Viruses attach and adhere surface receptors to target host cells, and the receptors determine viral tropism of hosts and tissues. Cell surface glycans are used as the virus receptors. The enveloping viruses generally recognize surface receptors of hosts as the initial step in the viral infection cycle to host and further define the viral host range determinant. From the linkage diversity and carbohydrate structure motifs, which are apparently distinct from proteins, most pathogens or viral agents use glycans as infection receptors. The molecular structure of the virus receptors is topical in the field of virus research, but no concrete consensus logics have emerged yet. The viral interaction with the host cell surface is rather the complexed phenomenon with a multiple stage. Such multiple processes possess multiple recognitions and interactions with multiple cell surface molecules as well as accompanying conformational shifts in the virus-produced proteins. In the enveloping viruses, the adhesion-strengthening attachment strategy is affordable for virus-host receptor recognition and interactions to give higher affinities between the receptor and ligand interaction. In that meaning, gangliosides may function in virus-host cell interaction as primary receptors or co-receptors. The co-receptor concept has well been exemplified in the section HIV infection.

Still independent investigations implicated many candidates such as gangliosides, integrins, and other membrane proteins for viral binding, attachment, and entry into host cells. The SA-containing glycans as attachment receptors have been elucidated for a broad range of DNA/RNA viruses and enveloping and nonenveloping virions. Because SAs are present on all the cells in vertebrates and sialylation is a terminator of glycosylation in lipids and proteins, sialic acids are frequently recognized as physiological candidates for host cell attachment via target receptors. For example, numerous pathogens are reported to attach to SA-linked receptors to penetrate cells, as in examples of various viruses and pathogenic bacterial exotoxins. Although multiple pathogens including viruses, parasites, and bacteria recognize SA residues on molecules in host cell PMs, certain pathogens cause tumors, too. Among SAs, the well-known binding counter is a NeuAc form, while NeuGc form and 9-O-acetylated SA residues are also used as recognition receptors. Viral pathogens which
recognize gangliosides as receptors are known for many infectious viruses including simian virus SV40, influenza virus, and polyomavirus. Some bacterial pathogens are also known to recognize gangliosides and the actual binding components are bacterial toxins and adhesins as well as the SA-binding adhesin from the *Helicobacter pylori*. Apart from the pathogens, upon Singlec-7 binding, gangliosides are also known to modulate immune-related cells like NK cell cytotoxicity.

The viral attachment, recognition, interaction, and adhesion to the host receptors expressed on the cell surfaces determine the fate of the virus, because such interaction contributes to virus attachment, adhesion, penetration, entry, and infection to the host cells or some cases contribute to the destruction of cytosolic region in the infected virus. Carbohydrate interactions are precisely controlled in the viral life cycles in attachment on the cell surface and cellular trafficking [317]. Virus attachment and entry are multistep process including binding to the cells and attachment receptor for internalization. Carbohydrate moieties of host cell surfaces are used to recognize and infect epithelial cells. In most instances, among carbohydrates, SA and SA linkages determine virus host range, host specificity, tissue tropism, host cell propagation, and pathogenesis, where most SA receptors consist of terminal SA linked to the penultimate Gal or GalNAc by a SA\(\alpha_2,3\) or SA\(\alpha_2,6\) linkage. With these biological functions of SA, gangliosides are the better receptors than SA alone for many viruses due to the longer lengths of glycan chains. Viruses are regularly subclassified into multiple families, depending on their nucleic acid presence and content with RNA virus or DNA virus, capsid symmetrical conformation with helical virus, icosahedral virus, or complex virus, and the lipid envelope existence with enveloping virus or nonenveloping virus. Each specific characteristic displays each distinct replication method, even though all they belong to obligate intracellular viruses that absolutely depend on the intracellular machineries in their life cycle. Viral infection into their host cells is multiply processed with cell surface receptor binding, PM fusion for enveloping viruses, endocytosis, membrane internalization or penetration for enveloping and nonenveloping viruses, replication site delivery and replication and virion progeny production. Virus recognition and binding an attachment to the host cell surfaces are the initial steps and events for their host infection. In virus cases, they infect their target cells of susceptible hosts by attachment of the virus. Such interaction is therefore a valuable target for antiviral therapy. Host cell receptors expressed on the PM surfaces adhere viruses. The receptors function as determinants of viral infection and tropism. Receptors are flexible for attachment, host decision, and infection to specific cells as host. For some viral families, large progress on understanding the molecular mechanism involved in viral penetration and entry into host cells has been made. Viral infection needs its entry to host cells, which consist of virus recognition, binding, and attachment to the host cell surfaces. Thereafter, the virus penetrates into the interior membranous side and is followed with the next viral disassembly of the capsid proteins of virus in host cells.

In the host cells, the genetic amplification and propagation of viruses is progressed in a programmatic way. Such serially continued steps are crucial for viral agents in trafficking to the extracellular environmental matrix for the
cytoplasmic compartments, where viral multiplication takes place in the host cells. Viral penetration and entry mechanisms are also adapted to express viral diseases and pathogenesis as the events frequently direct selective determination of target cells within the host toward the virus-induced disease site. In addition, penetration-entry steps trigger signaling pathways to influence the host cells antiviral state or apoptosis. Viral attachment is monophasic and multiple receptors are involved, depending on adhesion strength. Viruses are capable of binding different cell surface molecules such as lipids, proteins, and carbohydrates. Some groups are just simply attachment-related factors, concentrating virus on the cell surfaces. But other groups are used as direct accessible co-receptors or receptors to mediate host attachment of virus and penetration to host cells. Some viruses alternatively utilize certain receptors with a cell type specificity. Virus-binding molecules on the cell surface are typically glycoconjugates such as GSLs, glycoproteins, and proteoglycans [318].

In the cells, most glycan portions are extended to the extracellular area. Therefore, viruses cannot interact with the distal membrane apical glycans. Thus, viruses bind to the proximally located protein cores of PM of host cells. In general, a virus interacts with an attachment molecule named receptor, generally a carbohydrate glycan for adherence via low-affinity recognition and recognition to the cell surface, indicating an additional receptor is required for stable recognition with high binding affinity. In most cases, glycan binding is displayed by electrostatic forces to anionic charged SA residues-carrying glycan moieties or glycosaminoglycans. In rare cases, the glycan moieties are attached on both glycoproteins and glycolipids. Some viruses just bind to cell surface glycans only to facilitate viral entry, although the relationship between the glycan structures and status of viral disease incidence is incompletely explained. Virus recognition and interaction with cell surface receptors frequently enhance intracellular signaling cascades to enhance virus entry potential [319]. The first step of infection of virus to host cells is attachment of viral particles. The cell surface receptors are targeted by viruses, depending on cell type, and receptor specificity, as expressed for host cell tropism, specificity, pathogenic incidence, and virulent direction. Therefore, the knowledge how a virus recognizes, binds to, and interacts with their typical receptors is a primary step to prevent virus infection and spread.

Many enveloped viral pathogens decorate their surfaces with glycans. Examples of envelope proteins are the envelope glycoprotein (Env) of HIV-1, HA of influenza virus, coronavirus spike (S) glycoprotein, Ebola virus glycoprotein, Lassa virus glycoprotein complex (GPC), and flavivirus envelope (E) glycoprotein of dengue and Zika (Fig. 4.1). On the other hand, secreted viral proteins are also glycosylated. Examples include the nonstructural protein-1 (NS1) of flaviviruses, the secreted GP of Ebola, and the secreted glycoprotein G of HSV. To date, eight different virus families are known to use sialoglycoconjugates for attachment. They are enveloping and nonenveloping viruses as well as RNA and DNA viruses in structures. The eight viruses include Adenoviridae, Coronaviridae, Orthomyxoviridae, Paramyxoviridae, Picornaviridae, Parvoviridae, Papovaviridae, and Reoviridae. Among them, many viruses including the adeno-associated bovine virus, human parainfluenza virus as respirovirus, influenza virus, murine norovirus, Newcastle disease virus,
paramyxoviruses, Polyomaviridae family, Rabies virus, rotavirus, and Sendai virus [320–338] use gangliosides as receptors. Several viruses including influenza virus are known to use carbohydrate glycans as a principal receptor [339], whereas some other viruses including herpes simplex virus [340] and reovirus [341] use carbohydrate glycans as just initially adhesive molecules prior to binding specific molecular receptor for the real attachment, which is called adhesion strengthening event. Virus-carbohydrate recognition designates cell type susceptibility; however, the detailed information of each glycan to viral infection is very limited for most cases of carbohydrate-recognition and binding viruses.

### 4.1 GM3/Gb3-HIV Infection

In 1981 acquired immunodeficiency syndrome (AIDS) was first reported [342] (caused by HIV). The HIV genomic material comprises two plus (+) sense single RNA strands. Viral envelope contains the glycoprotein, gp120. HIV virions enter their host cells by viral host-cell membrane fusion [343]. HIV expresses a coat adhesin protein, gp120, which is composed of trimeric proteins with their high glycosylation. The virus-borne gp120 protein interacts with the CD4 protein expressed on Th cells. In addition, the gp120 protein has been known to recognize chemokine co-receptors, which are named CCR5 for R5 HIV1 strains and named CXCR4 in the X4 HIV1 strains with heterogeneity. The gp120 recognizes GalCer, which is the first identified, and also GalCer 3′ sulfate ester named sulfatide [344]. GalCer seems to act as an alternative or replacing receptor in CD4-deficient cells such as neural and GI epithelial cells [345] or reproductive epithelium. This raises a question whether epithelial cells are actually infected by HIV. However, if not, such cells can probably serve as a reservoir or latent cells for viral DNA for a longer latency. In fact, GalCer mimics prevent HIV infection to both CD4 positive and negative cells. GM3, GD3, and Gb3 are bound to gp120. The gp120s expressed from dual tropic phenotype of R5X4 HIV strains recognize specifically GM3, while gp120s expressed from the X4 HIV strains bind predominantly to Gb3.

In view of GSLs expressed on viral membrane, HIV-1 interacts with DCs and is disseminated to CD4+ T cells through so-called trans-infection pathway by virion membrane incorporation of the GSLs such as GM3 produced by host cells. This
DC-driven trans-infection pathway contributes to multiple CD4+ T cell infection, as the mechanism has been appreciated. DC-ligand interaction elicits type I IFN signaling to activate DC-enhanced T cell trans-infection. The type I IFN-elicited Siglec-1, named CD169, acts as the DC receptor to GM3-dependently capture HIV. DCs capture HIV-1 membrane incorporation of the α-2,3-SA gangliosides. The candidates are GM1 and GM3 that have α-2,3-SA residues, but GM3 is effective for viral particle capture. Virions are captured through Siglec-1, CD169 on the PM. GM3 depletion from viral membranes or Siglec-1 depletion from DCs lost HIV-1 activity of capture and internalization as well as T cell trans-infection by DCs. Siglec-1 on macrophages captures murine leukemia virus [346] and HIV [347]. In the case of MLV, Siglec-1-mediated capture by macrophages is followed by migration to lymphoid follicles and trans-infection of B cells. Siglec-1 drives virion capture in HIV-infected macrophages. Siglec-1 recognition captures virions and forms the virus particles. Siglec-1 captures virus particles because it attaches to gangliosides on the virus envelope. Therefore, Siglec-1 is also an important receptor in retroviral particle capture and transmission. Thus, pathogen parasitization of host produced attachment tool like the GM3-Siglec-1 interaction enables DC-mediated HIV dissemination.

On the other hand, classically, the viral envelope gp120 first recognizes its primary receptor on host cells, CD4, using a binding motif in its second constant (C2) region with a conformational shift in HIV gp120 which exposes its third variable loop (V3) which contains a consensus motif that binds to a seven transmembrane-spanning chemokine co-receptor [348]. HIV-1 infection and cell susceptibility are still unclear, because the receptor functions of GSLs during HIV infection are rather complex. HIV recognizes and interacts with the GSLs as viral receptor expressed on the cell surfaces of hosts when the host cells are negative for expression of the canonical receptor CD4. GSLs that gp120 recognition domain recognizes include GalCer, 3'-sulfogalactosyl-Cer, GM3, and Gb3 globoside [349]. Gb3 and Gb3 analogues are thus HIV inhibitors [350].

GSL recognition to the HIV-1 gp120 has been a main issue for a long time with regard to the HIV infection to non-lymphoid cells, CD4+ T cells and monocytes. The Pk blood group antigen (or globotriaosylceramide Gb3) has been evaluated as an inhibitor against infection of HIV-1. Seemingly, the Gb3 synthase or α-galactosyltransferase (A4GALT) is an indicator for the HIV-1 resistant phenotype of the host cells. HIV susceptibility is linked with the natural devoid of Gb3 expression because individuals with the P1k phenotype expressing Gb3 are resistant to HIV infection [351]. This is probably due to Gb3 capacity to interact with and compete for the co-receptor-binding region of chemokine present on the V3 loop of gp120. This status prevents gp120 interaction with the co-receptor of chemokines and consequently inhibiting HIV-host cell fusion [352]. Rather, other series GSLs of GM3 and GalCer were regarded as helpers of HIV infection. Gb3 is not expressed in human T cells, but HIV infection induces T cell Gb3 synthesis [283]. Gb3 has several GSL receptor ligand interactions in the interaction of Gb3 as a receptor for the E. coli verotoxin (VT) [353]. For the HIV infection, several cofactors facilitate gp120 binding to cells. Membrane GSLs including GalCer constitute one well-
known group of cofactors [354] and this information is helpful in the combinatorial
design of therapeutic GSLs derivatives lipid raft targets. Moreover, binding mech-
anism of pathogenic viral infection in cells can be easily explained.

V3 loop domain in the HIV gp120 glycoprotein basically interacts with the host
GSLs. The V3-loop region of membrane glycoprotein gp120 in HIV-1 is suggested
as a ligand for viral attachment, although its precise recognition to target cells is
unclear. In the experiment that T cell and macrophage tropically infectious HIV-1
such as X4 and R5 strains, respectively, were used, V3 peptide binds to host cell PM
GSLs and exhibited to inhibit the infection even in the absence condition of gp120-
CD4 recognition [349]. When the synthetic peptides mimicking the sequence
Corresponding to the 15–21 amino acids in the V3-loop domain in HIV-1 X4 strain
and HIV-1 R5 strain were prepared, the peptides competed and consequently
inhibited the infectious entry of the two HIV isolates. The surface GSL-binding
HIV V3 peptides potentiate the HIV-1 entry and can be used as a target for the HIV
viral entry blocking. It has previously been evidenced that the GSL-binding motif of
gp120 carries the amino acid motif of XXXPGGRAFXXX [355]. Interestingly, such
similar sequences are also found in some other soluble proteins like synucleins and
galectins [356] as well as transmembrane receptor proteins mentioned above. The
gp120-type recognizable domain is also found on the extracellular region of the
TNF-α receptor super-family with a hairpin structure that contains two aromatic
residues (F133 and F134) exposed to the solvent for PCI [357]. The two aromatic
amino acid residues directly bind to Gb3 and LacCer, while lowly interact with Gb4
and GD3, too. Therefore, gp120-type GSL-recognizing domains are conserved, as
the amino acid region is nested in two α-helices. In addition, the key amino acid Phe
residue functions to make docked sugar residue of the GSL glycan with phenylal-
anine. The carbohydrate-aromatic amino acid residue interaction is noncovalent and
observed at axial CH groups on the carbohydrate residue’s cyclic structure. If the
aromatic amino acid residues are substituted with alanine residue, their binding
capacity is greatly reduced [251]. The best glycan residue, giving the high binding
affinity to the target protein at aromatic amino acids, is specified to the second Gal
residue on the Cer-Glcβ1–4Gal sequence in LacCer [358]. Nonetheless, the
GSL-recognizing gp120 V3 loop preferentially binds to Gb3 rather than GM3,
although both Gb3 and GM3 are synthesized by the common structure of “Cer-
Glcβ1–4Gal”-core, indicating the specific glycan linkage specificity (Fig. 4.2). If
GSLs lack for carbohydrate head group like just SM, its binding to the protein region
is defective. As a similar case, the extracellular domain of serotonin-1a receptor
carries the LNKWTLGQVTC amino acids sequence, which is the well conserved
sequence in the serotonin receptor family. The motif amino acid sequence carries the
basic amino acid at Lys101, aromatic amino acid at Trp102, and turning amino acid
residues at Gly105 in the GSL-binding region.
4.2 GM1/GD1b/GT1b-Polyoma Virus Infection

Due to the characteristic linkage diversity of carbohydrates, virus entry and infection use such glycans. This is carbohydrate-specific nature with the broad structural motifs, now seen in proteins and nucleic acids. In addition, although polyoma virus and JC virus recognize SAs, each virus exhibits each distinct specificity. The *Polyomaviridae* for polyomaviruses (PyV) is a member of icosahedral, nonenveloped, dsDNA viruses that cover human BK polyomavirus (BK-PyV), JC polyomavirus (JC-PyV), polyomavirus of Merkel cells (MC-PyV), murine polyomavirus (mPyV), and SV40 [359]. The capsids are composed of 72 pentamers to form the icosahedral capsid structure that is composed of VP1, VP2, and VP3 proteins. Polyomavirus capsid protein VP1 is a pentameric protein consisting of 360 proteins. Thus, the main capsid protein is the VP1. Each VP1 pentamer recognizes a VP2 or VP3 in the capsid interior and encases dsDNA genome. Upon virion interaction with a receptor in the cell surfaces, the virions are subjected to internalization and transportation to the cellular organelle, ER. For example, SV40 or BK-PyV entry into cells is quite similar together and they use caveolae-mediated endocytosis to the ER for uncoating. Polyomaviruses recognize sialylglycans on the cell surfaces. BK-PyV as an opportunistic pathogen isolated in 1971 causes severe immunosuppression. Immunosuppressive patients receiving organ or bone marrow transplantation exhibit lytic propagation like polyomavirus-associated nephropathy and hemorrhages. BK-PyV as an opportunistic pathogen isolated in 1971 causes severe immunosuppression. Immunosuppressive patients receiving organ or bone marrow transplantation exhibit lytic propagation like polyomavirus-associated nephropathy and hemorrhages. BK-PyV recognition to cellular receptors is not clearly understood. The current knowledge of polyomavirus is based mainly on Rhesus monkey-infectious SV40 and mPyV. Polyomavirus penton contains a minor VP-2 protein copy, which is a capsid protein and a main VP-1 capsid protein with 360 copy numbers or VP3 variant truncated at the N-terminal region of VP2. Polyomavirus capsid pentons bind to surfaced sialylglycans on the host cells. The main BK-PyV penton receptors are GD1b and GT1b. However, BKV VLPs can bind to GAGs on target cells [360] in a manner of GAG-capsomer recognition.

Because BKV genome lacks any polymerase genes, BK-PyV DNA replication exclusively relies on the host system for virus replication. Therefore, BK-PyV
intracellularly traffics the ER region through host factors. GD1b or GT1b-bound BK-PyV on the PM of host cells enters host cells through a caveolin-driven endocytosis pathway. The endocytosed BK-PyV enters the endosomal organelle. Apart from BKV, others such as SV40, mPyV, and CTx toxin are also known to penetrate to the cell through receptor-caveolin-endocytosis. Cytosolic BK-PyV is further delivered to nucleus region by the minor capsid proteins through the α/β pathway. VP1 protein pentamers interact directly with the carbohydrate part of gangliosides, GSLs with SAs [361]. SV40-expressed VP1 protein preferentially recognizes the GM1, which is a binding receptor for viral attachment and entry, while mPyV strain requires other ganglioside receptors of GD1a and GT1b as binding receptors, and BK-PyV recognizes GD1b and GT1b as receptors. Interesting, affinity for the GM1 ligand-recognition site as a single site is relatively at a low level (Kd 5 mM). NeuGc and NeuAc are able to serve as receptors of SV40 [362]. The SV40 VP1 showed higher affinity for NeuGc-GM1 than NeuAc form and this indicates SV40’s preference to non-human cells such as monkey cells rather than NeuAc-expressing human cells. JCV can bind to a pentasaccharide of glycolipids and glycoproteins as an attachment and infection receptor [363]. The pentasaccharide usage, but not gangliosides, indicates the different preference of viruses as in the entry way of JCV and restriction of cell tropism for infection. Also, it was reported that JCV receptors utilize GM1 and GD1b as the receptors, but also the α2,6-linked Lac-series tetrasaccharide C receptor [364]. JC-PyV utilizes either an SAα2,3 or SAα2,6-attached glycans to interact with glial cells for infection [365] and a GT1b is also potentially involved [366]. But, BK-PyV utilizes the SAα2,3-linked glycans only to enter and infect cells. The GD1b and GT1b are also functioned for BK-PyV infection to kidney cells [367].

The binding pocket domains on VP1 of all the mouse polyomavirus of MC-PyV and mPyV, B-lymphotropic polyomavirus (LpyV) as well as polyomavirus 9 of human (HPyV9) bind to SAs [368–370]. The mPyV recognizes α2,3-linked SA. These sialylglycans were later demonstrated to present in the GD1a and GT1b carbohydrates as the glycan receptors for mPyV infection. In contrast, if the distinct region on the extended VP1 surface loops has the broken Neu5Ac-binding site, human polyomaviruses HPyV6 and HPyV7 bind to non-sialylglycan receptors [369]. Among JC-PyV strains, WT3 VLP strains are known to recognize Gt1b, GD2, GD1b, GD1a, GM2, GM1, and asialo-GM1, while Mad-1 VLP strains recognize the related forms of GM3/GD1a/GD1b/GD2/GD3/GT1b/GQ1b gangliosides; however other GM1/GM2 are not recognized [364]. Despite the research information of JC-PyV ganglioside recognition, the relevant recognition of JC-PyV to carbohydrate structures in the JC-PyV infection is still unknown in detail.

mPyV binds to host cell surface gangliosides and the α4-integrin receptor via the VP1 capsid protein. Receptor binding by mPyV suggested that mPyV binds to α2,3-linked sialylated oligosaccharides present in the GD1a and GT1b as the biologic receptors for mPyV. As an increasing knowledge on viral recognition proteins with sialylcarbohydrates is available in recent years, ganglioside and integrin are the receptor for mPyV infection and required for activation of the PI3K pathway [370]. However, how do gangliosides and α4-integrin play their roles in mPyV
infection is not answered. Gangliosides are needed for PyV trafficking to the ER [371]. Human Trichodysplasia spinulosa-associated polyomavirus (TSPyV) known as a causing agent of a skin disease in immunocompromised individuals interacts for attachment and infection with glycolipids, but not N- and O-linked glycoproteins [372, 373]. Whether TSPyV directly causes a human pathological infection through molecular binding, recognition, attachment, viral entry, penetration, and intracellular transportation in TSPyV infection is unclear. Using the high-resolution technology of X-ray crystal structures on the VP1 capsid protein complexed with the GM1 glycan, α2,3-sialyllactose (SL) and α2,6-SL, the terminal SA has been demonstrated to bind to the TSPyV VP1. This SA binding sites are commonly conserved in other polyomaviruses [373]. Mutation of SA-binding amino acid residues contributes to reduced cell recognition, attachment, and infection, indicating TSPyV VP1-glycan interaction as a PCI type. GM1 is easily converted by enzymatic desialylation of gangliosides of GD1a/b and GT1b by a neuraminidase as a molecular switch for local function. This may be linked with the GM1 utilization as such viral receptors.

4.3 GM2-GD1a-GT1b-GQ1b-Neolacto-Series GSLs-Paramyxoviruses (Newcastle Disease Virus, Respirovirus, Mumps Rubulavirus, and Avulavirus)

Paramyxoviridae belongs to a family of the order Mononegavirales. This family includes Aquaparamyxovirus, Avulavirus, Ferlavirus, Henipavirus, Morbillivirus, Respirovirus, and Rubulavirus. Paramyxoviruses are nonsegmented and enveloped viruses as the negative-strand RNA viruses. The viruses attach and enter the host cell through surface receptor by glycoprotein (G), hemagglutinin (H), or hemagglutinin-neuraminidase (HN). The attachment and recognition proteins of G, H, HN, and fusion protein (F) are used as the target antigens of neutralized Abs. The MuV consists of genomic 15,384 bp nucleotides. SA-containing glycolipids are also involved in the first stages of the paramyxovirus life cycle and a key viral biomarker of host range. The paramyxovirus belongs to the enveloped viruses and enters to the host cells via specific recognition of the host cells and consequent PM fusion. The paramyxovirus receptors are mainly elucidated in molecular and chemical levels from the Sendai virus (murine parainfluenza).

SA-bearing glycans are involved in the initial entry step of life cycle of the enveloped paramyxovirus. The attachment and entry to the target cells commence with their recognition to the host cell surfaces and followed by PM fusion. Virus recognizes the surface receptors by its attachment proteins resided on paramyxoviruses. HN glycoprotein recognizes cellular SA molecules and exerts sialidase activity and fusion activity. For example, the Sendai virus attachment and recognition proteins are called hemagglutinin-neuraminidase (sialidase), HN glycoprotein,
which recognizes and binds to host cell PM, although the virus-induced membrane fusion is still not well understood yet. For example, Sendai virus uses SAα2,3-linked gangliotetraosides such as neolacto-series gangliosides, GQ1b, GT1b, and GD1a as the attachment receptors, but not SA-lacking GM1 or GD1b on the terminal Gal residue. However, limited information is available for the entry receptors for currently reported other paramyxoviruses, compared to those of Sendai virus. However, in the human parainfluenza virus, type 1 virus or type 3 virus binds to N-acetyllactosamine-bearing glycan terminated with SAα2–3Gal.

For a model of other paramyxoviruses, GM3 is a Newcastle disease virus (NDV) receptor but not for Sendai virus receptor [332]. Sendai virus recognizes gangliosides, which terminally contain the NeuAc residues attached to Gal residue. As the Sendai virus receptor, GD1a influences lipid polymorphism and isotropic structure formation in monomethyldioleoyl phosphatidyl ethanolamine. Therefore, Sendai virus fusion with membrane liposomes is ganglioside dependent [333]. Apart from Sendai virus, although the receptors for other paramyxoviruses remain unknown, some sialylated glycoproteins, not gangliosides, are used as specific paramyxovirus receptors. For example, human parainfluenza viruses recognize Neu5Acox2–3Gal-attached N-acetyllactosaminio-glycans. For specifically, the human parainfluenza viruses of type 1 of hPIV1 as well as type 3 of hPIV3 have been specified in the carbohydrate recognition, where they preferentially recognize LacNAc-glycans with terminally attached NeuAcx2–3Gal. hPIV3 binds to NeuAco2–6Gal- or NeuGcox2–3Gal-carrying glycans [331]. As a quite similar type of viruses, Orthomyxoviruses, which are influenza viruses, also recognize SA-carrying glycans, although each influenza strain needs each specific SA linkage (α2–3 or α2–6) or SA type (NeuAc, NeuGc, or 9-O-Ac-NeuAc) [330]. Paramyxovirus NDV is not restricted to only gangliosides for binding to the host cells, but potentially binding to SA-containing glycoconjugates and sialoglycoproteins of host cell PM [374].

For NDV case which is relatively well established to date, two transmembrane glycoproteins, HN and fusion (F) protein [323], are expressed in viral envelope. The NDV uses the NeuAc form. HN recognizes cell surface SA-attached receptors and F protein facilitates the viral envelope fusion into the host cell PM [324]. The binding of HN to the host cell receptors induces changes in conformational structures of the protein HN and consequently induces the fusion capacity of F protein. In experimental binding assay between NDV and glycolipids, several GSLs including GM3, GM2, GM1, GT1b, GD1b, and GD1a are bound to the NDV virus [335]. In molecular level, NDV recognizes the terminally attached SA residue in GM3, GD1a and GT1b as well as the internally linked SA residue in GM2, GM1, or GD1b. However, GQ1b is not bound to NDV, although GQ1b occupies both terminal and internal di-SA residues. In contrast, GQ1b is known to bind to Sendai virus [334]. Apart from the ganglioside recognition, NDV binds to sulfatides and asialo-GM1, but not to Gal-Cer or Lac-Cer.

Mumps rubulavirus or Mumps virus (MuV) is a deafness, encephalitis, meningitis, and parotitis-causing human pathogen. MuV is the genus Rubulavirus of a family of Paramyxoviridae. Mumps vaccination is combined with measles-mumps-rubella (MMR) vaccine at ages of 12–15 months and measles-mumps-rubella-varicella
MuV HN binds to α2,3-SA linkages and prefers a SAα2,3-linked trisaccharide as a receptor. Additionally branched α2,3-SA-containing glycans are not strong to bind to MuV HN. (MMRV) vaccine at the ages of 4–6 years. MuV is an aerosol-transmitted human pathogen, belonging to Paramyxoviruses as enveloped and non-segmented negative-strand RNA viruses. The MMRV includes four genotypes (A, B, H, and N). Viral proteins include hemagglutinin-neuraminidase (HN), hemagglutinin (H), glycoprotein (G), and fusion protein (F). Each genotype conveys conserved α-helix domain in C-terminal receptor-binding region. MuV HN binds to α2,3-SA linkages and prefers a SAα2,3-linked trisaccharide as a receptor for mumps virus. The branched α2,3-SA oligosaccharides are weak in binding to viral HN, when compared to α2,3-SA trisaccharide (Fig. 4.3). Among 3SL and 6SL, 3SL prefers to bind to MuV-HN domain. Aromatic amino acid residue is crucial for 3SL recognition. Only α2,3-SA glycans are the MuV-HN receptors. N-/O-glycoproteins and GSLs are utilized as receptors for MuV HN.

MuV tropism specifies glandular tissues and CNS through carbohydrate receptors. MuV hemagglutinin-neuraminidase (MuV-HN) protein recognizes sia1 LewisX (SLex) and the GM2 carbohydrate structure. SLex and GM2 carbohydrates share the common trisaccharides of 3’SL. Thus, the binding site of MuV-HN recognizes both SLex and GM2. GM2, SLex, and 3’S-L sugar block MuV entry to host cells [375]. The α2,3-SA glycans such as SLex and SA-LacNAc are abundantly present in most tissues and GM2 is present mainly in neuronal cells, tissues, or adrenal glands, indicating MuV tropism. The diverse MuV recognition of the different carbohydrate receptors indicates its tropism and pathogenesis. MuV-HN favors SAα2,3-linked glycans over SAα2,6-linked glycans. The MuV-HN binds to both sugar types of GSLs and glycoproteins as receptors. Amino acid residue at Tyr369 position of MuV-HN recognizes 3’S-L and the SAα2,3-linked trisaccharides in all MuV species of the genotypes A, B, G, and L.

The specifically designed SA mimics can inhibit the HN or receptor-recognizing capacity of viral proteins can be designed as a future antiviral therapeutic strategy. For example, the precise information of paramyxovirus entry receptors and the viral entry to the cell surface will create a new vision.
4.4 GT1b/GQ1b-Rabies Virus (RABV) Interaction

Virus infection of cells is carried out as a stepwise process with initial binding to receptors in host cell surfaces, PM fusion of enveloped viruses or PM internalization and PM penetration, trafficking and replication, progeny production of virion, and extracellular release of viral particle into the cytoplasmic spaces. Therefore, receptors determine viral pathophysiology and tropism with tissue specificity. Rabies virus (RABV) is a neurotropic pathogen to neurons in the nervous system of mammals. RABV is an enveloped and negative sense ssRNA virus. This virus belongs to the Lyssavirus genus of Rhabdoviridae family. This Rabies-causing agent raises 100% fatality, indicating neurotropic RABV threat. A variety of candidate receptors are known for carbohydrates, gangliosides, NCAM, nicotinic acetylcholine receptor (nAChR), metabotropic glutamate receptor subtype 2 (mGluR2), and p75 neurotrophin receptor (p75NTR). After attachment to host cells, RABV is endocytosed via clathrin-mediation and transported through retrograde trafficking endosomal vesicles. The RABV consists of five viral proteins such as matrix protein (M), nucleoprotein (N), phosphoprotein (P), RNA polymerase (L), and glycoprotein (G). The linear RNA encapsulated by N proteins yields the nucleocapsid complex. The nucleocapsid-RNA complex with the P and L proteins forms a ribonucleoprotein (RNP). The viral coat membrane protein M wraps the RNP and protein G-based spikes bind to receptors and fused with PM with the protein M, potentiating entry of virus into host cells.

RABV is transmitted via animal bites. Virus particles enter the nervous system through a sensory nerve, the neuromuscular junction, and motor axons of the muscle surface. The viral envelope contains two G and M proteins. RABVG has a form of a trimeric type I glycoprotein structure with a 505 amino acid. The G trimer of RABV recognizes cells. RABVG mediates the virus transport to the CNS via the retrograde pathway. nAChR has been known as a receptor for muscle infection. RABV then uses nAChR, NCAM, p75 neurotrophin receptor named p75NTR, and sialyl GSLs for infection to host cells. Muscular nAChR binds to RABVG for RABV infection. Rabies virus adhered and attaches to the PM as the first step. For the cellular components in the attachment, gangliosides have also been known in rabies virus infection to chick embryo cells. Upon treatment with neuraminidase, the cells were desialylated without viral infection. However, cells treatment with gangliosides restored ganglioside incorporation and access to rabies virus infection. Among gangliosides, GT1b and GQ1b have been reported as the best receptor candidates and GD1b was the moderate candidate, whereas GM1 or GM3 was the poor one [337]. In the neuron membranes, polysialogangliosides are the receptor of rabies virus infection. In the healthy foxes and rabies virus-infected foxes of brain, glycoproteins and polysialylated gangliosides have been reported to be related [338].
Adenoviruses are human pathogens. Human adenoviruses are problematic with immunocompromised patients in healthy individuals at the eye, gastrointestinal, and respiratory system. Adenoviruses have seven species of A to G group with 57 to 90 types. The severe infectious species include adenovirus AdD56, AdB7d, and AdE4 through epidemics of infection. Receptor tropisms of adenoviruses are based on coxsackie and adenovirus receptor (CAR), CD46, desmoglein 2, or SA-containing carbohydrates [376]. Adenoviruses D species is mostly common with 35 of 57 canonical types. The species D causes epidemic keratoconjunctivitis (EKC) disorders. The EKC adenoviruses include Ad-D8, Ad-D37, and Ad-D64 in humans. Adenovirus is the dsDNA and feasible for genetic modification and therefore, this species is attractive for genetic applications to regress cancers through genesis of oncolytic viruses and vaccination vectors. Virus attachment, recognition, binding, and interaction with the cell surface indicate determinant property for tropism. Understanding of the precise recognition and interaction opens a new and potential therapeutic strategy to target for antiviral therapy. Attachment factors on the surfaces of host cells contribute to viral concentration. Certain viral receptor or co-receptors facilitate virus interaction with the host cell surfaces, recognition, binding, entry, and infection to host cells. Adenoviruses such as CAV-2, Turkey adenovirus 3, and human Ad-G52 interact with SA residue through the fiber knob lateral region. Human Ad-D8, Ad-D19p, Ad-D37, and Ad-D64K viral fiber knob proteins also recognize SA through their apical regions.

A residues as Neu5Acβ2,3-D-Galβ1,3-D-GalNAc1,4-(Neu5Acα2,3)-D-Galpβ1,4β-D-Glcp1,1-Cer. Previously, GD1a was reported to modulate the cytokine, GM-CSF, or leukocyte growth factor function and it has been known to modulate GM-CSF-induced cell proliferation [377]. GD1a regulates the expression level of the GM-CSF receptor (GMR). GMR is a heterodimeric protein and consists of a ligand-binding region for GMRα chain and a constant β chain (βc). GMRα is composed of two distinct isoforms and is polymorphic. Two distinct isoforms are known: (1) transmembrane dimerizes with βc and leads to signaling and (2) soluble variants lack the transmembrane domains. GMRα isoforms are synthesized by C/EBPα transcription factor upregulation by GD1a. GD1a colocalizes with GMR in monocytes. Exogenous GM3 or GD1a stimulates GM-CSF-activated cell growth, as monocytic cells synthesize predominantly GD1a and GM1. Reduced ganglioside synthesis decreases in GM-CSF-stimulated proliferation. The carbohydrate part of GD1a is used as a receptor responsible for adenovirus infection of adenovirus type 37 (Ad37) causing EKC disorders [378]. EKC is a highly contagious ocular disorder raised by three D adenovirus species. General pathological symptoms of EKC involve edema, pain, lacrimation, and vision regression. Other types of adenoviruses interact with host cells through binding to CD46 or the CAR named Ad37 protein, which recognizes SA-linked molecules. Using glycan microarray technology, the receptor-binding knob, which is present in the Ad37 fiber protein, has been found to bind a branch type of hexasaccharides in the GD1a having two terminal SAs. GD1a or GD1a-
specific Mabs block virus Ad37 binding capacity and infection to eye corneal cells. GD1a carbohydrate structure, but not the ganglioside GD1a itself, was organized with glycoproteins. Many D adenovirus species such as the EKC bind to the GD1a glycan–contacting residues. Heterogeneity in GD1a-depending infectiousness of several related adenovirus types is shown, although the adenoviruses, which cause the EKC, utilize the carbohydrate part in GD1a as an infectious receptor on the cell surfaces, further defined conclusions seem to be needed to finely define the function of GD1a as a receptor for non-EKC-causing D virus species or the relatedness. Using other experimental technologies including molecular imaging, modeling, NMR, and X-ray crystallography diffraction, the two terminal SAs were shown to dock into three SA recognition sites on the trimer molecule of Ad37 knob. The knob-GD1a glycan binding shows a high affinity as confirmed by surface plasmon resonance, giving a solution toward the future development of SA-based antiviral agents against EKC. The discovered GD1a carbohydrate glycan as an infection receptor for Ad37 may give a clue for inhibitory drugs. Drug compounds can be remodeled on SAs likely to Tamiflu in influenza A viruses, as highly useful antiviral therapy of influenza [377, 379]. The PCI between Ad37 knob and GD1a resembles with the influenza hemagglutinin (HA)-sialylglycan motif interaction [363]. For example, the SA-recognizing region is present on the influenza HA tip, but the SA-recognizing site on the knob is present around the central cavity.

4.6 GalCer-Adeno-Associated Virus (AAV)-Bovine AAV (BAAV) Interaction

Adeno-associated virus (AAV) belongs to the genus Dependovirus that uses a helper virus for replication. AAV is the ssDNA virus of family Parvoviridae, which functions as a replication helper for either herpesvirus or adenovirus. They are not toxic and are not related to any disease. AAV capsids consist of 60 protein subunits with VP1, VP2, and VP3 proteins, as alternatively spliced forms, which are the same gene regions. AAV utilizes diverse structures of cellular carbohydrate for attachment and infection. Type 2 AAV (AAV2) binds cell surface heparan sulfate proteoglycans. Bovine AAV (BAAV) also utilizes PM gangliosides for attachment and infection. AAVs are reported to recognize diverse glycan structures of proteoglycan heparan sulfate, N-type and O-type sialylglycans, and GSLs expressed on cell surfaces for recognition, attachment, binding, entry, penetration, and infection, but also for the virus life cycle. BAAV requires gangliosides and β1,4-GlcNAc residues on glycoprotein gp96 for virus attachment [325, 380]. Apart from the infection, BAAV uses carbohydrate interactions for infection or transcytosis, because it can also pass through epithelial barriers and endothelial barriers using cell surface carbohydrates-utilizing transcytosis. Transcytosis has been known for macromolecules and pathogens, where transcytosis event is similar to the macromolecular
movements from one side of a cell to the other sides [381]. In HIV and *S. pneumoniae* transcytosis, surfaced Gal-Cer and poly(IgA) are receptors, respectively [380]. The carbohydrate interaction for BAAV transduction movement or transcytosis movement requires a trimeric β1,4GlcNAc residue. A β1,4GlcNAc containing membrane glycoprotein gp96 is a receptor for BAAV transcytosis.

### 4.7 GM3/GM1a/Histo-Blood Group-Rotavirus Infection

Rotavirus belongs to the Reoviridae family as dsRNA viruses and causes animal and human infantile gastroenteritis. Small intestinal epithelial cells are targeted by the viral infection. The rotaviral particle or virion is composed of triple layers with a core segmentation genome, protein shell in internal region, and 7-trimeric virus protein (VP) capsid in which VP4 spike is penetrated. The serotype-dependent VP7 and protease-sensitive VP4 determine the rotavirus classification to type P rotavirus (VP4) and type G rotavirus (VP7). The VP4 protein of type P virus is subjected to the proteolytic cleavage to generate the N-terminal VP8 protein and C-terminal VP5 protein. VP8 attached to the dimeric VP4 spike head recognizes surface glycans of host cells at the early stage of life cycle. VP5 recognizes the α2β1 integrin to elicit infectious event and VP7 recognizes the integrins of αxβ2 and αvβ3. The α4β1 and α4β7 integrins are crucial for rotavirus entry to some host cells. Integrins-glycan receptors clustering in lipid rafts microdomains with HSP70 also are involved in the entry of rotavirus to host cells. The rotavirus receptors are also topical trend of rotavirus research, because entry of rotaviruses to host cells is called complexed process. The host receptors determine virus tropism to infection. Several events during evolution, including gene rearrangement, point mutation, rotavirus genome segmentation, and wide type G and P range potentiate gene reassortment, contributing to increase in genetic diversity. The most variable region of structural proteins is the VP8, allowing alteration in glycan receptor usage by rotaviruses as rotavirus entry mechanism.

The entry process involves sequentially interacted pathways with cell surface gangliosides with one or more SA residues. GSLs are a heterogeneous family of amphipathic lipids found on mammalian PMs. Rotaviruses are etiologically causing factors of gastroenteritis in animals and humans through the entry and infection to intestinal epithelial cells [382]. The *Reoviridae* family is dsRNA viruses and the rotaviral particle is layered in triple sets and made of an inner shell protein and an outer SP-4 with spike capsid VP 7 trimers. Thus, the outer layer contains VP4 spike proteins projecting through an inner VP7 shell protein. From the serotype-based VP7 glycoprotein and protease-susceptible VP4, rotaviruses can be further subclassified to G type virus for VP7 and P type for VP4 virus. VP4 is associated with rotavirus-cell attachment, while VP4 and VP9 determine serotype specificities of rotavirus P and G serotypes, respectively [383]. VP8 located on the dimeric VP4 head spike binds to carbohydrates on cell surfaces. VP5 is conformationally shifted affordable for host cell membrane penetration [384]. VP4 cleavage by proteolytic degradation
is important for conformational shifts for virus entry. VP5-carrying rotaviruses thus bind to the \( \alpha 2\beta 1 \) integrins and potentiate infection to cells [385]. VP7 enters rotavirus cells via the \( \alpha x\beta 2 \) and \( \alpha v\beta 3 \) integrins, while the \( \alpha 4\beta 1/7 \) integrins in some rotavirus can be used for specific host cell types [386].

Rotavirus entry is initiated by attachment to a glycan receptor of a glycolipid head group with subsequent vesicle formation. Penetration is operated by the uptake vesicle. Gangliosides are involved in successful endocytosis at the PM, and the mobile movement of the GSLs in the cell PM may further potentiate rotavirus entry. The rotavirus infection glycan receptors are specially interesting in recognition, attachment, endocytosis, host tropism, and pathogenic disease. The SA binding is decided by the VP8 amino acids at 187 and at 157 as a Sia binding site. Rotavirus also uses the histo-blood group antigens (HBGA) for its recognition receptor molecules. The HBGA is indeed asialo-glycans appeared on epithelial cells and erythrocytes, and also in certain mucosal secretions [387]. The diversity of the host receptor glycans is recognized by rotaviruses, and the GM1a and HBGA are the major receptors. Then, the question how the ligand spike protein of rotavirus interacts with the GM1a receptor at the molecular structure and mechanistic levels is interesting in understanding innate intestinal immunity and provides rationale to treat and prevent the rotavirus infection. The VP8 of human rotavirus P subtype [388] recognizes Le\(^b\) and H-type I HBGA, whereas VP8 of P6 subtype of neonatal ST-3 strain binds to only the H type I HBGA but not others [389]. Rotaviruses enter and penetrate host cells by disrupting membrane structure. RRV VP8 binds to GM1a and GM3 as a crucial step for binding of VP8 of the known many rotavirus strains.

Their host cell receptor and attachment ligand are glycans and the virion spike protein, respectively, toward precise host infection by rotaviruses. Rotaviruses prefer ganglioside to enter the host cells. Rotavirus receptors utilize sialic acids, gangliosides, and HBGA and the receptor-glycan binding induces VP8 protein conformational change. VP8 mediates virus entry to host receptors via the co-localized integrins-glycan receptors complex with additional membranous lipid rafts and cognate HSP70 [390]. NeuAC and NeuGc and \( \alpha 2\beta 1 \) integrin are known as rotavirus cellular receptors by recognition with virion spike protein, VP-4 [391]. Rotavirus’s HBGA engagement can be influenced by commensals, fucosylation, and immune system. Engagement of both gangliosides and HBGA receptors can further potentiate their adaption to emerging new hosts. SA residues present in terminal and internal sites in gangliosides and HGBA can be easily bound by VP8 in rotavirus host cell invasion. Human rotaviruses commonly engage GM1a as their receptor while outstanding variations identified in human-infectable rotaviruses determine their capacities to utilize A-type of HBGA, indicating the specific tropism. Therefore, viral host tropism derived from each distinct glycan receptor usage will direct the applied research. The plastic variation in VP8 conformational structure and preference to glycan receptors will clarify the rotavirus adaption acquisition to newly accessible hosts, regarding non-human-to-human transmission via gene reassortment events.

Human rotaviruses utilize GM1a ganglioside as a receptor, although GM1a engagement is not restricted to the human-infectable rotaviruses and there is also
not a species specificity in this rotavirus type. Some rotaviruses in humans and UK rotaviruses in bovine utilize GM1a form of GSLs during host cell infection and invasion by human rotaviruses. Therefore, GM1 tropism of human rotaviruses is not certain in its mechanism. The only assumption is that terminal SA residue and internal (or branched) SA residues may be the determinants for rotavirus species. The infection level of animal rotaviruses in host cells is decreased when neuraminidases are treated. The rotavirus VP8 binds to SA residue. The amino acid position 187 of VP8 determines SA-binding preference and the amino acid position at 157 determines SA-binding affinity. Animal-infectable rotaviruses use two different SA types of Neu5Gc and Neu5Ac as their binding carbohydrates. However, RRV known as a simian strain prefers Neu5Ac type, while swine type CRW-8 strain and bovine type NCDV strain prefer Neu5Gc type as a recognition SA type. Rotavirus VP4 binds α2,3-SA linked to the GM1 glycan chain, as the Wa rotavirus VP4 recognizes the internally located NeuAc on GM1 oligosaccharide in an independent mode of α2β1 and internal SA recognition. Another cholera toxin B (CTxB) known as a specific GM1-binding ligand diminishes rotavirus Wa infection to host cells, because the GM1 is the receptor both of CTB and VP4. GM3 gangliosides are also suggested to be appropriate receptors for the porcine rotavirus OSU strain [392, 393].

On the other hand, among the rotaviruses, the two types of sialidase-sensitive, simian SA11 and bovine NCDV as well as sialidase-insensitive, bovine rotavirus strains are known. The infection by the simian rotavirus SA11 strain is mediated by the cell surface SAs species. GM1 ganglioside blocks the rotavirus SA11 infection to the host cells [394, 395]. The known three subtypes including SA11, NCDV, and UK rotavirus strains recognize non-acidic GSLs of gangliotetraosyl-Cer (GA1, another name of asialo-GM1) and gangliotriaosyl-Cer (GA2, another name of asialo-GM2), giving the distinct binding specificities. The viruses also bind to sialylneolactotetraosyl-Cer and GM2 and GD1a. Among them, strain UK rotavirus only can bind to NeuAc-GM3 and GM1. Sialyl-Gal (NeuGc/NeuAca2–3-Galbeta) is the binding site. Instead, only SA11 and NCDV rotavirus strains can bind to NeuGc-GM3. Thus, sialidase-susceptible strains bind to externally located SA residues in gangliosides, while sialidase-insensitive strains bind to internally located SAs of gangliosides [396, 397].

4.8 GM1/GM2-Reovirus Infection of Reoviridae Family

Several viruses primarily use glycans as a receptor, while some viruses like reovirus use glycans as an initial attachment site prior to recognition of a protein receptor to strengthen the binding level. Reoviruses bind to surface carbohydrate receptors as the first infectious step. Mammalian reoviruses sero-typically disseminate with the host by specific glycan recognition. Each reovirus serotype recognizes each specific glycan but the molecular function of each glycan is not well understood in pathogenesis. Reoviruses bind to surface carbohydrate receptors as the first infectious
step. Each reovirus serotype recognizes each specific glycan but the molecular function of each glycan is not well understood in pathogenesis. In mammals, reoviruses infect in a serotype-dependent manner in the murine CNS. Mammalian orthoreoviruses (reoviruses) display broad cell and tissue tropism in vivo and also are well-designed to study virus-receptor recognition and interactions. Reoviruses are double-stranded RNA (dsRNA) viruses with ten segments encapsidated in the protein shells of the two types, outer capsid and core capsid, infectious for the mammalian gastrointestinal and respiratory tracts, rarely causing systemic disease in the newborn [398]. For the reoviral pathology, apoptotic events are developed in cells and infected mice. Therefore, inhibition or perturbation of viral disassembly by endosomal acidification inhibitors or proteolytic enzymes abrogates apoptosis or death signaling. Reoviral disassembly activates NF-κB to induce apoptotic signaling in cells [384]. Reoviruses prefer to infect tumor cells, giving a possibility of being used in cancer treatment [399]. Reovirus serotype 1 (T1) propagates through hematogenous infection with a tropism to specifically target hydrocephalus-causing ependymal cells. However, reovirus serotype 3 (T3) propagates through hematogenous and neuronal tropisms to target lethally encephalitis-causing CNS neurons. However, the molecular mechanism underlying the serotype tropism in neuropathogenesis remains unexplained. Reassortant reovirus strains that are evolved to have gene segments of two independent reoviral strains exhibited that the attachment protein σ1-encoding S1 gene gains serotype-specific CNS infection through different receptor recognition. The three reovirus serotypes are currently known. For example, type 1 Lang reovirus is named T1L virus and type 2 Jones reovirus is called T2J virus as well as type 3 Dearing reovirus named for T3D virus, depending on distribution of σ1 capsid protein. T1L can target to infect ependymal cells, causing hydrocephalus [400], where the σ1 150 kDa protein homotrimers are involved in the virus attachment to target cells.

Reovirus T1 and T3 use the identical receptors as proteins, which include Nogo receptor 1 (NgR1) and junctional adhesion molecule (JAM)-A. However, the reovirus T1 and T3 recognize distinct glycans as carbohydrate receptor. Reovirus T1 recognizes GM2, which has terminally α2,3-Neu5Ac and β1,4-GalNAc residues. The carbohydrate structure in GM2 glycan is present in both glycoproteins and the ganglioside GM2 (Fig. 4.4). Therefore, α2,3sialylglycans are used as the carbohydrate glycan receptors for reovirus T3, but not for other reovirus types of glycan

Fig. 4.4 Structures and synthetic pathways of GM2 and GM1 as well as the related gangliosides
binding in T1 reovirus infection. The interaction between reovirus T1 and GM2 occurs in the physiological condition. In contrast, reovirus T3 recognizes the GM3-containing carbohydrate of the SAα2,3-Gal-Glc linkage, SAα2,6-linkage, and SAα2,8-linkage of sialylglycans. Exogenous treatment with GD3, GM3, and 3SL blocks reovirus T3 adherence and attachment to host cells. Because exogenous GM3 carbohydrate does not block reovirus T1 infection to cells, it is suggested that reovirus binding to glycan receptors depends on serotypes. Thus, the SA-binding reovirus T3 can target neurons more effectively than the SA-unrelated strains [401]. The glycan-binding region of T3D σ1 interacts with α2,8-NeuAc, α2,3-NeuAc, and α2,6-Neu5Ac as its carbohydrate receptors, indicating that carbohydrate-recognizing potentials contribute to each specificity of the viral pathogenesis [401, 402]. Binding is made by a bidentate salt bridge type, connecting Arg202 with the carboxylic acid of Neu5Ac residue. The additional lactose backbone additionally recognizes T3D σ1, utilizing a distinct glycan sequence appeared on the surface PM of the host cells [361]. Reovirus hemagglutination shows each serotype dependency. T1 reoviruses can specifically agglutinate human erythrocytes, but not erythrocytes of bovine. In contrast, T3 reoviruses can agglutinate bovine red blood cells, too [403], indicating the distinct carbohydrate-recognizing regions present on the T1 and T3 reovirus are quite different.

Because human erythrocytes express Neu5Ac and bovine erythrocytes express mainly Neu5Gc type, less amount of Neu5Ac, the hydroxyl group of Neu5Gc, has been suggested to favorably recognize a hydrophobic pocket in the type 1σ1 carbohydrate-recognition region. Using structure-guided mutagenesis, a GM2 receptor non-binding T1 reovirus mutant has been made [395]. The mutant virus does not cause hydrocephalus, compared to the wild-type virus in GM2-deficient mice and in cultured ependymal cells of the brain ventricles. Recently, from the glycan microarray, GM2 has also been found as a carbohydrate receptor for T1L reovirus [398], binding to the T1L σ1 head region. GM3 can also bind to T1L σ1; however, GM2 glycan is preferentially used as a carbohydrate receptor for T1L reovirus probably due to the extra-containing GalNAc residue of GM2. The reovirus σ1 is a trimeric fiber composed of three domains of body, tail, and head. The T1 σ1 head region protein recognizes the Neu5Ac and GalNAc residues of the GM2 carbohydrates. Amino acids of Val354, Ser370, and Gln371 residues interact with GM2, because the S370P or Q371E mutation in reovirus σ1 protein is impaired for agglutination of human erythrocytes and infection to host cells. The S370P and Q371E double mutants created for the reovirus T1L σ1 attachment protein do not recognize GM2 but wild-type T1L σ1 does bind to GM2. In the infection of the wild-type T1L and the S370P-Q371E double mutants, glycan-recognition capacity is likely not to largely influence replication in the infected murine. However, the hydrocephalus level of the T1L infection of wild type is high compared to the S370P-Q371E double mutant, indicating that T1L is a less symptom type of murine of hydrocephalus, which GM2 is not produced. Therefore, GM2 recognition is a key factor of each serotype reovirus symptom and is an essential factor of serotype-dependent infection of reovirus. Branched glycan GM1 has been suggested to be essential from the competition with cholera toxin [404]. Except for the GM2 glycan-recognition region
in T1Lσ1, another specific T1L σ1-binding site is the JAM-A, although the D1 region of human JAM-A N-terminal region does not contain any glycosylation site [403]. Thus, the carbohydrate receptor is independently engaged and the reovirus-host cells infection may use multiple steps, postulating that the virus first recognizes GM2 with low affinity and thereafter it recognizes JAM-A with relatively higher affinities than the previous one [405], following integrin-mediated uptake of virus.

As well described for the reovirus, entry strategies displayed by the enveloped viruses need multiple recognition, binding, and interaction between virus and cell surface receptors, accompanying with conformational changes in virus-recognizing proteins. Such enveloped viruses involve a recognition-binding-adhesion-enhancing strategy during initial virus-cell interactions, where show the lower affinities between virus and surface carbohydrates [406]. Then, this event is followed by the increased affinities between virus and secondary receptor, eventually viruses entering via membrane fusion or receptor-mediated endocytosis [407]. With regard to the increased affinities, SA is often used as a coreceptor toward virus attachment and tropism. Binding to SA is the first initial step of viral infection. For the next step, high-affinity binding to a secondary receptor is required for the further interaction with host cells, giving a conceptionally such tropism, implies for the attachment-enhancing mechanism for reovirus interaction because sialic acid and head receptor expressions determine the virus-specific infection [408]. Higher expression of AS and head receptor indicates more efficient infection. Currently, because reovirus is under investigation for a vaccination vector as well as oncolytic agent development, reovirus-glycan recognition can be applied to the therapeutic trials of reovirus glycan-binding mechanism. Understanding reovirus binding to glycan may allow some clue to apply for development of therapeutic agents.

4.9 Gb4-Parvovirus Infection

The Parvovirinae has five genera as a subdivision. They are amrovirus, bocavirus, dependovirus, erythrovirus, parvovirus, aleutian mink disease virus, bovine parvovirus, AAV2, human parvovirus B19, and mice minute virus, which are based on genome structure and protein structure. Their capsid open reading frame (cap) encodes two or three (depending on the virus) overlapping structural viral proteins (VP) which assemble the T = 1 capsid. The non-pathogenic dependovirus replication depends on co-infection with a helper virus of adenovirus, herpes simplex virus, or papillomavirus. The other genera replicate by themselves without helper virus cooperation. AAV1 recognizes both SAα2–3 and SAα2–6 N-glycans. AAV2, AAV3, and AAV13 recognize heparan sulfate proteoglycan (HSPG). AAV4 and AAV5 recognize SAα2–3 O-glycan and SAα2–3 N-glycan, respectively. Bovine AAV binds to gangliosides and chitotriose of β1,4-GlcNAc for infection. GSL globoside/globotetraosylceramide (Gb4Cer) is an infection receptor for B19 parvovirus of humans. Virus-like particles directly recognize globo-series Gb4Cer.
Human B19 parvovirus type belongs to a small nonenveloped virus as an ssDNA type and belongs to the genus *Erythrovirus*, the subfamily *Parovirinae*, and the family *Parvoviridae*. As a human pathogen, the B19 type species of human *Parvo-virus* is known to cause the childhood disease erythema infectiosum [341, 409]. B19 is also problematic in pregnancy of humans and infection of fetal ages causes fetal death with hematological disorders. The viral capsid is composed of two VP1 and VP2 proteins, forming an icosahedral symmetry with 60 structural protein subunits. The two proteins differ in terms of the additionally repeated N-terminal sequence, 227 amino acids, on VP1. B19 virus has an extremely narrow tissue tropism and infects most of human erythroid progenitor cells. B19 virus replicates in the erythroid lineage BFU-E and CFU-E cells. For the B19 virus receptor and internalization, virus infects erythroid progenitor cells targeting its receptor, the P antigen of blood groups [410]. In fact, B19 parvovirus infects the erythroid progenitors in the BM by recognizing the GSL globoside Gb4. Virus binding to Gb4Cer has been demonstrated on thin-layer chromatogram (TLC) data, confirming that the Gb4 globoside carbohydrate structure is crucial for viral attachment, interaction, and entry to the host cells. Anti-Gb4 antibodies specifically inhibit the B19 virus infection to bone marrow-derived mononuclear cells; exogenous Gb4Cer treatment blocks the viral binding to erythroid cells.

Tropically, Gb4 species is present in many different cell types, but predominantly present in erythroid lineage progenitor cells of humans in the bone marrow. B19 virus hemagglutinates but soluble or Gb4 treatment inhibits the hemagglutination. However, the current issue of B19 virus tropism is that B18 virus pathogenicity and erythroid tropism are directly not related with Gb4 expression. The Gb4 level in host cells is not related to B19 viral recognition with the cells but its level seems to be necessary but not exclusively for replicative infection. This indicates that PM molecules may affect the GB4 binding with other GSLs to B19V.

The fact that the Gb4Cer is not recognized in the phospholipid bilayer membrane indicated that Gb4Cer is not the direct actor like a bona fide receptor to B19. Then, it has been known that B19 binding to the cellular receptor globoside (Gb4Cer or Gb4) indeed leads to structural and conformational shifts and alternation of the capsid proteins. This allows the viral accessibility via the N-terminal region of VP1 (VP1u) to host cells. The Gb4 is called P-antigen due to its synonym and its synthesis is well known in certain carcinomas in the testicular tissues. P antigens is the site for B19 infection in cells, as the tumor cells can be infected by B19 infection [411]. However, co-receptors are required for infection in erythroid progenitor cells. During internalization to the host cells of humans, B19 virus binds to the erythroid progenitor cells through Gb4 interaction or blood group P antigen interaction [35]. Because P antigen is largely expressed in erythro-lineage types, surface P antigen binds to the virus. But the process is not sufficient for complete entry and infection. It indicates the existence of some co-factors including α5β1 integrin, and Ku80 is required for the complete infection of B19 [412]. The Gb4Cer as receptor has been reconfirmed for B19 [413, 414]. The oligosaccharide epitope is functioned as a B19 ligand, and this knowledge regarding the viral binding with Gb4Cer opens a new B19-Gb4Cer interaction as the PCI type for virus infection [415]. B19 virus also binds to
membrane-associated Gb4Cer, which is a form of the reconstituted Gb4Cer, as a receptor [416].

4.10 SA/GM3-Influenza a Virus Infection

4.10.1 SA-Specific Influenza a Virus

Influenza A belongs to a negative-sense ssRNA virus as an enveloped type of the Orthomyxoviridae family and causes epidemics in humans. Enveloped type of viruses is featured with a lipid bilayer. The lipid bilayer is easily embedded or merged with the target cell lipid bilayer. This is a first step for the viral entry into host cells. The influenza virus is classified depending on the virus-host infectious similarity. Three different Influenza virus species are classified. A, B, and C virus types are known for the influenza. Human influenza virus A/B types are the causative factors of seasonal influenza emergence in humans. Zoonotic influenza A type virus infects humans with pandemics. From the vaccination limitation by optimal efficiency rates and mismatched effect, antiviral agents are emerged as anti-influenza infection, as the commercial inhibitors of N-acetylneuraminidase (NA) are available for oseltamivir and zanamivir on the market. However, the NA inhibitor resistance of H1N1 virus desires a new generation of antiviral drugs.

Influenza virus A and B type viruses comprise two distinct glycoproteins. They are viral spike proteins that are known for hemagglutinin (HA), a sugar receptor-binding protein, and for NA that destroys the binding receptor or cleaves the terminal sialic acid. For HA activation, the inactive precursor HA0 is cleaved to the disulfide-bond HA1 and HA2 by host enzymes. HA1 has an α-SA glycan-binding domain. The two spike proteins of HA and NA are particularly characteristic of influenza virus during the influenza viral life cycle. They directly function in the virus processes, including host interaction, adhesion, host plasma membrane penetration, endosome arrangement, ER-Golgi trafficking, viral particle maturation, viral assembly, and burst-out release, of influenza virus A and B. Among them, influenza type A virus is subtyped into antigenically distinct 16 HA and 9 NA subtypes and thus theoretically 154 subtypes are calculated as possibly emerging viral candidates. The NA spike glycoprotein is enzymatically active sialidase enzyme to cleave NeuAc or SA from viral receptor carbohydrate chains. The NA activity is, therefore, essential for virus budding, cleaving off them, from the host cell membranes. In general, influenza virus NAs are classified into two major groups. Group-1 virus NAs include four different types such as N-1, -4, -5, and -8, while the Group-2 virus NAs include other five types of N-2, -3, -6, -7, and -9 for each NA. Contrary to influenza virus A and B types, the C type influenza virus is known to cause relatively mild respiratory symptoms upon infection in humans and differs from the other influenza virus types of A and B. Interestingly, the host specificity of the influenza virus type C is in its preferred receptor sugar structure, where N-acetyl-9-O-NeuAc being the binding substrate. Therefore, the host receptor sugar structure is destroyed by the C type viral
enzyme that is the acetylesterase specific for \( N \)-acetyl-9-O-NeuAc only, but not other substrates such as NA.

Cell surface receptors are directly utilized to bind by a virus and they are generally glycoconjugates such as GSLs, glycoproteins, and proteoglycans. GSLs can directly be used as receptor molecules such as hormones, interleukins, interferons, lymphokines, and cytokines or microbes such as viruses, bacteria, and microbial exotoxins through their carbohydrate glycan structures. Glycan moieties extend into the extracellular outwards, and therefore, virus binding targets the membrane distal carbohydrate and the proximal proteins of membranes. Terminal, internal, or non-terminal carbohydrate regions of GSLs are used as the minimal carbohydrate recognition site. Short GSLs closely located in proximity to the membrane lipids are used as virus receptors. Amino acid sequence around the receptor recognition site defines its binding specificity. Host cell carbohydrate receptor-microbial ligand interactions are low in affinity and are enhanced by multivalency of receptors. Glycan binding is performed by electrostatic forces to negatively charged SA-based carbohydrates or glycans. Such carbohydrate motif is found in glycolipids and glycoproteins.

SA residues are essential for influenza virus receptors [326], but the fine definition of the receptor molecules is not settled down. Sialic acid residues linked to gangliosides are cell entry receptors of many viruses. In pathogenic agents, mumps, influenza, corona, parainfluenza, noro, rota, and DNA tumor viruses use such SA residues linked to gangliosides. Receptor-binding molecules are located on viral envelopes or on the nonenveloped virus surface. In order to de-attach the burst viral particles from host cells, sialidase or sialyl-\(O\)-acetyl-esterase is expressed as the receptor-destroying enzymes. The enzymes function to release virus particles from infected cells and protect sialyl-conjugates interfering during viral attachment. The roles of gangliosides in virus entry into target cells remain unclear. SAs are linked in linkage modes of \( \alpha2,6 \) or \( \alpha2,3 \) glycosidic bonds to a Gal residue and also of \( \alpha2,8 \) bonds to a pre-existing internal \( \alpha2,3\)-SA. Diverse glycosidic linkages and substitutions are found on the pyranoside ring or SA side linkages produce SA structure diversity. Viruses preferentially bind to SA attached carbohydrates, and this binding specificity determines ranges of virus hosts, tissue-specific tropism of virus, cell type specificity, and viral pathogenic progression. In fact, the influenza A virus of humans and avian prefers to \( \alpha2,6\)- and \( \alpha2,3\)-SA residues, respectively, and thus the sugar type distribution of each SA linkage type correlates with the glycosidic linkages of the different host cells. The respiratory tract epithelial cells in humans and intestinal epithelial cells of birds express their species-specific SA type for each viral preference [417]. The interactions between viruses and SA linkages of carbohydrate contribute to host range and tissue tropism.
4.10.2 Attachment, Endocytosis, and Influenza Virus Host Tropism

From the three influenza virus types, influenza A virus type is a causing agent, frequently raising for tropically threatened pathogenicity in humans and animals. The environmentally primary infection hosts of influenza A virus are avian (birds or chicken) and occasionally transmit to other animal species via changes in SA-recognizing capacity of HA. The changes in host range occur by genetic shift events caused by homologous recombination in swine host. Although the origin of influenza B virus is not clearly known, influenza B virus is considered as a descendant of avian influenza A virus and this type causes mainly respiratory infections in humans, but the detailed mechanism remains unknown yet. During the last two decades, influenza virus A type has been pandemic in specific local areas from Asian countries [418]. The infection of influenza A virus requires its interaction with the cell surface glycan receptors using the surfaced HA and NA. The virus envelope HA and NA are also used for protection in host antibody response, too. Influenza A virus infects the host species with high specificity due to their HA specificity to SA residues. The pandemic influenza A virus globalization spends a hugely social and economic expenditures. Influenza virus A is reported to be particularly susceptible to environmental responses, provoking the genetically homologues mutation through interspecies and eventually spreading out with an avian-swine-human crossed trans-infection. The avian-swine-human crossed trans-infection is accelerated by interspecies hyper-genetic recombination. Therefore, it is assumed that influenza A viruses are specifically diverse in their wide host ranges from avian to humans with widely evolved adaptation in natural selection [419]. Interaction between influenza viral HA and NeuAc residues in carbohydrates causes host infection. Attachment to NeuAc is performed by HA receptor-recognizing proteins as components of viral envelopes or nonenveloping viruses. Changes in the receptor specificities determine virus tropism, specificity, and transmission of viruses. Historically, NeuAc or SA was the first example of the virus receptor (Fig. 4.5) [420]. SAs are modified to the active substrate form, CMP-NeuAc (SA), by a specific CMP-SA synthetase enzyme in nucleus. The sugar nucleotide, CMP-SA translocation to the Golgi apparatus, is mediated by a specific enzyme, CMP-NeuAc translocase, once CMP-SA synthesis is ready, and they are used as the donor substrate of

![Fig. 4.5](attachment:figure45.png)
sialyltransferases. Hirst and McClelland and Hare, for the first time, described that influenza virus can hemagglutinate erythrocytes [418, 421]. The NeuAc or SA-recognizing viruses include many animal and human pathogenic viruses including influenza, corona, mumps, noro, parainfluenza, rota, and tumor viruses in orthomyxovirus or paramyxovirus, or etc.

Influenza A virus exhibits each specific species and tissue specificity, which is derived from HA sequence differences, because HA displays the preference of human influenza A for α2,6SA in human and avian virus form for α2,3SA in avian. Through the interchangeable hypermutation susceptibility of the virus, three worldwide influenza pandemics were present with the specificities having H1H1, H2N2, and H3N2 types to date [422]. The first pandemic outbreak of the lethal avian influenza A H5N1 type virus was spread in 1997, and the virus continues to adapt and evolve, yielding global sanitary concerns. During the past decade, a few cases of emerging viral strains were recorded for the 2009 H1N1 swine-origin virus [422] and the H7N9 2013 Chinese virus [423] received much attention from the health controls due to the serious public panic. The avian influenza A virus H5N1 is the lethal and pathogenic influenza virus, and it was considered a powerful threat to domestic animals and humans, although the avian-human inter-mutation is not well understood yet. The recent issue why the pandemic and the widespread of avian influenza H5N1 virus in birds leads to the increased bird-to-human transmission get reached global concern. To prevent the influenza viral respiratory diseases, Flu vaccination is considered an effective strategy to prevent virus infection, but annual reformulation to match antigenic variations is needed for application.

Influenza viruses selectively bind to NeuAc- or SA-linked Gal whereas avian type influenza viruses bind to NeuAc- or SA-linked Gal [424]. The human virus infection is through airway epithelium of the upper respiratory tract. For infection of influenza A viruses via receptor-mediated endocytosis, surface glycoproteins HA, for the first step of virus entry, binds to the terminal NeuAc or SA-linked receptors present in the target cell surfaces through the HA molecule interaction with α-sialoglycoproteins or α-sialoglycolipids [425, 426]. NeuAc or SA bound to gangliosides and glycoproteins are receptors of a variety of viral entries. In complex glycoconjugates, NeuAc or SAα2,3-/SAα2,6-Gal, SAα2,3-/SAα2,6-GalNAc, SAα2,6-GlcNAc, or SAα2,8-SA residue is the sialylated structural patterns. Influenza viruses can not recognize α2-8Neu5Ac in ganglioside GD3 series or polysialyl glycoproteins, but recognize only α2,3- or α2,6-bound NeuAc or SA residues. For example, Neu5Acα2,3/Neu5Acα2,6GalNAc, Neu5Acα2,3/Neu5Acα2,6Gal, and Neu5Acα2,6GlcNAc are the cases [420]. The next step is that NA cleaves the SA-linked receptor present in the target cell surfaces. Some viruses carry sialyl-O-acetyl-esterase instead of NA. The enzyme destroys the receptor to potentiate the infected virus to release from infected host cells.

Mutations in HA lead to the influenza A adaptation ability to the respiratory tract of humans because α2,6-SA is synthesized on columnar epithelial cells of human airway respiratory tract. SA linkage specificity is also observed in papovaviruses with the tropism and pathogenicity. The virus host specificity is derived by the differences in SA- or NeuAc-binding specificity [421, 427], where the human-type
receptor has α2,6-linked NeuAc- or Sia-sugars and the avian receptor carries α2,3-linked NeuAc- or Sia-sugars, respectively [428]. Human-type influenza virus-binding NeuAcα2,6Gal carbohydrates are broadly expressed in most of the tissues including duodenum, heart, ileum, lung, liver, and spleen. In contrast, avian influenza virus-binding NeuAcα2,3Gal carbohydrates are found in the distinct tissues such as trachea and lungs. For attachment and entry, human virus has to bind to α2,6SAs, and α2,6-SA cleavage is required for virus release. Therefore, the NeuAc- or SA-sugar receptor-binding specificity is the key player and the most important barrier in H2N2 and transmission [429, 430]. For species transmission of avian virus type to humans, swine plays a key role as the intermediate hosts, because the swine trachea and airway epithelium have both of α2,6-linked NeuAc- or Sia-sugars and α2,3-linked NeuAc- or SA-sugars, respectively. The events of homologous genetic recombination take place between the human-type and avian-type influenza viruses [431, 432].

Respiratory tract epithelial cells of human airway are the first infection line of influenza virus in humans [433]. However, the infectivity of each avian influenza virus to human respiratory track is not well defined yet, and the relationship between the viral infection and transport behavior of NeuAc or SA receptors is not also well explained. In addition, there is no detailed mechanism(s) for the distribution of SA-linked receptors to date. The distribution of avian and human-type SA receptors can indirectly be analyzed in various organs by SNA lectin, which is isolated from Sambucus nigra, and MAA-II lectin, which was isolated from Maackia amurensis agglutinin (MAA), respectively. Lectins SNA and MAAII recognize the α2,6−/α2,3-NeuAc or SA-containing receptors present in the epithelial cells and organs of the respiratory tract [434, 435]. Basically, human beings synthesize the sugar linkages of NeuAc- or SA-α2,8−/α2,6−/α2,3-Gal residue through its catalytic enzymes such as α2,8, α2,6-, and α2,3-sialyltransferases specific for glycoprotein or glycolipids through the endoplasmic reticulum/cis-media-trans-Golgi apparatus (ER-Golgi system), as shown in Table 4.1. The representatives of NeuAc- or SAα2,3-, α2,6- and α2,8-glucosidic glycoprotein include N-glycan or O-glycans on glycoproteins expressed on the cell surfaces (Fig. 4.6). In glycolipids, the examples of NeuAc- or Sia-α2,3-linked lipids are the ganglioside GM3, which is NeuAc- or Sia-α2,3Galβ1,4Glc-ceramide. Thus, three sugar residues of sialyllactose are exposed as hydrophilic moiety on the cell surfaces of host cells or viral enveloped coats.

Influenza virus attaches its host cells via the viral HA binding to sialylglycans of host cell surfaces. A series of viral life cycle includes endocytosis, endosomal acidification, M2-driven uncoating and HA-driven viral-endosome fusion, and viral genome transportation to nucleus. Influenza virus also undergoes clathrin-mediated endocytosis as well as dynamin-2 GTPase or caveolae-driven endocytosis. Also, the dynamin-independent micropinocytosis also occurs via the receptor tyrosine kinase (RTK) activity. The FGFR2 and FGFR4 with the RTK are involved in influenza virus entry as the first cases, because the influenza virus is associated with the EGFR. The PDGFRβ-GM3-interacted influenza viral endocytosis involves PDGFRβ phosphorylation but the RTK inhibitor Ki8751 inhibits PDGFRβ
Although influenza virus neuraminidase cleaves SA residue to yield desialylated PDGFRβ. Virus entry involves the Raf-MEK-Erk signalings. During cell attachment and endocytosis, influenza virus swindles the GM3-associated PDGFRβ signaling cascade. RTK function is modulated by gangliosides in the lipid raft microdomain. Ganglioside species positively or negatively regulate diverse RTKs. Although gangliosides are associated with influenza virus attachment and entry, their intracellular roles after virus endocytosis are not studied. Recently, an RTK inhibitor showed the downstream involvement in the post-entering behavior of the cells [436]. The RTK inhibitor Ki8751 inhibited endocytosis of influenza virus via anti-influenza A and B virus activity in host cells that PDGFRβ-expressed RTK associates with GM3-embedded lipid rafts. Upon influenza virus treatment, viruses are attached to GM3-embedded PDGFRβ-associated endosomal vesicle of the host cells.

For more complex gangliosides, the NeuAc- or SA-containing gangliosides including GM1, GM1b, GM2, GD3, GD1α, GD1b, GT3, GQ3, or related glycolipids are expressed on humans (Fig. 4.6). For examples, the CTxB subunit of V. cholerae binds to the GM1, which has the NeuAc- or SAα2,3-linked structures in the enterocytes surface of the intestinal tract [437]. In addition, considering the ABO blood type in humans, the question is raised why are the so-called human type NeuAc or Siaα2,6Gal receptors for influenza virus binding specifically expressed on the human respiratory cells only, but not NeuAc or Siaα2,3Gal receptors? [438]

### Table 4.1 Sialyltransferases for α2,3-, α2,6-, and α2,8-sialyl linkages

| Sialyltransferases | Abbreviation |
|-------------------|--------------|
| Galβ1,3GalNAcα2,3-3ST | ST3Gal I |
| Galβ1,3GalNAcα2,3-ST(second type) | ST3Gal II |
| Galβ1,3(4)GlcNacα2,3-3ST | ST3Gal III |
| Galβ1,4(3)GlcNacα2,3-3ST | ST3Gal IV |
| GM3 synthase | ST3Gal V |
| Galβ1,4GlcNAcα2,3-3ST | ST3Gal VI |
| Galβ1,4GlcNAcα2,6-6ST | ST6Gal I |
| Galβ1,4GlcNAcα2,6-6ST | ST6Gal II |
| GalNAcα2,6-6ST | ST6GalNAc I |
| Galβ1,3GalNAcα2,6-6ST | ST6GalNAc II |
| NeuAcα2,3Galβ1,3GalNAcα2,6-6ST | ST6GalNAc III |
| NeuAcα2,3Galβ1,3GalNAcα2,6-6ST (second type) | ST6GalNAc IV |
| GD1α synthase | ST6GalNAc V |
| GD1α/GT1α/GQ1βα synthase | ST6GalNAc VI |
| GD3 synthase | ST8Sia I |
| Polysialic acid synthase | ST8Sia II |
| NeuAcα2,3Galβ1,4GlcNAcα2,8-8ST | ST8Sia III |
| Polysialic acid synthase (PST-1) | ST8Sia IV |
| α2,8-sialyltransferase | ST8Sia V |
| α2,8-sialyltransferase | ST8Sia VI |
Furthermore, neutrophil homing and rolling is also associated with normal glycosylation with the expression of carbohydrates with the NeuAc- or SAα2,3-linked structures on glycoproteins or glycolipids [439]. Expression of the sialylated-type 1 or 2 chain with SAα2,3-Galβ1,3/4[Fucα1,4/3]GlcNAc structure for sialyl Lewis A (SLA) or SLX is enhanced by α2,3-sialyltransferases of ST3Gal I-VI in those functional cells, because SLA and SLX act for the E-selectin adhesion to endothelium. The avian HAs adaptation to human host cells is thus based on the switching-on affordable for binding specificity from avian virus type of α2,3-SA residues to human virus type α2,6-SA residues present in the upper airway respiratory epithelial cells. The typical SAα2,3-glycan structure represents the NeuNAc-α2,3Galβ1,3/4GlcNAc sequence motif. The SAα2,6 typical structure is the NeuNAc-α2,6Galβ1,4GlcNAc. The virus binding to sulfatides and neutral glycolipids has been known for influenza A and HIV.

**Fig. 4.6** Structures of sialyl α2,3, α2,6, and α2,8 linkages of ganglioside GM3 (a) and GD3 (b), as well as N- and O-glycan sugar chains (c) of glycoprotein
4.10.3 Neuraminidase (NA) Inhibitors as Influenza Virus-Inhibiting Drugs

The NA was isolated as a candidate for influenza virus-targeting drugs. NA-specific inhibitors of zanamivir, oseltamivir, and peramivir are used for the influenza. Oseltamivir was the first drug against influenza. Recently, due to emerging influenza virus strains including H1N1 and avian H5N1 [440], effective drugs are required to cope with influenza viruses. In 2009, peramivir was opened in America against swine-origin H1N1 (A) resistant to oseltamivir. H5N1 virus threatens humanity. Although multiple neuraminidase inhibitors were developed for influenza infection, the rapid occurrence of drug-resistant viral variants needs multifunctional anti-influenza drugs. The resistance issue of influenza virus-inhibiting drugs reflects the development of the new antiviral agents. One anti-entry agent of virus endocytosis is a solution in blocking of virus-host cell recognition in the entry event. At present, the neuraminidase inhibitory drugs have been approved in many countries, but it is mainly administered intravenously, which is very inconvenient for patients. To replace such drugs, recently, many plant-derived polyphenols exert antiviral effects against influenza virus and anti-NAs with strong activities against N2 and N1. For example, the 3,4-dihydroxyphenyl group from caffeic acid was essentially interacting with the NA active site according to the docking analysis, whereas several CA derivatives acted as non-competitive inhibitors. Potent NA inhibitors from caffeic acid derivatives can be designed to cope with influenza virus. CA inhibits and eclipses the multiplication of influenza A virus in vitro, while the progeny virus yield was markedly decreased in the presence of CA [441]. They are diverse in their structures, as shown in flavonoids, xanthones, and diarylheptanoids [442–445]. CA derivatives had potent anti-influenza virus activity and NA inhibitory activity in vitro. The potential pharmacological use of CA or its derivatives is admitted for an antiviral drug against influenza A virus. Unlike the classical structure, these structures are many and act as noncompetitive NA inhibitors, in such a way that they inhibit the NA activity by binding on non-active sites of the enzyme. They could be new candidates for developing new anti-influenza agents. Small natural compounds can be used for new therapeutic agents using fused chemobiological synthesis in NA biology without side effects. Chemobiological fusion science using the natural compounds as the chemical scaffold will create new drugs for influenza. We remember that the discovery of low molecular weight Aspirin with its ingredient acetylsalicylic acid upgraded the human health and welfare during the twenty-first century. A part of the present description has previously been published in the Editorial, Journal of Glycomics and Lipidomics, which is not a cited journal.
4.11 GD1a-Porcine Sapelovirus Infection

The word Sapelovirus comes from Simian, Avian, and Porcine entero-specific viruses. The Picornaviridae family consists of 29 genera with nonenveloped and positive-sense ssRNA viruses. Picornaviruses are known to be various infection diseases-causing agents for intestinal, respiratory, neurological, and cardiac diseases in both humans and animals. The genus Sapelovirus is composed of two species of Sapelovirus A that is formerly termed Porcine sapelovirus and Sapelovirus B that is formerly termed simian sapelovirus. Although a third species called Avian sapelovirus known as duck picornavirus TW90A was previously classified, the species is currently reclassified to a family of a new genus termed Anativirus A. The two sapelovirus species are distinguished by several parameters including difference in host species and virus diversity in sequences. In fact, VP1 gene has about 50% homology in amino acid sequences of viral species. The simian type of sapeloviruses, which are suggested to three sero-types, is currently progressing their inter-species recombination, as found in several genomic sequences. The porcine sapelovirus (PSV) is a causative agent of reproduction disorder, diarrhea, pneumonia, and polioencephalomyelitis in pigs. PSV recognizes SAα2,3-linkage on GD1a glycolipids as a receptor, although the role of GD1a is still not clear in viral pathogenesis. However, PSVs are not bound to histo-blood group antigens (HBGAs). PSV belongs to the Picornaviridae family, characteristic of small, nonenveloped, single-stranded, and positive-sense RNA genomes. Sapelovirus genus as a picornavirus includes simian, avian, and porcine picornaviruses [446]. PSV is widely distributed with prevalence and the receptor for PSV has recently been defined, as PSV uses SAα2,3-glycan on GD1a for recognition and entry into target cells.

4.12 GD1a/Gal-Cer/HBGA-Murine As Well as Human Norovirus Infection of Caliciviridae Family

Terminally linked SA-Gal or SA-GalNAc residue by an SAα2,3 or SAα2,6 linkage is determined as receptors for viral host range, pathogenesis, and tropism. Enterovirus, reovirus, and rotavirus have their host tissue tropisms of the intestinal tract. Gangliosides having one or more SAs in the α2,3 and α2,8 linkages like GD1a are attachment receptors for Noroviruses (NoVs) during the entry of the virus cycle. NoVs belong to the Norovirus genus in the Caliciviridae family. As the shape of small, round-structured and ssRNA, positive sense RNA virus with about 7.7 kb length. This infects both animals and humans. Human NoVs are a causative agent of epidemic acute gastroenteritis. No effective vaccine or antiviral therapeutic drugs are developed for human use. They are nonenveloped RNA virus group and thus a NoV capsid protein encapsulates the ssRNA. From lack of this viral culture model, norovirus-like particles have been employed with recombinant capsid protein
(VP1). The single viral protein VP1, comprised of the two shell (S) and protruding (P) regions, is organized to 90 dimers. The P domain has two subdomains of P1 and P2, where the subdomain P2 recognizes its host cells. The HBGAs are known as human NoVs attachment factors or receptors in a strain-dependent way [447, 448], although binding of P-domain to gangliosides is also a basic step for various non-human caliciviruses or non-human noroviruses. Primary infection depends on P domain binding to the HBGA. However, HBGAs always do not account for tropism of all human NoVs and entry into the hosts. HBGAs attached to PM glycolipids or glycoproteins are largely present in surfaces or erythrocytes and mucosal epithelial cells as well as present as free carbohydrate forms of fluids like saliva. Similarly, heparan sulfate and secreted oligosaccharides of human milk are also engaged. NoVs bind to HBGA in all ABO, Lewis, and secreted fluids. Certain human NoVs like Noda485 are not bound to the known HBGAs, implying that HBGAs are not the solely binding and entry targets for human NoVs. Caliciviruses of FCV, MNV, PoSaV, and TV specifically interact with sialylated glycans and HBGAs.

Gangliosides are also another ligand for NoVs in human and murine [327, 447]. Human NoVs recognize GM3 as the highest affinity ligand [410]. Among the GM3 structure, the Sia-Gal-Glc moiety has been demonstrated as the dominant recognition site for the NoV in the ESI-MS assay. The additionally glycosylated gangliosides such as GM1, GM2, GD1b, GD2, or GD3 exhibited reduced recognition capacity, when compared to GM3. The SAα2,3-linkage in ganglioside has been confirmed in norovirus infection. Human Nov P domain directly binds to α2,3-linked SA residue on 3′-sialyllactose (3SL). From saturation nuclear magnetic resonance (MRI), surface plasmon resonance (SPR), and mass spectrometry (MS), ligand-binding epitopes, binding affinity, and stereochemistry in the ganglioside carbohydrate-VP1 interaction have been reported [449]. In addition, GD1a is an attachment and recognition receptor for MNV strains, which was isolated from the brains of immunocompromised mice [35]. Although GM2, asialo-GM1 (named GA1), and GD1a are the normally expressed gangliosides of murine macrophages, MNV-1 strain recognizes a terminal SA-Gal linkage of GD1a and this is the reason why the MNV-1 does not bind to asialo-GM1 or GM1 gangliosides. The lack of ganglio-specific GSLs in murine macrophages through GlcCer synthase inhibitors decreases the binding and infection levels of MNV-1 to murine macrophages. However, the GD1a treatment rescues the defected phenomenon of viral infectivity.

HBGAs attached to membrane proteins or glycolipids on the membrane surfaces of erythrocytes and mucosal epithelial cells are frequently found in saliva or milk. Human NoV-HBGA interactions are observed in ABO or Lewis antigens [450]. Rabbit hemorrhagic disease virus (RHDV) is also a positive-sense ssRNA virus of the Lagovirus genus in the Caliciviridae family [451]. RHDV of caliciviruses targets HBGAs of the host cell for attachment. The lack of HBGA ligands in rabbit exhibits resistance to RHDV infection. RHDV VLPs recognize the A type 2 HBGA glycans, B type 2 HBGA glycans, and H type 2 HBGA carbohydrates expressed on the erythrocytes and epithelial cell surfaces. Some NoVs do not bind any HBGAs,
suggesting the HBGA-independent interaction, and claiming that HBGAs are not always the sole receptor for human-type NoVs. Then, GSLs and acidic oligosaccharides have been recognized as human NoV receptors. It was known that galactosylceramide and HBGA glycosphingolipids interact with a VLP of human NoV [447, 452]. VLPs also recognize heparan sulfate, sialyll(S) LeX, SLeα, S-lacto-N-neotetraose, S-lacto-N-tetraose, and S-lacto-N-fucopentaose but not 3'-sialyllactose (SL) and 6SL. Therefore, apart from gangliosides, histo-blood group antigens, α2,3-sialylated chain such as SLeX, and glycosaminoglycan heparan sulfate are also recognized as the attachment receptors for human NoV strains [452].

4.13 GD3/GM2-Zika Virus Infection

In the genus Flavivirus in the Flaviviridae family, flaviviruses have specific envelop and positive ssRNA. A group of Dengue, Japanese encephalitis, West Nile, yellow fever, and Zika virus are included. Viruses are typically transmitted by arthropod vectors. Zika virus has a specific tropism to neuronal infection causing CNS dysfunction. The neurotropic properties of Flaviviruses are similar to those of Dengue, encephalitis, and West Nile virus like Zika virus. Zika virus (ZIKV) is a causative agent of a global health emergency, implicating the virus as an emergent neuropathological agent in the human nervous system, including fetal and ocular brain diseases, neonatal microcephaly, and adult Guillain-Barré syndrome (GBS). The ZIKV is a positive-sensed and ssRNA Flavivirus transmittable to humans via mosquitoes [453].

ZIKV infection leads to the development of autoimmune response, GBS, which occurs in the peripheral nervous system [406]. The GBS symptom basically originates from an infectious agent of bacterium C. jejuni and also several others including Epstein–Barr virus (EBV), influenza A virus, hepatitis E virus, H. influenza, cytomegalovirus, and Mycoplasma pneumonia. Its pathologic mechanism is explained by carbohydrate antigen in peripheral nerve tissue and production of the anti-ganglioside antibodies caused by carbohydrate similarities [454]. ZIKV infection increases in GD3-specific autoantibody, because GD3 is largely expressed in neural SC to maintain the self-renewal.

Are gangliosides related to ZIKV GBS and microcephaly? Currently, the clue is in that the virus obtains host membrane glycolipids and incorporates them. For example, molecular mimicry between pathogenic and neuronal ganglioside antigens is a driving force for GBS upon Campylobacter infections [455]. Antibodies to glycolipids (GM1, GA1, GM2, GDla, GDlb, or GQlb) were detected in the onset of GBS. Neurological complications occurred at ZIKV infection are thus caused by autoimmune antibodies, targeting gangliosides. ZIKV easily crosses the placenta barriers because the Zika virus is found in pregnant women’s amniotic fluid. In addition, ZIKV infection suppresses human neural progenitor cell proliferation [456]. Autoantibody production against gangliosides such as GD3 in ZIKV infection is interesting. Treg/Th17 imbalance is suggested as the neuropathogenic factor.
during Zika infection [457] with blood–barrier disruption. For GD3 acquisition in Zika virus in the process of viral shedding of infected neural SC that expresses GD3, ZIKV acquires the membrane lipids from the host cells in the course of the virus budding stage. The GD3 is therefore recognized and targeted as an autoantigen by the host immunity because the pathogen-associated molecular pattern (PAMP) can break peripheral tolerance to GD3 [458]. Such produced GD3 autoantibodies suppress the neural cell functions regulated by GD3 functions.

The flaviviruses have similar entry tropism to hosts through GAG-binding site of envelop protein to cell surfaces. Also, high-Man N-glycosylated envelop protein of flaviviruses binds to DC-SIGN of host immune cells. Flavivirus binds to DC-SIGN or GAG on host immune cells (Fig. 4.7). Another marker of ZIKV infection is GM2 ganglioside [459], known for other viruses such as polyomavirus and HIV [460], as GM2 was also elected as an infection biomarker. As a pathogen receptor at membrane’s outer layer, the GM2 is important for ZIKV infection with brain tropism.

**Fig. 4.7** Zika virus-host cell interaction via DC-SIGN and GAGs on host immune cells

4.14 GM1/GD1b-Varicella-Zoster Virus (VZV) As Well as GM2-Cytomegalovirus (CMV) Interaction during Infection in GBS

Gangliosides are abundantly expressed in the nervous system, mainly in axons of neuron. Anti-ganglioside antibodies are observed in various autoimmune diseases, including GBS, Miller-Fisher syndrome, chronic idiopathic ataxic neuropathy, multifocal motor neuropathy, and IgM paraproteinemia neuropathy [461–465]. GBS-displaying infection, including *C. jejuni*, varicella-zoster virus (VZV),
and Cytomegalovirus (CMV), leads to the production of anti-gangliosides antibodies, allowing many auto-immune diseases. CMV is known as an antecedent infectious agent with its high immortal rate, which phenotypically expresses the demyelination variant of GBS. The fibroblastic cells infected with CMV produce GM2. CMV-infected GBS patients exhibit sensory function deficits with anti-GM2 antibodies as IgM subtype. The known Fisher syndrome is a type of GBS variants having anti-GQ1b antibodies as an IgG type. The produced antibodies reactive for the GSLs cause neuropathies, because such antibodies against GSLs injure and damage motor neurons or sensory neurons, block ion channels, inhibit remyelination, and induce axonal degeneration [465–468].

In GBS patients with chicken pox/VZV infection, IgM types of anti-GM1 antibody as well as anti-GD1b antibody are produced in infected patients [469]. GM1-specific antibody titers and GD1b-specific antibody titers are easily detected in such patients with the GBS after VZV infection, because the antibodies are specific for terminal Galα1,3GalNAc-carrying glycolipids. In Herpes simplex virus and CMV, anti-GM2 antibodies in patients with GBS are frequently detected in the GBS patient sera with CMV [360]. GM2-specific antibodies are also detected in the GBS patients during CMV infection. However, CMV infection does not seem to be related with anti-ganglioside GM2 antibodies. CMV-infected fibroblasts produce GM2 epitopes specific for GM2-specific antibodies. Thus, GM2-specific antibody production in GBS patients infected with GBS is increased by molecular mimics of GM2 and CMV-mediated antigenic epitopes. Antibodies specific for GT1b, GD1b, GD1a, GM3, GM2, and GM1, but not anti-GQ1b antibodies, are detected as IgM and IgG class [470].

### 4.15 GD1a-Norovirus Interaction

Norovirus (NoV) belongs to the Norovirus genus of the family Caliciviridae. They contain small and round forms of RNA viruses. The virus infects humans and animals [471]. Human NoV (HuNoV) causes epidemic acute gastroenteritis [472]. NoVs are nonenveloped, capsid, ssRNA, positive sensed RNA viruses with 7.7 kb genome size. The capsid VP1 protein of NoVs is a single protein form. NoV-like particles (VLPs) have an icosahedral symmetric structure with VP1. VP1 is classified into P and S domains. The S domain has a specific structure of an interior and icosahedral shell form. But the P domain is featured by the protrusion of dimeric forms [449]. The P domain part is re-classified as P-1 and P-2 subdomains, where the subdomain P-2 occupies the outer surface of capsid protein, which is crucial for the host recognition of virus and immunological recognition of NoVs. It has been implicated that GSLs and SA-based acidic carbohydrates function as human NoV-binding ligands. Binding to gangliosides is crucial for the life cycles of many nonhuman caliciviruses and noroviruses. Human NoVs recognize HBGAs and glycosaminoglycan (GAG) heparan sulfate (HS); α2,3-sialylglycans in the type 2 chain and SLeα are carbohydrate for the receptor for the attachment and infection
of HuNoV [473]. HBGA carbohydrates are attached to membrane glycoproteins or glycolipid. Such HBGA carbohydrates are frequently found on the erythrocytes and mucosal epithelial cells [474]. Human NoVs interact with HBGAs. The NoV-HBGA interaction is found in ABO blood types, Lewis antigens, and secretor/nonsecretor antigen types [475]. The molecular basis of such interactions is studied using NoV P dimers complexed with HBGA carbohydrates. Some NoVs of VA115 type named GL3 type do not bind to HBGAs, and therefore HBGAs is suggested not to be the sole receptor for infection of human NoVs. GM3 was the highest affinity ligand for the VA387 P and VLP. Interestingly, the affinity for NoV VA387 is relative to HBGA carbohydrate structure [476]. Gangliosides, acidic GSLs, consist of one or multiple SA residues via SA$\alpha_2,3$ and SA$\alpha_2,8$ linkages and are utilized as the receptors of norovirus attachment and infection [452]. HBGA and gangliosides bind to GII.4 human NoV with binding epitopes and affinities. The binding has been demonstrated by 3SL, the GM3 carbohydrate, and B antigen [477]. Recently, human NoVs have been demonstrated to recognize gangliosides using catch-and-release ionization MS (CaR-ESI-MS) tool [471], in the experiment of the human NoV VA387-P (GII.4)-binding GSL.

In murine norovirus (MNV), the GD1a was bound to MNV-1; however, MNV does not bind to GM1 or GA1 named asialo-GM1 [449]. SA residues in gangliosides are a recognition site and receptor for MNV. MNV-1 recognizes SA sequences appeared on cell lines and cultured murine primary macrophages. Recognition of terminal GD1a SA is the initial step in viral infection during the life cycle. Epitope analysis using 3'-sialyllactose suggested the direct recognition of SA$\alpha_2,3$-linkage to the P domain. The human NoV and carbohydrates interaction emphasizes the multivalent binding property of norovirus. Direct recognition of a human NoV GII.4 P dimer to SA$\alpha_2,3$-linkage further supports a functional role of ganglioside-recognition specificity in norovirus. VLPs of GII.4 also bind to HBGA and GalCer [327, 451]. GII NoVs VLP binds to surfaced heparan sulfate of the host cells, while GII.4 VLPs recognize SA-carrying glycans including sialyl Le$^X$, sialyllacto-N-neotetraose, sialyllacto-N-fucopentaose, and sialyllacto-N-tetraose with similar binding affinities to HBGA glycans [471]. Recognition of GII.4 VLPs to the SA part of sialyl Le$^X$ and sialyl Le$^a$ was demonstrated [478]. Sialyl carbohydrates, but not fucose, including 3'- and 6'-sialyllactose, bind to the VLP, implying that SA-carrying carbohydrates are also used as ligands of human NoVs. SA-containing oligosaccharides are known to interact with certain caliciviruses (CVs) of animals, such as feline calicivirus (FCV), murine NoV 1, and pig sapovirus [327, 479, 480]. However, the ligand status of SA for human sapoviruses as a calicivirus group still remains further evidenced.

Although genus Sapovirus like Norovirus genus, which is belonged to Caliciviruses cause acute and chronic gastroenteritis in animals and humans are also involved in gastroenteritis outbreaks in pediatrics [481]. The genus Sapovirus includes five genogroups G-I/II/-III/-IV/-V. From them, G-I/G-II/G-IV/G-V groups target human beings for infection and GIII group targets swine species. Although virus-to-host cell recognition via receptors is the initial cycle of infection, the precise receptors for the Sapovirus genus have not been clear yet. However, HBGA is a
target molecule present on host cells for Calicivirus attachment. Similarly, Tulane
virus, a monkey rhesus calicivirus, also utilizes HBGA antigen as its infection
receptor [482]. Virus particles or virions obtained from sapovirus G-I and G-V
group of humans do not recognize salivary HBGAs or synthetically related glycans
[483]. The noroviruses and sapoviruses are genetically and closely related groups in
humans and animals, and the possibility of zoonotic inter-transmission is of concern
[484]. The porcine sapovirus recognizes carbohydrates of both SAα2,3-/SAα2,6-
linked glycans as entry receptors. Because porcine SaV does not stably agglutinate
erythrocytes that carry the SAα2,3 and SAα2,6-linkages, porcine SaV is suggested
to stabilize interaction between the sialyl glycans.

On the other hand, FCV as the calicivirus family also recognize GD1a for its
receptor. FCV is a group of the calicivirus family and is well studied for its binding
and entry. Caliciviruses are classified into the genus lagovirus (LaV), nebovirus,
NoV, sapovirus, vesivirus, and recovirus [485]. Only genus NoV and sapovirus
group enter the human host. Vesivirus also includes the FCV, a respiratory disease-
causing agent in cats. Rabbit hemorrhagic disease virus as a LaV group causes a
necrosis-type hepatitis with hemorrhage leakage and mortality. Rhesus macaques
Tulane virus and newbury agent-1, a bovine enteric virus named Newbury1, belong
to recovirus and nebovirus groups, respectively. The FCV recognizes the airway
tract in the upper tract via the α2,6-SA linkage and the junctional adhesion molecule-
1, following viral endocytosis [486, 487]. HBGAs, type 2 chain α2,3-sialylglycans
(sialyl-Lex), and heparan sulfate are also known to function as the infection receptor
for human NoV [488]. SAs in carbohydrates of host cell glycoproteins and glyco-
lipids are widely used as viral attachment receptor to epithelial cells [481]. Termi-
nally linked SA to the penultimate Gal residue, linked by an SAα2,6 or SAα2,3
linkage, is the general character of SA-containing receptors [475]. SA specifies for
virus host range and tissue tropism, as most viruses, bacteria, and toxins are reported
to utilize gangliosides.

### 4.16 nLc4Cer-Dengue Virus Interaction

Dengue virus (DENV) is a viral disease worldwide with dengue fever (DF) such as
capillary leakage and hemorrhagic manifestations [489, 490]. DENV causes mor-
bidity and mortality in the world. It is transmitted by mosquitoes such as Aedes
aegypti and A. albopictus strains. Thus, DENV belongs to a mosquito-carrying
Flavivirus, endemic in tropical and subtropical area. Infection with one serotype
produces serotype-specific antibodies. Dengue-causing viruses include four anti-
genic serotype viruses named DENV-1, -2, -3, and -4. As DENV belongs to the
Flavivirus genus of Flaviviridae, other Flaviviridae family is also known for Zika,
Yellow Fever, Japanese Encephalitis, and West Nile viruses. The virus is an
enveloped spherical virion with an 11 kb and positive-sensed and ssRNA strand,
consisting of an icosahedral symmetry and a nucleocapsid core [491]. Each serotype is antigenically distinct due to 30% or difference in their amino acid sequences. The RNA genome consists of 10 genes on an open reading frame (ORF), encoding for three capsid, precursor membrane (prM), and envelope protein with the additional seven nonstructural proteins (NS) including NS-1/NS-2a/NS-2b/NS-3/NS-4a/NS-4b/NS. The NS proteins are involved in viral replication and host immune evasion. NS1 is dimeric in early stages of infection and hexameric form of NS1 protein is secreted [492]. The NS1 dimer is present on the lumen side of the ER. NS1 recognition with NS4a and NS4b enhances the viral replication complex formation.

Macrophages and mononuclear phagocytes such as DCs are major targets of dengue virus. The Dengue virus envelope glycoprotein (E) as a class II fusion protein has a receptor binding domain [493]. E protein has three distinct domains. Among them, the domain I is located on the hinge region and domain II having hydrophobic amino acid residues is located in a fusion loop with dimerization capacity. Domain III is host receptor molecule-recognizing region. Dengue virus E protein recognizes lectin and also the surface molecules of the host cells. Surface carbohydrates of host cells recognize dengue virus because the carbohydrate recognition is a key point of DENV propagation. Dengue glycoprotein E binds to the target carbohydrates as receptor molecules including HS GAGs and GSL neolactotetraosylceramide (nLc4Cer) on the host cells [494]. Dengue viruses recognize nLc4Cer of the host cell surface. Dengue virus type 2 binds to neutral glycosphingolipid nLc4Cer, Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ1–1–Cer, on TLC plates [495]. Also, Galβ1,3GalNAcβ1,4Galβ1,4Glcβ1–1–Cer, Gg4Cer, and neutral glycosphingolipid L-3, Gg3Cer, GalNAcβ1,4Galβ1,4Glcβ1–1’Cer have been recognized by the virus to a lesser extent. The virus has no affinity to LacCer. Neutral glycosphingolipids are the binding sites of virus and the β-GlcNAc residue seems to be linked to binding to the host cell surfaces. From the fact that DENV propagates between mosquitoes and humans, GSLs acting as receptors including L-3 and nLc4Cer may support virus transmission [496]. In lectins, DC-SIGN mediates entry of DENV to DCs as the primary target. DC-SIGN binds to N-Glycan at position 67. MBP binds to amino acid positions 67 and/or 153. Mannose receptor is a target site of DENV entry into macrophages [497]. The C-type lectin-like protein CLEC5A (MDL-1) as a macrophage receptor is a receptor for dengue virus [498]. Blockade of CLEC5A can be used in a therapeutic avenue of dengue virus disease (Fig. 4.8).

Host PRR binding triggers type I IFN response. Therefore, the DENV PRRs include the above heparan sulfate, nLc4Cer, DC-SIGN and Man residue, CD14, retinoic acid (RA)-inducible gene I (RIG-I) in the cytosolic region, melanoma differentiation-associated protein 5 (MDA5), and endosomal TLR-3 and TLR-7 [318]. In addition, protein folding effectors like heat shock proteins, HSP70/90, and chaperones also interact with DENV serotype 2 (DENV-2) [499]. Laminin receptor, CD14-associated protein, and uncharacterized proteins also interact with DENV [500], [266]. Immature virus has a protruding prM protein trimer with envelope glycoprotein E. Secondary heterotypic infection causes more severe disease than primary infection by antibody-dependent enhancement (ADE). ADE
Fig. 4.8 Macrophage receptor, C-type lectin-like protein CLEC5A (MDL-1) as a receptor for dengue virus displays a virus-antibody complex phagocytosis through FcγIIa receptor [501]. DENV E glycoprotein recognition of the host receptor potentiates virus entry via clathrin-mediated endocytosis. In the Golgi apparatus, the prM is cleaved by furin protease to yield the mature virion and release by exocytosis [502]. RIG-I and MDA5 respond to phosphate-containing RNA and long dsRNA [503]. They translocate to the mitochondrial membrane. Consequently, mitochondrial antiviral signaling (MAVS) protein via the caspase activation recruitment domains (CARD) and MAVS is activated. Thereafter, downstream of TANK-binding kinase 1 (TBK1), IκB kinase-ε (IKKe), phosphorylating IFN regulatory factors (IRF3), and IRF7 are activated to increase the gene expression level of type I IFN. In the TLRs, TLR3 recognizes DENV dsRNA and TLR7 recognizes ssRNA in endosomes. TLR3 phosphorylates TIR-domain-containing adapter-inducing IFNβ, interacting
with TNF-receptor-associated factor 3 (TRAF3) and TBK1/IKKe to induce IFNα/β-stimulating genes (ISGs) and chemokines [490]. TLR3 acts synergistically with RIG-I and MDA5 in producing an antiviral state against DENV infection. TLR7 uses the MyD88 signaling. The PRR recognizes cyclic GMP-AMP synthase and stimulator of IFN gene (STING) pathway that recognizes cytoplasmic DNA [504]. DENV damages the mitochondria and mitochondrial DNA activates cGAS-STING. Then, cGAS nucleotidyl transferase releases cyclic GMP-AMP that recognizes STING and induces TBK1, IRF3, and production of type I IFNs. The mtDNA activates endosomal TLR9 that recognizes nonmethylated CpG motifs-containing DNA [505]. In complementation, MBL binds to Man glycans on the DENV surface [506] and induces cleavage of C4 and C2 by MASP-2 and depositing C4b and C2a, forming the C3 convertase, C5 convertase, and C5b-9 membrane attack complex (MAC) to lyse.

Apart from GSLs, heparan sulfate proteoglycans (HSPGs) are used as the primary receptors for dengue virus as well as both HSV type 1 and HSV type 2 but each virus recognizes different structures [498]. Sulfated HSPG is also a receptor for dengue virus [499]. Like DEGV and HSV, adeno-associated virus type 2 (AAV2) binds cell surface HSPGs [500]. Also, as an enveloped RNA virus, Lassa virus (LASV) is an Old World arenavirus known in West African countries. LASV belongs to the family Arenaviridae and is a negative-sense and ssRNA virus. LASV entry needs bindings to O-glycans expressed on the dystroglycan (DG). In addition, the phosphatidylserine (PtdSer)-binding receptors TIM-1, Axl, and Tyro3 as well as CLRbs are the DG-independent entry sites for the LASV. TIM-1 potentiates entry. In the absence condition of DG, TIM-1 helps the entry of LASV pseudovirions (Fig. 4.9). Other enveloped viruses such as HCV and Zika virus also use the PtdSer receptors in the viral envelope as GP-independent receptors.
Lassa virus (LASV) binds to O-glycans on the dystroglycan (DG) and phosphatidylserine (PtdSer)-binding TIM-1, Axl, and Tyro3 as well as CLRs.