Glycosylation of dengue virus glycoproteins and their interactions with carbohydrate receptors: possible targets for antiviral therapy

Fakhriedzwan Idris · Siti Hanna Muharram · Suwarni Diah

Introduction

Dengue virus (DENV) is a member of the family Flaviviridae and has four distinct serotypes, designated DENV1–4. Fifty million people are estimated to become infected with dengue virus annually, and approximately 500,000–1,000,000 of these infections lead to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), with mortality rates of 5 %–30 % [1]. With no licensed vaccine or specific antiviral treatments available to prevent dengue infection, it is considered a major public health problem in subtropical and tropical regions.

Dengue virus infection is characterized by headache, biphasic fever, prostration, rash, pain in various parts of the body, leukopenia, and lymphadenopathy [2]. In severe cases, the infection can progress to DHF, which occurs when infection with one serotype causes individuals to suffer more-severe disease after subsequent infection with a different serotype. DHF is a severe febrile disease characterized by abnormalities of hemostasis and increased vascular permeability, which in some instances results in hypovolemic shock syndrome, DSS, when immune cells are enhanced by preexisting non-neutralizing antibodies directed against dengue viral proteins, although the exact mechanism is yet to be resolved [3, 4]. However, host and viral factors, such as the genetics of the infecting viral strain, have been implicated in contributing to disease severity [5].

DENV, like other flaviviruses, has three structural proteins. These structural proteins are originally encoded as a polyprotein by a single long open reading frame (ORF), which is later cleaved co- and posttranslationally by both cellular and viral proteases to produce the C, M, and E proteins from the amino terminus of the polyprotein. The virus also expresses seven nonstructural proteins derived...
from the carboxyl terminal sequence of the polyprotein, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [6, 7]. The positive-stranded RNA viral genome is packaged in a lipid bilayer [8]. The functions of the proteins are summarized in Table 1. The ORF is flanked by 5′ and the 3′ untranslated regions, which comprise the conserved cis-acting RNA secondary structural elements required for miRNAs translation and replication [9, 10].

### Glycosylation of DENV glycoproteins

DENV does not encode its own glycosyl transferase enzymes, so it requires the host cell to provide most or all of those necessary for the complete synthesis of the viral glycoproteins, including the glycosylation process. To date, N-glycosylation sites have been identified in only three of the 10 DENV proteins (prM/M, E, and NS1) [8]. The biosynthesis of these viral glycoproteins requires the transfer of a Glc3Man9GlcNAc2 oligosaccharide precursor to the budding protein, after which the terminal glucose residues on the core unit are trimmed [25]. This involves the sequential trimming of three glucose residues attached to the oligosaccharide, which is catalyzed by the endoplasmic reticulum (ER) α-glucosidase I and II enzymes [26]. α-Glucosidase I cleaves the outer α-1,2-linked glucose residue, whereas α-glucosidase II cleaves the two inner α-1,3-linked glucose residues, leaving the proteins monoglycosylated. This action allows the glycoproteins to bind to the ER chaperones calnexin and/or calreticulin to be properly folded downstream. The reglycosylation process is initiated on the incompletely folded proteins by UDP-glucosyltransferase 1, which acts as a sensor of correct protein folding. Once correctly folded, the proteins are then released from the re- and deglycosylation cycle and transported to the Golgi complex for further processing [27].

### Glycosylation of E, prM/M, and NS1 glycoproteins

The E glycoprotein monomer has three structural and functional domains. Domain I is the hinge region, which contains the amino terminus; domain II is responsible for the stabilization of the dimer and contains the fusion peptide; and domain III is an immunoglobulin (Ig)-like domain containing putative receptor-binding motifs [28, 29]. The glycoprotein has one or more potential N-linked glycosylation motifs in the form N–X–T/S, where X is any amino acid except proline [30]. Studies have shown that the numbers and locations of the motifs vary significantly among the DENV subtypes [31, 32]. Glycosylation motifs have commonly been observed in domain I at the residue asparagine 153 (N153) or N154, but the addition of a glycan to these residues is not an absolute requirement for infectivity. Although glycosylation is not essential at these residues, several studies have shown that mutations that occur at N-linked glycosylation sites in the E glycoprotein can affect virus-mediated membrane fusion and neurovirulence [33–35]. Glycosylation mutants have been described for glycoproteins E. Differences in amino acids were noted between the four wild-type DENV serotypes at amino acid 154, which is glutamic acid in both DENV 1 [36] and DENV 3 [37], and aspartic acid in both DENV 2 [38] and DENV 4 [39]. Another N-linked glycosylation motif of DENV occurs at N67, which mediates the viral infection of dendritic cells bearing DC-SIGN receptors and is essential for viral assembly and exit [40, 41]. Removal of

| Protein | Size     | Subunits | Functions                                                                 | References |
|---------|----------|----------|---------------------------------------------------------------------------|------------|
| C       | 100 amino acids | None     | Facilitates capsid assembly                                                | [11]       |
| prM/M   | 21 kDa/8 kDa  | None     | prM interacts with and stabilizes the E protein.                           | [12]       |
| E       | 53 kDa    | None     | Forms outer protein envelope                                              | [12, 13]   |
| NS1     | 48 kDa    | None     | Cleaves the NS1–NS2A junction                                              | [14–16]    |
| NS2     | 22 kDa    | NS2A, NS2B | NS2A plays a role in RNA synthesis and virion maturation                  | [7, 17]    |
|         |           |          | and as an activator of correct NS1 processing                            |            |
| NS3     | 618 amino acids | None     | Acts as a protease when combined with NS2B                                 | [18, 19]   |
| NS4     | 16 kDa    | NS4A, NS4B | Functions as an RNA helicase and RTPase/NTPase                            | [20–22]    |
| NS5     | 104 kDa   | None     | Essential for the replication and transcription of the viral genome       | [23, 24]   |
the N67 glycan has been shown to severely affect viral fitness and to reduce cell infectivity [42].

The glycosylation of the DENV prM/M glycoprotein has not been extensively studied. The prM protein is fused to the ectodomain of the E glycoprotein [43] with a glycosylation modification at asparagine N69. Other potential N-linked glycosylation sites have also been identified at residues 7, 31, and 52. The glycosylation of prM may allow it to act as a chaperone for E protein folding in the ER, permit its association with the membrane, and allow it to act in the assembly of the E protein [44].

The glycosylation of NS1 begins when the monomer is altered within the ER lumen by the addition of high-mannose-type glycans at both N-linked glycosylation sites and its subsequent rapid dimerization [45, 46]. Pryor and Wright [47] demonstrated that when secreted NS1 was treated with endoglycosidase H, complex glycans attached at N130 and high-mannose glycans at N207, so both N130 and N207 are N-linked glycosylation sites. Further alterations of the carbohydrate moieties in the NS1 dimer occur in the Golgi apparatus before the protein is transported to the cell surface and released from the infected cell. Interestingly, the presence of a polymannose-type sugar on the dimer may protect one of the N-glycans from further maturation in the Golgi, without which the stability of the dimer and the secretion of the glycoprotein would be reduced [48]. The glycosylation of NS1 is essential for viral processes ranging from replication to virulence, but it is unclear at this point how glycosylation affects these processes [15, 49].

Overview of glycans: virus–glycan interactions

Carbohydrate chains, also known as glycans, are one of the four basic components of cells. They are remarkably structurally diverse [50] and usually exist as covalent linkages with saccharides that are conjugated to proteins (glycoproteins) or to lipids (glycolipids) on the cell surface. The glycans of mammals are well conserved, although species-specific variations occur. Because of these variations, glycans that are involved in pathogen–receptor interactions may determine the susceptibility of specific organisms to infectious pathogens [51]. Different types of glycans can be produced during glycosylation depending on the type of residues that are attached to the cellular proteins or lipids. The glycosylation of proteins involves N-glycans, O-glycans, and glycosaminoglycans, frequently known as proteoglycans. N-glycans are formed when they bind to a specific subset of N residues in proteins, located in the N–X–S/T motif, whereas O-glycans attach to subsets of serine and threonine residues [52, 53]. The linear glycosaminoglycans are also serine- and threonine-linked but are usually highly sulfated [54]. Lipid glycosylation is also a common modification in the secretory pathway that produces glycolipids, also known as glycosphingolipids, which include the sialic-acid-bearing gangliosides [55].

The propagation of a virus and disease progression depend on the direct interactions between the virus and the host cell receptors. Different viruses may have preferences for differ glycan moieties for their attachment. These can be charged glycan moieties, such as sialic acid, which are readily recognized by orthoreoviruses [56], rotaviruses [57], and influenza viruses [58]; heparan sulfate, recognized by herpes viruses [59] and paroviruses [60]; or neutral glycans, such as histo-blood group antigens, which are bound by rotaviruses [61, 62] and noroviruses [63, 64]. The significant diversity in the recognition of specific glycans within a particular viral species, which arises from genetic differences, dictates the cell tropism, host specificity and adaptation, interspecies transmission, and pathogenesis of the virus.

Glycan interactions with DENV glycoproteins

Four major types of receptors in mammals are believed to be targeted by DENV. These are 1) carbohydrate molecules, 2) carbohydrate-binding proteins (also known as lectins), 3) factors related to protein folding, such as chaperones [65, 66] and heat shock proteins [67], and 4) other proteins, such as a high-affinity laminin receptor [68] and a CD14-associated protein [69]. However, in this review, we are focusing on molecules containing carbohydrate moieties (Table 2), so only carbohydrate and carbohydrate-binding protein receptors will be discussed.

Carbohydrate molecules as receptors

This group of receptors includes sulfated glycosaminoglycans (GAGs) and glycosphingolipids, which can act as coreceptor molecules, enhancing the efficiency of viral entry. Heparan sulfate is among the sulfated GAGs that are involved in the initial attachment of DENV to the cell surface, when the E glycoprotein binds to the molecule. Recently, Okamoto et al. [81] showed that a specific heparan sulfate proteoglycan, called syndecan-2, is a membrane heparan sulfate proteoglycan utilized by DENV as a receptor. Heparan sulfate is a linear repeating copolymer with variably sulfated uronic acid and glucosamine carbohydrate residues, and it is highly negatively charged [82]. It is found not only on the cell surface but also in the extracellular matrix [72].

Another type of carbohydrate molecule that has been reported recently is neolactotetraosylceramide (nLc4Cer), a glycosphingolipid with no sulfation. This carbohydrate
molecule may be a coreceptor of DENV and assist in the attachment of the virus to the host cell [73]. DENV interacts with this glycosphingolipid at the nonreducing terminus of Gal\(\beta\)1-4GlcNAc\(\beta\), which is expressed on the surface of susceptible cells, such as human erythroleukemia K562 and baby hamster kidney BHK-21 cells.

**Carbohydrate-binding proteins**

These proteins are commonly grouped with the C-lectins, which are expressed on dendritic cells and macrophages located under human skin. They are known to play an important role in the initial contact with DENV, after it is introduced by a mosquito bite. The best-characterized lectin involved in the interaction between the virus and dendritic cells is dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN). DC-SIGN is classified as a member of the calcium-dependent C-type lectin family and has affinity for the high-mannose glycans found on different pathogens, including Ebola virus [83], hepatitis C virus [84], human immunodeficiency virus (HIV) [85], and several parasites, yeasts, and bacteria [86]. The interaction between DC-SIGN and DENV occurs through the high-mannose N-linked glycans found on the E glycoprotein [40, 87]. This interaction has been confirmed in studies by Navarro-Sanchez et al. [70] and Tassaneetrithep et al. [71] in which both groups successfully inhibited DENV infection by introducing soluble DC-SIGN and antibodies directed against DC-SIGN. However, the internalization of DC-SIGN is not essential for DENV infection [88], so the lectin may not function as a specific receptor but may allow the attachment and concentration of the virus on the cell surface.

This phenomenon can be explained by studying the attachment of the E glycoprotein to the carbohydrate moiety of DC-SIGN. When the E glycoprotein binds to the carbohydrate-recognition domain of DC-SIGN, there is no conformational change in the E glycoprotein of the mature virus, even though such changes are observed when full-length DC-SIGN molecules bind to the glycoprotein. However, this binding leaves a single E glycoprotein in the asymmetric unit vacant, and the putative receptor-binding domain III present in each E glycoprotein is free to bind the receptor. It has been proposed that the interaction of carbohydrate moieties with DC-SIGN mimics normal cellular processes and therefore protects the receptor-binding domain of the E protein from the host’s immune surveillance and neutralization.

**Lectin receptors on macrophages**

Macrophages bear other C-type lectin receptors, the mannose receptor and C-type lectin domain family 5 member A (CLEC5A). The mannose receptor has been shown to bind DENV, Japanese encephalitis virus, and tick-borne meningoencephalitis virus by a mechanism similar to the mechanism of DC-SIGN binding. However, the ligand specificity of the mannose receptor differs from that of DC-SIGN in that its ligands are terminal mannose, fucose, and N-acetyl glucosamine rather than high-mannose oligosaccharides and fucosylated glycans. It has been hypothesized

---

**Table 2 Carbohydrate receptors targeted by DENV on mammalian and insect cells**

| Molecule                 | Type                  | Cell type                      | Serotype               | References |
|--------------------------|-----------------------|--------------------------------|------------------------|------------|
| DC-SIGN                  | C-type lectin         | Monocyte-derived dendritic cells | DENV 1, 2, 3, and 4    | [71]       |
| Heparan sulfate          | Glycosaminoglycans    | Vero                           | DENV 2                 | [72]       |
| nLa4Cer                  | Glycosphingolipid     | K562                           | DENV 2                 | [73, 74]   |
| Mannose receptor         | Protein               | NIH3T3                         | DENV 1, 2, 3, and 4    | [75]       |
| High-affinity laminin receptor | Protein               | HepG2                          | DENV 1, 2 and 3        | [68, 76]   |
| CLEC5A                   | C-type lectin         | Macrophages                    | DENV 1, 2, 3, and 4    | [77]       |
| L-3                      | Glycosphingolipid     | AP-61                          | DENV 2                 | [74]       |
| 40- and 45-kDa glycoproteins | Glycoprotein           | C6/36 cells                    | DENV 4                 | [78–80]    |
that the mannose receptor is more than just an attachment molecule for DENV because it is internalized into cells during infection and is found mainly in the endocytic pathway, unlike DC-SIGN, which is mainly restricted to the plasma membrane [75].

CLEC5A, also called myeloid DAPI2-associating lectin (MDL-1), is classified as a type II transmembrane receptor and is found on the surface of macrophages and monocytes [89]. The receptor contains a C-type lectin-like domain in its C-terminal extracellular region and has only four amino acids in its predicted N-terminal cytoplasmic region. The receptor is recognized as a potential DENV receptor. The binding of DENV to CLEC5A contributes significantly to the mortality associated with DENV infection by triggering excessive macrophage activation. Chen et al. [77] demonstrated that the survival rate in mice increased when CLEC5A was blocked. Both human and mouse CLEC5A are reported to bind DENV and the interaction is inhibited by mannose and fucose in vitro.

Viral glycosylation processes as therapeutic targets

Understanding the mechanisms that contribute to the invasion of host cells, as discussed above, may clarify how specific agents that interrupt or modify viral replication or expression can be used to limit the damage caused during infection. The synthesis of N-glycans may be interrupted at several stages by the inhibition of specific enzymes. In the rough ER, tunicamycin inhibits the transfer of the tri-mannose core to asparagine residues. The removal of terminal glucose residues may be blocked by nojirimycin or castanospermine [90], and the phosphatase-mediated conversion of the dolichylpyrophosphate released by glycosidic cleavage is inhibited by bacitracin. Brefeldin A inhibits the transport of the resultant high-mannose N-glycans to the Golgi apparatus, and further processing by mannosidase enzymes may be affected by deoxymannojirimycin or swainsonine [91, 92]. Deoxynojirimycin is an inhibitor of ER α-glucosidase and disrupts the trimming of

| Acronym | Source of lectin | Common name | Major specificity | Virus | References |
|---------|-----------------|-------------|------------------|-------|------------|
| HHA     | Hippeastrum hybrid | Amaryllis bulbs | α1,3-mannose and/or α1,6-mannose | SIV, HCV, DENV1–4 | [94–96] |
| GNA     | Galanthus nivalis | Snowdrop | High-mannose structures, multiple terminal mannose α1,3-mannose | HCV, HIV-1, DENV1–4 | [94, 96] |
| ConA    | Canavalia ensiformis | Jack bean | Terminal mannosyl residues | DENV2 | [97] |
| NTL     | Narcissus tazetta var. chinensis | Chinese daffodil | Similar to GNA | RSV, various strains of influenza virus A and B | [98, 99] |
| NPA     | Narcissus pseudonarcissus | Daffodil or Lent lily | α-D-mannose | HIV-1 | [100] |
| CA      | Cymbidium hybrid | Cymbidium (orchid) | Mannose-specific, α-mannose | HIV-1 and 2, CMV, influenza A, HCV | [94, 101] |
| EHA     | Epipactis helleborine | Broad-leaved helleborine | Mannose-specific, α-mannose | HIV-1 and 2, CMV, influenza A | [101] |
| TDL     | Typhonium divaricatum (L.) Decne | Rodent tuber | Mannose-specific | HSV-2 | [102] |
| SGM2    | Smilax glabra | Sarsaparilla | Mannose and/or mannan | HSV-1, RSV | [103] |
| PpL     | Parkia pendula | Acacia (male) | Terminal mannosyl residues, glucose | CMV, herpes virus 6 | [104] |
| PCL     | Polygonatum cyrtomena | Giant Chinese Solomon’s seal | Mannose and sialic acid | HIV-1 and HIV-2 | [105] |
| BanLec  | Musa acuminate | Banana | High-mannose structures | HIV-1 | [106] |
| WGA     | Triticum vulgaris | Wheatgerm | GlcNAc oligomers, N-acetyl lactosamine, some sialic acid residues | DENV2 | [107] |
| UDA     | Urtica dioica | Stinging nettle root | GlcNAc oligomers, Galβ1,4-GlcNAcβ1 | CMV, coronaviruses, SIV, DENV1–4 | [95, 96, 101, 107] |
| JFL     | Artocarpus heterophyllus | Jackfruit | N-acetyl-α-D-galactosamine | HSV-2, VZV, CMV | [108] |

SIV = simian immunodeficiency virus; CMV = cytomegalovirus; DENV = dengue virus; HIV = human immunodeficiency virus; RSV = respiratory syncytial virus; HSV = herpes simplex virus; VZV = varicella-zoster virus
terminal glucose, thus affecting the subsequent folding pathways of DENV glycoproteins prM and E [44, 93].

Targeting virus–carbohydrate receptor interactions with plant lectins

Plant lectins, also known as carbohydrate-binding agents (CBAs), have recently emerged as potent agents for treatment of DENV infection, targeting the initial attachment of the virus to cells. Several CBAs with unique properties and specificities (Table 3) have been identified and used to block viruses. Most of these lectins bind specifically to mannose moieties, ranging from terminal mannose residues to high-mannose glycans, which are targeted by a wide range of viruses [94–106].

The antiviral activities of these CBAs against DENV have recently been explored, particularly the lectins of *Hippeastrum* hybrid (HHA), *Galanthus nivalis* (GNA), and *Urtica dioica* (UDA), which were initially found to inhibit the interaction between HIV and DC-SIGN-expressing cells [100]. The same CBAs have also been shown to dose-dependently inhibit the interaction between all four serotypes of DENV and DC-SIGN in Raji/DC-SIGN+ cells and monocye-derived dendritic cells [96]. Binding assays have shown that these CBAs do not interact with cellular membrane proteins but instead interact directly with the viral glycosylated envelope proteins [109]. Concanavalin A (ConA) and wheat-germ agglutinin reduce the development of plaque induced by DENV in BHK cells [97]. A competition assay using mannose showed that the inhibitory effect of ConA results from its binding to α-mannose residues on the viral protein. However, these lectins do not completely inhibit DENV when introduced individually to infected cells. Therefore, further studies of the effects of combinations of CBAs are required.

Concluding remarks

The importance of DENV glycosylation and its interaction with carbohydrate receptors warrants further investigation to develop an efficient treatment for DENV-related diseases. Available drugs that disrupt protein glycosylation will result in the incomplete maturation of DENV. In cases of severe dengue infection, where DENV replication can be enhanced by an antibody-dependent enhancement mechanism [110], these drugs are anticipated to be effective in limiting viral replication, thus reducing the viral load, which may lessen the severity of the disease. The aforementioned drugs are designed to inhibit human glycosylation enzymes, but whether the same drugs can elicit similar effects on the glycosylation enzymes of DENV vectors must be explored further. To date, no studies have specifically explored the DENV protein glycosylation processes in mosquitoes. However, Mason [111] demonstrated the ability of mosquito cell lines to release the mature and glycosylated E glycoprotein and NS1 of Japanese encephalitis virus, another member of the family *Flaviviridae*. Therefore, it is predicted that DENV also undergoes glycosylation in the mosquito, and glycosylation inhibitors are anticipated to be effective to some degree.

Although drugs that disturb the glycosylation process during the formation of viral glycoproteins may seem useful in this context, the same drugs may also disturb the glycosylation of cellular glycoproteins. However, CBAs or lectins act directly on the viral interaction at the cell surface and need not fuse with the cell to exert their antiviral activities. Therefore, it is anticipated that CBAs will not interfere with the synthesis of glycans on cell-surface glycoproteins. The potential utility of lectins as antiviral agents seems promising. The antiviral potency of lectins against each virus may differ because their three-dimensional conformations differ, or because the availability of glycan conformations with the proper carbohydrate moieties differs across viral proteins. Several challenges must still be addressed, such as the high cost of the purification and mass production of lectins, their storage and stability, bioavailability, and cellular toxicity. However, these issues may be resolved by designing synthetic CBAs that are structurally similar to the natural molecules but stable and nontoxic. Several synthetic molecules are already available [112, 113], providing a basis for the further exploration of potentially therapeautic CBAs. Drug delivery using especially designed capsules or nanocarriers may also be a useful strategy to combat bioavailability problems. Based on these factors, CBAs should be considered a valuable class of antiviral agents that warrants further investigation and eventual application in the clinical setting.

Acknowledgments This work is part of the Neuroinfectious Diseases in Brunei Darussalam Region project UBD/BRC (1), funded by the Brunei Research Council, Department of Economic Planning and Development, Brunei Darussalam, and supported by Universiti Brunei Darussalam. F. Idris is a recipient of a Graduate Research Scholarship, Universiti Brunei Darussalam.

Funding This work was funded by the Brunei Research Council, Department of Economic Planning and Development, Brunei Darussalam (UBD/BRC(1)).

Conflicts of interest Idris F declares that he has no conflict of interest. Muharram SH declares that she has no conflict of interest. Diah S declares that she has no conflict of interest.

Ethical approval This study contained no experiments involving human participants or animals.
References

1. Gubler DJ (2002) Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol 10:100–103

2. Oishi K, Saito M, Mapua CA, Natividad FF (2007) Dengue illness: clinical features and pathogenesis. J Infect Chemother 13:125–133

3. Halstead SB (2003) Neutralization and antibody-dependent enhancement of dengue viruses. Adv Virus Res 60:421–467

4. Dejnirattisai W, Jumnainsong A, Tellez Y, Zody MC, Saborio S, Nunez A, Lennon NJ, Birren BW, Gordon A, Henn MR, Harris E (2011) Dynamics of dengue disease severity determined by the interplay between viral genetics and serotype-specific immunity. Sci Transl Med 3:114ra128

5. OhAinle M, Balmaseda A, Macalalad AR, Tellez Y, Zody MC, Saborio S, Nunez A, Lennon NJ, Birren BW, Gordon A, Henn MR, Harris E (2010) The medical chemistry of dengue fever. J Med Chem 52:7911–7926

6. Lindenbach BD, Rich CM (2001) Flaviviridae: the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (eds) Fields virology, 4th edn. Lippincott Williams & Wilkins, Philadelphia, pp 991–1041

7. Holden KL, Harris E (2004) Enhancement of dengue virus translation: role of the 3′ untranslated region and the terminal 3′ stem-loop domain. Virology 329:119–133

8. Chiu WW, Kinney RM, Dreher TW (2005) Control of translation by the 5′- and 3′-terminal regions of the dengue virus genome. J Virol 79:8303–8315

9. Ma L, Jones CT, Groesch TD, Kuhn RJ, Post CB (2004) Solution structure of dengue virus capsid protein reveals another fold. Proc Natl Acad Sci USA 101:3414–3419

10. Zhang W, Chipman PR, Corver J, Johnson PR, Zhang Y, Mukhopadhyay S, Baker TS, Strauss JH, Rossman MG, Kuhn RJ (2003) Visualization of membrane protein domains by cryoelectron microscopy of dengue virus. Nat Struct Biol 10:907–912

11. Kuhn RJ, Zhang W, Rossman MG, Pletnev SV, Corver J, Lenches E, Jones CT, Mukhopadhyay S, Chipman PR, Strauss EG, Baker TS, Strauss JH (2002) Structure of dengue virus. Implications for flavivirus organization, maturation and fusion. Cell 108:717–725

12. Falgout B, Markoff L (1995) Evidence that flavivirus NS1- and 3′-terminal regions of the dengue virus genome RNA do not contain the replicase function. J Virol 73:4611–4621

13. Bazan JF, Fletterick RJ (1989) Detection of a trypsin-like serine protease domain in flaviviruses and pestiviruses. Virology 171:637–639

14. Gorbolena AE, Donchenko AP, Koonin EV, Blinov VM (1989) N-terminal domains of putative helicases of flavi- and pestiviruses may be serine proteases. Nucleic Acids Res 17:3889–3897

15. Lindenbach BD, Rice CM (1999) Genetic interaction of flavivirus non-structural proteins NS1 and NS4A as a determinant of replicase function. J Virol 73:4611–4621

16. McMahon HT, Gallop JL (2005) Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 438:590–596

17. Jao CC, Hedge BG, Gallop JL, Hegde PB, McMahan HT, Haworth IS, Langen R (2010) Roles of amphipathic helices and the bin/amphiphysin/rvs (BAR) domain of endophilin in membrane curvature generation. J Biol Chem 285:20164–20170

18. Egloff MP, Benaroch D, Selisko B, Romette JL, Canard B (2002) An RNA cap (nucleoside-2′-O-)methyltransferase in the flavivirus RNA polymerase NS5: crystal structure and functional characterization. EMBO J 21:2757–2768

19. Selisko B, Dutartre H, Guillemot JC, Debarnot C, Benaroch D, Khromykh A, Despres P, Egloff MP, Canard B (2006) Comparative mechanistic studies of de novo RNA synthesis by flavivirus RNA-dependent RNA polymerases. Virology 351:145–158

20. Ruddock LW, Molnari M (2006) N-glycan processing in ER quality control. J Cell Sci 119:4373–4380

21. Hebert DN, Foelmer B, Helenius A (1995) Glucose trimming and reglucosylation determine glycoprotein association with calnexin in the endoplasmic reticulum. Cell 81:425–433

22. Hammond C, Braakman I, Helenius A (1994) Role of N-linked, glucose trimming, and calnexin in glycoprotein folding and quality control. Proc Natl Acad Sci USA 91:913–917

23. Modis Y, Ogata S, Clements D, Harrison SC (2003) A ligand-binding pocket in the dengue virus envelope glycoprotein. Proc Natl Acad Sci USA 100:6986–6991

24. Ishak H, Takegami T, Kamimura K, Funada H (2001) Comparative sequences of two type 1 dengue virus strains possessing different growth characteristics in vitro. Microbiol Immunol 45:327–331

25. Gavel Y, von Heijne G (1990) Sequence differences between glycosylated and non-glycosylated Asn-X-Thr/Ser acceptor sites: implications for protein engineering. Protein Eng 3:433–442

26. Johnson AJ, Guirakhoo F, Roehrig JT (1994) The envelope glycoproteins of dengue 1 and dengue 2 viruses grown in mosquito cells differ in their utilization of potential glycosylation sites. Virology 203:241–249

27. Ishak H, Takegami T, Kamimura K, Funada H (2001) Comparative sequences of two type 1 dengue virus strains possessing different growth characteristics in vitro. Microbiol Immunol 45:327–331

28. Guirakhoo F, Hunt AR, Lewis JG, Roehrig JT (1993) Selection and partial characterization of dengue 2 virus mutants that induce fusion at elevated pH. Virology 194:219–223

29. Kawano H, Rostapshov V, Rosen L, Lai CJ (1993) Genetic determinants of dengue type 4 virus neurovirulence for mice. J Virol 67:6567–6575

30. Sanchez IJ, Ruiz BH (1996) A single nucleotide change in the E protein gene of dengue virus 2 Mexican strain affects neurovirulence in mice. J Gen Virol 77:2541–2545

31. Chu MC, O’Rourke EJ, Trent DW (1989) Genetic relatedness among structural protein genes of dengue 1 virus strains. J Gen Virol 70:1701–1712

32. Osatomi K, Sumiyoshi H (1990) Complete nucleotide sequence of dengue type 3 virus genome RNA. Virology 176:643–647
38. Deubel V, Kinney RM, Trent DW (1988) Nucleotide sequence and deduced amino-acid sequence of the nonstructural proteins of dengue type 2 virus, Jamaica genotype: comparative analysis of the full-length genome. Virology 165:234–244

39. Zhao B, Mackow E, Buckler-White A, Markoff L, Chanock RM, Lai CJ, Makino Y (1986) Cloning full length dengue type 4 viral DNA sequences: analysis of genes coding for structural proteins. Virology 156:77–88

40. Pokidysheva E, Zhang Y, Battistij AE, Bator-Kelly CM, Chipman PR, Xiao C, Gregio GG, Hendrickson WA, Kuhn RJ, Rossmann MG (2006) Cryo-EM reconstruction of dengue virus in complex with the carbohydrate recognition domain of DC-SIGN. Cell 124:485–493

41. Mindt J, Lazzarini A, Amara A, Gammarn AV (2007) Essential role of dengue virus envelope protein N glycosylation at asparagine-67 during viral propagation. J Virol 81:7136–7148

42. Bryant JE, Calvert AE, Mesesan K, Crabtree MB, Volet KE, Silengo S, Kinney RM, Huang CYH, Miller BR, Roehrig JT (1998) Glycosylation of the dengue 2 virus E protein at N67 is critical for virus growth in vitro but not for growth in intrathoracically inoculated Aedes aegypti mosquitoes. Virology 366:415–423

43. Li L, Lok SM, Yu IM, Zhang Y, Kuhn R, Chen J, Rossmann MG (2008) The flavivirus precursor membrane-envelope protein complex: structure and maturation. Science 319:1830–1834

44. Courageot MP, Frenkiel MP, Dos Santos CD, Deubel V, Despres P (2000) α-Glucosidase inhibitors reduce dengue virus production by affecting the initial steps of virion morphogenesis in the endoplasmic reticulum. J Virol 74:564–572

45. Winkler G, Randolph VB, Cleaves GR, Ryan TE, Stollar V (1977) Evidence that the mature form of the flavivirus nonstructural protein NS1 is a dimer. Virology 162:187–196

46. Pryor MJ, Wright PJ (1993) The effects of site-directed mutagenesis on the dimerization and secretion of the NS1 protein specified by dengue virus. Virology 194:769–780

47. Pryor MJ, Wright PJ (1994) Glycosylation mutants of dengue virus NS1 protein. J Gen Virol 75:1183–1187

48. Flamand M, Megret F, Mathieu M, Lepautre J, Rey FA, Deubel V (1999) Dengue virus type 1 nonstructural glycoprotein NS1 is secreted from mammalian cells as a soluble hexamer in a glycosylation-dependent fashion. J Virol 73:6104–6110

49. Sommure P, Hauhart RE, Atkinson JP, Diamond MS, Avirutnam P, Hsu IC, Deng C, Lai CJ, Makino Y (1992) A link between dengue type 2 virus NS1 protein and modulates secretion, cell-surface expression, hexamer stability, and interactions with human complement. Virology 413:253–264

50. Ohsubo K, Marth JD (2006) Glycosylation in cellular mechanisms of health and disease. Cell 126:855–867

51. Gagneux P, Varki A (1999) Evolutionary considerations in relating oligosaccharide diversity to biological function. Glycobiology 9:747–755

52. Schachter H (2000) The joys of HexNAc. The synthesis and function of N- and O-glycan branches. Glycobiol 10:465–483

53. Yan A, Lennarz WJ (2005) Unravelling the mechanism of fucose incorporation into N-linked oligosaccharides. J Biol Chem 280:3121–3124

54. Esko JD, Selleck SB (2002) Order out of chaos: assembly of ligand binding sites in heparan sulfate. Annu Rev Biochem 71:435–471

55. Maccioni HJ, Giraud CG, Danniotti JL (2002) Understanding the stepwise synthesis of glycolipids. Neurochem Res 27:629–636

56. Reiter DM, Frierson JM, Halvorson EE, Kobayashi T, Demody TS, Stehle T (2011) Crystal structure of reovirus attachment protein σ1 in complex with sialylated oligosaccharides. PLoS Pathog 7:e1001216

57. Dormitzer PR, Sun ZY, Wagner G, Harrison SC (2002) The rhesus rotavirus VP4 sialic acid binding domain has a galectin fold with a novel carbohydrate binding site. EMBO J 21:885–897

58. Connor RJ, Kawaoka Y, Webster RG, Paulson JC (1994) Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. Virology 205:17–23

59. Shieh MT, Wu Dunn D, Montgomery RJ, Esko JD, Spear PG (1992) Cell surface receptors for herpes simplex virus are heparan sulfate proteoglycans. J Cell Biol 116:1273–1281

60. Hueffer K, Parrish CR (2003) Parvovirus host range, cell tropism and evolution. Curr Opin Microbiol 6:392–398

61. Huang P, Xia M, Tan M, Zhong W, Wei C, Wang L, Morrow A, Jiang X (2012) Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. J Virol 86:4833–4843

62. Hu L, Crawford SE, Czako R, Cortes-Penfield NW, Smith DF, Le Pendu J, Estes MK, Prasad BV (2012) Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. Nature 485:256–259

63. Marionneau S, Ruvoen N, Le Mouillac-Vaidye B, Clement M, Cailleau-Thomas A, Ruiz-Palacios G, Huang P, Jiang X, Le Pendu J (2002) Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. Gastroenterology 122:1967–1977

64. Huang P, Farkas T, Marionneau S, Zhong W, Ruvoen-Clouet N, Morrow AL, Altaye M, Pickering K, Newburg DS, Le Pendu J, Jiang X (2003) Noroviruses bind to human ABO, Lewis, and secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. J Infect Dis 188:19–31

65. Cabrera-Hernandez A, Thepparit C, Suksanpaisan L, Smith DR (2007) Dengue virus entry into liver (HepG2) cells is independent of hsp90 and hsp70. J Med Virol 79:386–392

66. Upanan S, Kuadkitkan A, Smith DR (2008) Identification of dengue virus binding proteins using affinity chromatography. J Virol Methods 151:325–328

67. Reyes-Del Valle J, Chavez-Salinas S, Medina F, Del Angel RM (2005) Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. J Virol 79:4557–4567

68. Tio PH, Jong WW, Cardosa MJ (2005) Two dimensional VOPBA reveals laminin receptor (LAMR1) interaction with dengue virus serotypes 1, 2 and 3. J Virol 80:1223–1227

69. Chen YC, Wang SY, King CC (1999) Bacterial lipopolysaccharide inhibits dengue virus infection in primary human monocytes/macrophages by blockade of virus entry via a CD14-dependent mechanism. J Virol 73:2650–2657

70. Navarro-Sanchez E, Altmeyer R, Amara A, Schwartz O, Fieschi F, Vireliez JL, Arenzana-Seisdedos F, Despres P (2003) Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. EMBO Rep 4:723–728

71. Tassaneinprithhe B, Burgess TH, Granelli-Piperno A, Trumpfheller C, Finke J, Sun W, Eller MA, Pattanapanyasat K, Sarabomth S, Birx DL, Steinman RM, Schlesinger S, Marovich MA (2003) DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med 197:823–829

72. Chen Y, Maguire T, Hileman RE, Fromm JR, Esko JD, Linhardt RJ, Marks RM (1997) Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. Nat Med 3:866–871

73. Aoki C, Hidari KIPJ, Itonari S, Yamada A, Takahashi N, Kasama T, Hasebe F, Islam MA, Hatano K, Matsuoka K, Taki T, Guo CT, Takahashi T, Sakano Y, Suzuki T, Miyamoto D, Sugita M, Terunuma D, Morita K, Suzuki Y (2006) Identification and characterization of carbohydrate molecules in mammalian cells recognized by dengue virus type 2. J Biochem 139:607–614
Dengue virus glycoprotein interactions with receptors

74. Wichit S, Jittmittraphap A, Hidari KL, Thaisomboonsuk B, Petmitr S, Aoki C, Itanon S, Morita K, Suzuki T, Suzuki Y, Jampangner W (2011) Dengue virus type 2 recognizes the carbohydrate moiety of neutral glycosphingolipids in mammalian and mosquito cells. Microb. Immunol. 55:135–140

75. Miller JL, Dewet BJ, Martinez-Pomares L, Radcliffe CM, Dwek RA, Rudd PM, Gordon S (2008) The mannose receptor mediates dengue virus infection of macrophages. PLoS Pathog. 4:e17

76. Thepparit C, Smith DR (2004) Serotype-specific entry of dengue virus into liver cells: identification of the 37-kilodalton/67-kilodalton high-affinity laminin receptor as a dengue virus serotype 1 receptor. J. Virol. 78:12647–12656

77. Chen ST, Lin YL, Huang MT, Wu MF, Cheng SC, Lei HY, Lee CK, Chiou TW, Wong CH, Hsieh SL (2008) CLEC5A is critical for dengue-virus-induced lethal disease. Nature 453:672–676

78. Salas-Benito JS, del Angel RM (1997) Identification of two surface proteins from C6/36 cells that bind dengue type 4 virus. J. Virol. 71:7746–7752

79. Yazi Mendoza M, Salas-Benito JS, Lanz-Mendoza H, Hernández-Martínez S, del Angel RM (2002) A putative receptor for dengue virus in mosquito tissues: localization of a 45-kDa glycoprotein. Am. J. Trop. Med. Hyg. 67:76–84

80. Reyes-del Valle J, del Angel RM (2004) Isolation of putative dengue virus receptor molecules by affinity chromatography using a recombinant B protein ligand. J. Virol. Methods 116:95–102

81. Okamoto K, Kinoshita H, Parquet Mdel C, Raekiansyah M, Kimura D, Yui K, Islam MA, Hasebe F, Morita K (2012) Dengue virus strain DEN2 16681 utilizes a specific glycochain of syndecan-2 proteoglycan receptor. J. Gen. Virol. 93:761–770

82. Griffin CC, Linhardt RJ, Van Gorp CL, Toida T, Hileman RE, Griffin CC, Linhardt RJ, Van Gorp CL, Toida T, Hileman RE, Po¨hlmann S, Zhang J, Baribaud F, Chen Z, Leslie GJ, Lin G, Schubert RL, Brown SE (1995) Isolation and characterization of hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGN. J. Virology 69:4070–4080

83. Marzi A, Möller P, Hanna SL, Rudd PM, Gordon S (2008) The mannose receptor mediates trans-infection of T cells. Cell 100:587–597

84. Po¨hlmann S, Zhang J, Baribaud F, Chen Z, Leslie GJ, Lin G, Granelli-Piperno A, Dom, RW, Rice CM, McKeating JA (2003) DC-SIGN, a dendritic cell-specific HIV-1 binding protein that enhances trans-infection of T cells. Cell 100:587–597

85. Alen MMF, Kapteijn SJF, De Burghgraeve T, Balzarini J, Neys J, Schols D (2009) Antiviral activity of carbohydrates-binding agents and the role of DC-SIGN in dengue virus infection. Virology 387:67–75

86. Bakker AB, Baker E, Sutherland GR, Phillips JH, Lanier LL (1999) Myeloid DAP12-associating lectin (MLD)-1 is a cell surface receptor involved in the activation of myeloid cells. Proc. Natl. Acad. Sci. USA 96:9792–9796

87. Elbein AD (1991) Glycosidase inhibitors: inhibitors of N-linked oligosaccharide processing. FASEB J 5:3055–3063

88. Cenci di Bello I, Fleet G, Namgung SK, Talano K, Winchester B (1989) Structure-activity relationship of swainsonine. Inhibition of human α-mannosidases by swainsonine analogues. Biochem. J. 259:855–861

89. Bertaux C, Daulemans D, Meertens L, Cormier EG, Reimus JF, Peumans WJ, Van Damme EJM, Igashiri Y, Oki T, Schols D, Dragic T, Balzarini J (2007) Entry of hepatitis C virus and human immunodeficiency virus is selectively inhibited by carbohydrate-binding agents but not by polyanions. Virology 366:40–50

90. François KO, Auwerx J, Schols D, Balzarini J (2008) Simian immunodeficiency virus is susceptible to inhibition by carbohydrate-binding agents in a manner similar to that of HIV: implications for further preclinical drug development. Mol. Pharmacol. 74:330–337

91. Wu SF, Lee CJ, Liao CL, Dwek RA, Zitzmann N, Lin YL (2002) Anti viral effects of an iminosugar derivative on flavivirus infections. J. Virol. 76:3596–3604

92. Kimura D, Yui K, Islam MA, Hasebe F, Morita K (2012) Dengue virus receptor molecules by affinity chromatography using a recombinant B protein ligand. J. Virol. Methods 116:95–102

93. Wu SF, Lee CJ, Liao CL, Dwek RA, Zitzmann N, Lin YL (2002) Antiviral effects of an iminosugar derivative on flavivirus infections. J. Virol. 76:3596–3604

94. Bakker AB, Baker E, Sutherland GR, Phillips JH, Lanier LL (1999) Myeloid DAP12-associating lectin (MLD)-1 is a cell surface receptor involved in the activation of myeloid cells. Proc. Natl. Acad. Sci. USA 96:9792–9796

95. Elbein AD (1991) Glycosidase inhibitors: inhibitors of N-linked oligosaccharide processing. FASEB J 5:3055–3063

96. Po¨hlmann S, Zhang J, Baribaud F, Chen Z, Leslie GJ, Lin G, Granelli-Piperno A, Dom, RW, Rice CM, McKeating JA (2003) DC-SIGN, a dendritic cell-specific HIV-1 binding protein that enhances trans-infection of T cells. Cell 100:587–597

97. van Kooyk Geijtenbeek TB (2003) DC-SIGN: escape mechanism for pathogens. Nat Rev Immunol. 3:697–709

98. Hacker K, White L, de Silva AM (2009) N-linked glycans on dengue viruses grown in mammalian and insect cells. J Gen Virol. 90:2097–2106

99. Lozach PY, Burlegh L, Staropoli I, Navarro-Sanchez E, Harriague J, Virélier JL, Rey FA, Després P, Arentsana-Seisedo F, Amara A (2005) Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. J Biol Chem 280:23698–23708

100. Bakker AB, Baker E, Sutherland GR, Phillips JH, Lanier LL (1999) Myeloid DAP12-associating lectin (MLD)-1 is a cell surface receptor involved in the activation of myeloid cells. Proc Natl Acad Sci USA 96:9792–9796
106. Swanson MD, Winter HC, Goldstein II, Markovitz DM (2010) A lectin isolated from bananas is a potent inhibitor of HIV replication. J Biol Chem 285:8646–8655
107. Van der Meer FJUM, de Haan CAM, Schuurman NMP, Hajjema BJ, Verheije MH, Bosch BJ, Balzarini J, Egberink HF (2007) The carbohydrate-binding plant lectins and the non-peptidic antibiotic pradimicin A target the glycans of the coronavirus envelope glycoproteins. J Antimicrob Chemother 60:741–749
108. Wetprasit N, Threesangsri W, Klamklai N, Chulavatnatol M (2000) Jackfruit lectin: properties of mitogenicity and the inhibition of herpesvirus infection. Jpn J Infect Dis 53:156–161
109. Alen MMF, De Burghgraeve T, Kaptein SJF, Balzarini J, Neyts J, Schols D (2011) Broad antiviral activity of carbohydrate-binding agents against the four serotypes of dengue virus in monocyte-derived dendritic cells. PLoS One 6:e21658
110. Guzman MG, Alvarez M, Halstead SB (2013) Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. Arch Virol 158:1445–1459
111. Mason P (1989) Maturation of Japanese encephalitis virus glycoproteins produced by infected mammalian and mosquito cells. Virology 169:354–364
112. Striegler S, Dittel M (2003) A sugar discriminating binuclear copper (II) complex. J Am Chem Soc 125:11518–11524
113. Mazik M, Cavqa H, Jones PG (2005) Molecular recognition of carbohydrates with artificial receptors: mimicking the binding motifs found in the crystal structures of protein–carbohydrate complexes. J Am Chem Soc 127:9045–9052