cell nucleus

endocytosis, or macropinocytosis, followed by membrane fusion between the viral envelope and the endosomal membrane. In this pathway, a low pH-dependent conformational change in the viral surface proteins is thought to be involved in membrane fusion (Yamauchi and Helenius 2013). Subsequently, the viral capsid enters the cytosol or, for some types of virus (mainly RNA viruses), the capsid contents are directly released into the cytosol in a process called penetration. The capsids of most DNA viruses are transported toward the nucleus. The host glycans play an important role in viral infection during these viral entry processes. Cell surface glycans mediate the attachment of viral particles to the cell surface, thus concentrating viral particles on the cell surface and enhancing the entry efficiency and/or serving as cellular receptors involved in the transmission of endocytosis-inducing signals to the cytoplasm or the induction of conformational changes in the viral surface molecules to induce membrane fusion (Yamauchi and Helenius 2013).

The monosaccharide sialic acid labels all eukaryotic cell surfaces and caps many different oligosaccharide structures on *N*-linked and *O*-linked glycoproteins as well as on glycolipids such as gangliosides. Some sialic acid-terminal glycans have emerged as attachment factors or receptors for an enormous number of viruses. Conformational changes in the envelope proteins are generally not triggered when carbohydrates serve as attachment factors. However, highly pathogenic influenza

viruses utilize sialic acids as entry receptors through an association of these receptors with the viral hemagglutinin antigen (HA). The binding of HA to sialic acid-containing cell surface molecules leads to the transduction of endocytosis signals in host cells and consequently induces macropinocytosis or endocytosis mediated by the clathrin, lipid raft, and caveolae (Yamauchi and Helenius 2013). Subsequently, a conformational change in HA is induced by the low pH conditions in the endosomes. In contrast, after the binding of HA to sialic acids, host cell proteases on the mucosal surfaces of the respiratory tract cleave and activate HA. Therefore, the susceptibility of HA to certain cellular proteases affects the tissue tropism and virulence of the influenza virus (Viswanathan et al. 2010). Furthermore, avian influenza viral HA prefers to bind to α2,3-linked sialic acids, whereas the human viral HA prefers to bind to α2,6-linked sialic acids, indicating that the sialic acid structure is important in the determination of influenza virus tropism and host specificity.

HA from the human parainfluenza viruses, which are classified as *Paramyxoviridae*, similarly attaches to sialic acids (Neu et al. 2011). Other *Paramyxoviridae* viruses including the measles virus (MV), Newcastle disease virus (NDV), mumps virus, Sendai virus, and respiratory syncytial virus (RSV) also interact with sialic acids via HA or HA-neuraminidase (NA; HN). The binding of MV and NDV HA or HN to sialic acid triggers membrane fusion via the induction of conformational changes in the fusion protein (F protein). Some enteroviruses such as the coxsackievirus A24 variant and enterovirus 70, which cause acute hemorrhagic conjunctivitis, are also known to attach to sialylated oligosaccharides. Other *Picornaviridae* viruses, the hepatitis A virus, and certain rhinoviruses that induce upper respiratory inflammation also interact with sialic acids. Human adenovirus type 37, an epidemic keratoconjunctivitis-inducing virus, was recently shown to bind to a disialylated GD1a that contained α2,3-linked sialic acids. The human JC and BK polyomaviruses (JCV and BKV) cause a fatal demyelinating disease and kidney graft loss, respectively, in immunocompromised hosts (Neu et al. 2009). JCV binds to α2,3- or α2,6-linked sialic acids as well as its receptor, GT1b, that contains α2,3-linked sialic acids. BKV binds to GD1b and GT1b, which contain α2,3-linked sialic acids. The recently identified Merkel cell polyomavirus, a human oncovirus, preferentially binds to the GT1b. VP1, the major capsid protein of other mammalian polyomaviruses, including the simian virus 40 (SV40) and murine polyomavirus (mPyV), also attaches to sialic acid-terminal glycans such as GM1 (SV40), GD1a, and GT1b (mPyV), which contain α2,3-linked sialic acids. Some strains of the human norovirus, which is a member of the *Caliciviridae* family and a causative agent of gastrointestinal illnesses, such as vomiting and diarrhea, bind to non-sialylated histo-blood group antigens whereas others bind to sialyl-Lewis X (Neu et al. 2011). Rotaviruses, which are members of the *Reoviridae* family, induce gastroenteritis in children and have long been classified into strains according to sensitivity to sialidase treatment. However, strain Wa, which was previously considered a sialidase-insensitive strain, was recently shown to bind to the ganglioside GM1 via the viral protein VP4. Because of its branched structure, GM1 is considered to be resistant to sialidases. *Parvoviridae*

viruses such as the adeno-associated viruses 4 and 5, *Herpesviridae* viruses such as the human and murine cytomegaloviruses (HCMV and MCMV), *Hepadnaviridae* viruses such as the hepatitis B virus, *Rhabdoviridae* viruses such as the vesicular stomatitis virus, and some of the *Coronaviridae* virus are also known to associate with sialic acids.

HSPGs function as attachment factors and/or entry receptors for some viruses such as the human papilloma viruses (HPV), which are the causative agents of squamous fibroepithelial tumors, condylomas, and malignant epithelial tumors (Bartlett and Park 2011). More than 150 types of HPV have been identified and classified into two categories according to tropism. Some of these, including the cervical cancer-inducing strains 16 and 18, preferentially infect the genital mucosa whereas others infect the skin. Some HPVs bind to HSPG on either epithelial cell surfaces or basement membranes via interactions with the L1 major capsid protein (Raff et al. 2013). After binding to HSPG, cyclophilin B alters the capsid conformation and proprotein convertase cleaves the minor capsid protein L2 to expose the N-terminus of L2. Subsequently, HPVs bind to L2-specific receptors, including the annexin A2 heterotetramer (HPV16) and syntaxin 18 (bovine papilloma virus), for entry. The herpes simplex virus (HSV) glycoprotein C also associates with HSPGs on the host cell surface. HSPG-bound HSV particles not only concentrate on the cell surface but also are transported from cell protrusions such as filopodia to the cell body, wherein viruses can easily enter into the cells (Tiwari et al. 2012). Similar viral entry processes have also been observed with respect to the human immunodeficiency virus (HIV), HPV, HCMV, and Kaposi's sarcoma-associated herpesvirus (KSHV). Endocytosis or membrane fusion is subsequently induced by other envelope glycoproteins such gB and/or gH through an interaction with their receptors such as nectins, herpes virus entry mediators, the paired immunoglobulin-like type 2 receptor α (PILRα) (Satoh et al. 2008), myelin-associated glycoprotein (Suenaga et al. 2010), non-muscle myosin heavy chains (Arii et al. 2010), or integrins (Connolly et al. 2011).

In the case of HIV infection, HSPGs not only serve as an attachment factor but also as a virulence factor-receptor. HIV gp120 is an envelope glycoprotein that binds to CD4 and HSPGs (Bartlett and Park 2011). The binding of gp120 to HSPGs is thought to increase the concentration of viruses on the cell surface to enhance infectivity. The HIV transcriptional activator protein (Tat) is released from HIV-infected cells and can be detected in the serum of HIV-infected individuals. The neurotoxic Tat is involved in the pathogenesis of AIDS encephalopathy as well as KSHV-induced tumorigenesis. Tat interacts with the αvβ3 integrin, VEGF receptors, and chemokine receptors (e.g., CCR2, CCR3, and CXCR4) as well as HSPG. Tat is oligomerized upon association with HSPG and is subsequently internalized to activate the transcription of HIV genes. In addition, the interaction between HIV and HSPG is involved in the sexual transmission of HIV infection. HIV virions can bind to the heparan sulfate expressed on spermatozoa and can thus be transmitted in the semen along with free virions. These spermatozoa-associated virions are then efficiently transmitted to dendritic cells (DCs), macrophages, and T cells.

Glycans on Viral Surface Glycoproteins

Viruses use cellular biosynthetic systems to generate components and take advantage of the cellular glycosylation pathway, particularly the *N*-glycosylation pathway, to modify viral proteins. Viral envelope proteins require *N*-glycosylation for proper folding and trafficking. In addition, envelope protein glycosylation affects the interactions of these proteins with cellular receptors (Fig. 2a). Glycosylation is also involved in the immune recognition of viruses (Fig. 2b), thus affecting viral infectivity, survival, and transmissibility (Vigerust and Shepherd 2007).

The influenza virus is among the most studied viruses with respect to viral component glycosylation, although the sialylated HA receptors expressed on host cells have also been well studied, as mentioned above (Fig. 1). The glycosylation of influenza viral HA and NA affects a variety of functions, including receptor binding and viral entry/egress. HA is posttranslationally modified via glycosylation in a host cell-dependent manner; this process is crucial for the proper folding and trafficking of HA during infection. On HA, the number of glycosylated sites range between 5 and 11, and these sites are primarily located on the globular head of HA (Vigerust and Shepherd 2007). Host proteases cleave and activate HA during viral entry into the cells. Therefore, the carbohydrates located near the cleavage site prevent protease access to HA. In addition, glycans near the receptor-binding site inhibit viral entry (Fig. 2a). Because sialic acids not only bind to HA during host cell entry but also during egress, HA must be dissociated from sialic acids via its own NA when the viral particles are released from the cells. Moreover, carbohydrates located around HA globular head serve to mask antigenic epitopes from immune recognition. This process is part of an influenza virus antigenic drift mechanism where glycans create a shield that prevents antibody (Ab) access and recognition (Fig. 2b).

HIV is a highly mutagenic and variable member of the *Retroviridae* family that contains multiple subtypes or clades. HIV gp120 is among the most heavily glycosylated proteins in nature (Vigerust and Shepherd 2007). gp120 possesses 18–33 possible *N*-linked glycosylation sites. Although gp120 associates with HSPG while attaching to host cells, as mentioned above (Fig. 1), the carbohydrates on mature gp120 molecules also play a direct role in the interaction with CD4. The loss of glycans diminishes the binding of HIV to CD4, resulting in reduced infectivity and cytopathicity (Fig. 2a). The CD4 binding site on gp120 is protected by glycans and a recessed structure. The glycosylation of gp120 is also involved in neutralizing Ab sensitivity (Fig. 2b). Because severe antigenic drift has been observed with respect to gp120, it is difficult to induce neutralizing Abs against HIV via immunization. Neutralizing Abs against HIV were recently reported to recognize certain gp120 glycosylation sites in a glycan-dependent manner (Klein et al. 2013). Furthermore, some drugs directly target the carbohydrate components of gp120 and thus potentially regulate viral infections (Vigerust and Shepherd 2007). Chloroquine and its derivatives have been successfully used in combination therapies to reduce the viral loads in HIV patients. Chloroquine accumulates in the endosome and prevents the acidification that is required for the membrane fusion

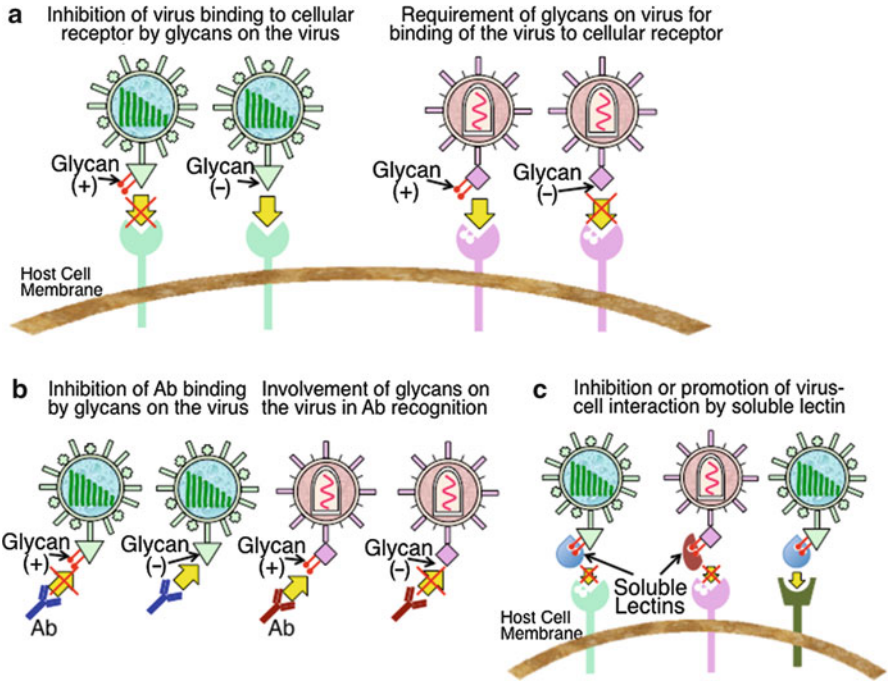

Fig. 2 Involvement of viral surface glycans in entry and antibody recognition. (**a**) Envelope glycans are required for binding to receptor(s) or attachment partner(s) such as lectins in certain viral strains. In contrast, envelope glycans of some other viral strains inhibit viral protein(s) binding to receptor(s). (**b**) These glycans act to shield the protein from host neutralizing antibodies. In contrast, some antibodies recognize glycans or specific configurations of glycans and glycan-binding proteins. (**c**) Soluble host factors such as soluble lectins associate with viral proteins via glycans and can either inhibit or promote the interaction between the virus and the host cell surface

and entry of HIV into the cytoplasm. Chloroquine also inhibits glycosyltransferases in the ER and Golgi, resulting in the insufficient glycosylation or misfolding of viral proteins.

Regarding glycans associated with other viruses, glycans on the envelope protein E and prM of flaviviruses, which includes the highly pathogenic West Nile virus (WNV), have been proposed to be involved in receptor binding, membrane fusion, virus assembly, and pathogenicity because the removal of glycans from either the prM or E proteins was found to reduce both viral entry and viral particle release (Vigerust and Shepherd 2007). The loss of E1 protein glycosylation in the hepatitis C virus (HCV) was also found to affect translocation of the E1 protein to the cell surface as well as protein folding, leading to reduced viral entry. *N*-linked glycosylation was also found to be involved in the pathogenicities of the Ebola, Hantaan, Newcastle, Hendra, and Nipah viruses as well as of the metapneumovirus and the severe acute respiratory syndrome coronavirus (SARS-CoV).

Viruses are also recognized by host immune factors other than Ab. There has been an increased interest in carbohydrate recognition molecules such as the mammalian lectins (Vigerust and Shepherd 2007). One notable family is the calcium-dependent lectins (C-type lectins) including the cell-associated macrophage mannose receptor (MMR), the dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN), the soluble lectin surfactant-associated proteins A and D (SP-A and SP-D), and the mannose-binding lectin (MBL). These C-type lectins participate in the sensing or clearance of pathogens during immune responses.

MMR participates in both endocytic and phagocytic uptake of proteins and particles. Influenza virus HA and HIV gp120 associates with MMR (Vigerust and Shepherd 2007). In particular, influenza virus bound to MMR is readily internalized and delivered through its association with an acidic vesicle. MMR may thus be involved in direct influenza viral infection (Fig. 2c). The DC-SIGN-mediated recognition of the HIV gp120 *N*-linked mannose-rich glycans leads to the transfer of virions from DCs to $CD4^+$ T cells (Fig. 2a). The HCV E2 protein associates with DC-SIGN and the related liver lectin L-SIGN via the high-mannose *N*-glycans on E2. The association between HCV and these lectins may be involved in HCV infection of the liver or DC. Glycans on the prM or E proteins of WNV interact with DC-SIGN or its receptor (DC-SIGNR). The glycosylation sites and glycan types found on the WNV virions will determine the molecules that they bind during infection (e.g., DC-SIGN or DC-SIGNR). DC-SIGNR is associated with many types of viruses, and those viruses that associate with DC-SIGN predominantly possess mannose-rich surfaces. Glycans on the envelope proteins of the Ebola virus, Marburg virus, HCMV, Dengue virus, and Sindbis virus also interact with DC-SIGN, L-SIGN, and DC-SIGNR via high-mannose *N*-linked glycans and are involved in infectivity. Interactions between the SARS-CoV S protein and filovirus glycoproteins with the lectin LSECtin (CLEC4G) enhance infection. The *LSECtin* gene is located in the same chromosomal locus as the *DC-SIGN* gene, and LSECtin is co-expressed with DC-SIGN in the liver and lymph nodes. LSECtin does not interact with either the HIV or HCV envelope proteins. Because MMR, DC-SIGN, and LSECtin are expressed on innate immune cells, an additional advantage of the association between viruses and these molecules may render the viruses invisible to the immune cells. Soluble lectins such as MBL bind to Ebola and Marburg envelope glycoproteins and block the association of both viruses with DC-SIGN. SP-A and SP-D similarly interact with the influenza virus to block the association of HA with sialic acids (Fig. 2c). The binding of MMR, MBL, or SP-D to the influenza virus involves the molecular carbohydrate recognition domain and high-mannose oligosaccharides on HA and NA. MMR and SP-D also bind to HIV and prevent the entry and replication of HIV (Fig. 2c). These lectins directly bind to various viruses and act as soluble effectors of innate immunity by interfering with the associations of these viruses with the targeted receptors.

Another mammalian lectin family member, galectin (also called the S-type lectin), has been reported to promote HIV and human T-lymphotropic virus (HTLV)-1 infections (Sato et al. 2009). In particular, the binding of galectin-1 to

β-galactoside-containing sugars enhances viral attachment and infection of target cells such as macrophages (HIV) and $CD4^+$ T cells (HIV and HTLV-1). In contrast, galectin-1 inhibits the fusion of the Nipah virus with its target cells. The P-type lectin cation-independent mannose-6-phophate receptor (MPR^{ci}) is ubiquitously expressed and primarily functions as a molecular chaperone that transports *N*-linked oligosaccharide-modified proteins from the trans-Golgi network to the early endosomes. MPR^{ci} has been reported to enhance varicella-zoster virus infections (Connolly et al. 2011; Suenaga et al. 2010). PILRα is primarily expressed on granulocytes and monocytes, where it recognizes ligand molecules in a sialic acid-dependent manner similar to that of the sialic acid-binding immunoglobulin-like lectin (Siglec; I-type lectin) (Connolly et al. 2011; Satoh et al. 2008). PILRα binds to HSV gB in a sialic acid-dependent manner and mediates membrane fusion during viral infection both in vitro and in vivo. Thus, the glycosylation balance is important for proper protein functions and life cycles of many viruses.

Concluding Remarks

Glycans play pivotal roles in both pathogens and hosts. Many viruses utilize the host protein synthesis and glycosylation machinery and undergo host-type glycosylation of their viral surface proteins along with the incorporation of host glycoproteins into virions. Viruses can thus exploit the host glycan recognition system to establish an infection and conceal themselves from the host immune system. Much has been learned about the importance of glycosylation with regard to proper viral protein function and the role of host glycans during viral infection. This knowledge can now be applied to the development of novel therapies and prophylactic measures against a wide range of viruses.

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