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Modulation of Cellular Tropism and Innate Antiviral Response by Viral Glycans

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Arbovirus · Dendritic cells · Immunity · Glycan · Glycosylation · Lectin · Viral attachment/entry · Virus-host cell interactions

Abstract
Arthropod-borne viruses (arboviruses) are a significant cause of human and animal disease worldwide. Multiple interactions between virus and the host innate immune system ultimately determine the pathogenesis and clinical outcome of the infection. Evidence is rapidly emerging that suggests viral glycans play a key role in viral pathogenesis by regulating host cell tropism and interactions with the host innate immune response. Glycan-mediated interactions are especially important for arboviruses which must adapt to variable glycosylation systems and cellular receptors within both vertebrate and invertebrate hosts. This review focuses on emerging evidence which supports a crucial role for viral glycans in mediating host cell tropism and regulating the innate antiviral response.

Introduction

Arthropod-borne viruses (arboviruses) are responsible for a large number of diseases worldwide. Infections with arboviruses such as Rift Valley fever virus, West Nile virus (WNV), Dengue virus (DEN), Ross River virus (RRV), Venezuelan equine encephalitis virus and chikungunya virus represent significant public health and economic burdens, especially in developing areas where these diseases are most prevalent. There are more than 500 known arboviruses and approximately 100 of them are known to cause human disease. During the past 20 years many factors have converged to cause a dramatic resurgence or emergence of epidemic arboviral diseases affecting both humans and domestic animals. Some of these factors include demographics, social changes, urban sprawl, changes in agricultural practices, genetic changes in pathogens and global climate changes.

To successfully develop prophylactic and therapeutic interventions to lessen the toll on human and animal health, key interactions between these viruses, their invertebrate vectors and their vertebrate hosts must be understood. Pathogenic viruses interface with a susceptible host at many points including viral entry, pathogen recognition by the host and engagement of effector molecules of the innate and adaptive immune systems. Glycan components of enveloped viruses have been shown to facilitate many of these pathogen-host interactions, making viral glycan-mediated interactions rational targets for therapeutic intervention. This review will provide a comprehensive overview of glycan-mediated interactions between arboviruses and their mammalian hosts.

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Glycosylation in Vertebrate and Invertebrate Hosts

Glycosylation is the enzymatic process by which saccharides are covalently linked to proteins and lipids co- and post-translationally. Glycosylation serves to increase protein diversity and function and this is particularly important for viruses, where different glycosylation states can increase the functional diversity of proteins encoded within a relatively 'simple' genome. Three types of glycosylation have been described thus far: N-linked, O-linked and C-linked. The most common form in viruses is N-linked glycosylation, where a high mannose core is attached to the amide nitrogen of asparagine within the conserved motif Asn-X-Ser/Thr. N-linked glycosylation of viral envelope proteins allows for proper folding and intracellular trafficking which facilitates efficient virion production and release. Viral N-linked glycans also interact with cellular receptors thus increasing viral infectivity and/or altering viral recognition by host immune cells. While both mammalian and insect cells produce N-linked glycoproteins, there are fundamental differences in the processing pathways, and these differences may be particularly important for arboviruses, which must successfully replicate within and make the transition between vertebrate and invertebrate hosts. Differences in glycosylation processes between vertebrate and invertebrate systems are briefly discussed below, and the reader is directed to the following excellent articles for more in-depth information [1, 2].

Mammalian Cell Glycosylation

In mammalian cells, attachment of the mannose occurs co-translationally followed by extensive trimming and remodeling that culminates in transit through the endoplasmic reticulum and Golgi. Initially the glycan chains have high mannose content and are referred to as 'high mannose' or 'simple' glycans. In the remodeling phase, different terminal monosaccharides are added to the mannose chain such that the overall effect is the production of glycans which exhibit a high degree of complexity, which are thereby termed 'complex' glycans. Another mammalian form, the hybrid glycan, occurs as an intermediate between simple and complex glycan end products. Furthermore, high mannose glycans can also be found on mature proteins, likely due to protein folding which shields high mannose glycans during protein transit through the endoplasmic reticulum/Golgi. This prevents glycan processing and allows high mannose chains to emerge as end product glycans [2]. Finally, the ability to add multiple glycan branches to a single core structure further increases the heterogeneity of mammalian complex glycans.

Insect Cell Glycosylation

Early studies revealed differences in both structure and function of viral N-linked glycans produced by mosquito versus mammalian cell lines. In general, glycans produced by mosquito cells are far less complex than mammalian-cell-derived viral glycans. Within insect cells, homologues of mammalian enzymes involved in trimming of N-linked glycans were present while only a few enzymes involved in remodeling or elongation were identified [2, 3]. Thus, the predominant viral glycoproteins produced in insect cells are high-mannose or paucimannose.

Mammalian Lectins

Lectins are glycan-binding proteins which bind specific glycan moieties via one or more carbohydrate-recognition domains. It has become evident that lectins play a role in key processes of the host innate immune response to pathogens: (1) pathogen recognition and internalization by antigen presenting cells; (2) initiating antigen presenting cell differentiation and maturation, and (3) interacting with Toll-like receptors (TLRs) to initiate pathogen-specific DC differentiation and immune responses via cytokine expression. There are 3 lectin receptor families involved in glycan recognition: galectins, sialic acid-binding immunoglobulin-like lectins (siglecs), and C-type lectin receptors (CLRs). For the purposes of this review only CLRs, glycan-binding receptors that act in a calcium-dependent manner, will be discussed. Many CLRs are expressed on antigen-presenting cells, such as macrophages and DCs, and some have also been identified on NK or endothelial cells [4–6]. Several key CLRs expressed on DCs, such as the mannose receptor, DEC205, and dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) are involved in glycan-mediated pathogen recognition and internalization of antigen for loading on MHC class II molecules [4]. Soluble lectins such as the mannose-binding lectin bind glycans on the surface of pathogens leading to complement activation or direct pathogen opsonization [7]. Furthermore, there are several examples of CLRs either modifying or suppressing TLR-mediated signals. The HIV gp120 protein has been shown to induce IL-10 production and prevent DC maturation in a glycan-dependent manner [8]. Ligation of DC-SIGN by the mycobacterial cell wall component Man-

Table 1. Arboviral glycan function and interaction with host lectins

Virus	Role for viral glycans	Host lectin	Ref.
Dengue	Attachment/infectivity	DC-SIGN (DCs)	17, 18, 52
	Blocks DC maturation	CLEC5A	33
	Specifically stimulates pro-inflammatory cytokines without altering INF-α production		34
Dengue[1]	Attachment/infectivity	MR (macs)	19
West Nile virus	Attachment/infectivity	DC-SIGN	20, 21, 53
	Evasion of DC maturation		
	Neuroinvasion		
West Nile virus[1]	Engages lectin complement pathway	Unknown	46
	Reduces IFN-α in plasmacytoid DCs		37
	Blocks dsRNA-induced type I IFN and proinflammatory cytokines		36
Alphaviruses (Sindbis, RRV, VEE, EEE, WEE)[1]	Attachment	DC-SIGN L-SIGN	14, 24
Alphaviruses (RRV, VEE, and Barmah Forest virus)	Mammalian glycans increase type I IFN, mosquito glycans increase infectivity	Unknown	38, 39
Sindbis[2]	Decrease complement activation via alternative pathway		45

dsRNA = Double-stranded RNA; MR = mannose receptor; EEE= eastern equine encephalitis; VEE = Venezuelan equine encephalitis; WEE = western equine encephalitis.

[1] Phenotype specific to virus propagated in mosquito cells. [2] Phenotype specific to virus propagated in mammalian cells.

LAM alters lipopolysaccharide (LPS) mediated TLR signaling [9]. Furthermore, ligation of the mannose receptor has been shown to inhibit pro-inflammatory cytokine induction, while signaling through BDCA-2, a CLR expressed on plasmacytoid dendritic cells, inhibits TLR-induced type I IFN induction [10, 11].

Arbovirus Glycan-Mediated Cell Tropism, Attachment and Entry

The interaction of virus with its host cell receptor is a critical factor in determining host and tissue tropism. Since arboviruses transmit between arthropods and vertebrates in nature, these viruses must either adapt to receptors conserved in both hosts or diversify to interact with multiple receptors in different hosts. Many arboviruses target DCs for viral replication after transmission from the mosquito vector [12–14]. In particular, immature DCs express a large variety of CLRs which can function as antigen uptake receptors, including DC-SIGN and mannose receptor. In addition to their role as antigen receptors, CLRs that facilitate productive infection by increasing the efficiency of virus binding, but whose presence is not absolutely required for viral entry, are often referred to as attachment factors [15]. DC-SIGN represents a common attachment factor for multiple viral pathogens, including HIV, Ebola, and arboviruses within the *Flaviviridae* and *Togaviridae* [13–15].

DCs and macrophages are the initial targets of DEN infections following delivery by the mosquito vector [12, 16]. DC-SIGN serves as an entry receptor for DEN and 2 putative glycosylation sites (Asn-67 and Asn-153) are required for full viral infectivity [17, 18]. DEN viruses lacking Asn-67 were able to infect mammalian cells and translate and replicate the viral genome but were unable to produce infectious particles. Further, loss of the glycan at position 67 reduced infection of immature DCs, suggesting interaction between this glycan and DC-SIGN. Viruses lacking Asn-153 were also impaired for infectivity of mammalian cells lines. In contrast, loss of one or both glycosylation sites had no effect on replication and propagation of viruses in mosquito cells. Further, mosquito cell-produced DEN but not mammalian cell de-

rived DEN virus binds the mannose receptor on macrophages and initiates a productive infection [19].

The E protein of WNV plays an important role in viral replication and maturation, receptor binding, membrane fusion and virus assembly. Strains of WNV are divided into two lineages (L1 and L2) which differ in their virulence such that L1 strains are more frequently associated with severe disease in humans, including more severe neurological disease. While most L1 strains have a conserved N-linked glycosylation site on the E protein, this motif is lacking in most L2 strains. Recently it was shown that glycosylated WNV infected DC-SIGN expressing DCs more efficiently and resulted in increased production of pro-inflammatory cytokines compared to non-DC-SIGN expressing cells [20]. It was suggested that the increased pathogenicity of L1 strains is linked to their ability to engage DC-SIGN which enhances viral infection and increases tissue damage mediated by increased pro-inflammatory cytokines. In support of this, naturally occurring variants of WNV with different amino acid sequence changes at an N-linked glycosylation site in the E protein were assessed for pathogenicity in a mouse model [21]. Mice infected subcutaneously with viruses displaying a glycosylated E protein developed lethal infections while nonglycosylated viruses produced minimal mortality. In contrast, all mice inoculated intracerebrally with the differentially glycosylated forms of WNV succumbed rapidly to the infection. Thus, glycosylation of the WNV E protein appears to be a determinant of neuroinvasiveness; however, it remains to be determined exactly which murine lectin(s) contribute to this process and whether this reflects a role for the E protein in viral binding/entry, modulation of the innate immune response, or avoidance of innate immune effector pathways such as the complement cascade.

In addition to DC-SIGN, WNV glycans are also recognized and bound by DC-SIGNR (L-SIGN or CD209L) a CLR expressed on microvascular endothelial cells, especially in the liver sinusoids and lymph nodes [13]. In fact, DC-SIGNR more efficiently promoted WNV infection than did DC-SIGN, especially when the virus was produced in human cell lines. Although a single N-linked glycosylation site on either the prM or E glycoprotein of WNV was sufficient for DC-SIGNR-mediated infection, preferential use of DC-SIGNR was a specific to the WNV E protein. While mannose-rich glycans on WNV were required for interaction with DC-SIGN, complex glycans mediated reporter virus interactions with DC-SIGNR [22].

Alphaviruses encode for two glycoproteins, E1 and E2, which are involved in cellular attachment and entry via glycan mediated interactions. The E1 protein directs the membrane fusion process, and E2 is postulated to function as a cell receptor-binding domain for several alphaviruses [23]. DC-SIGN and L-SIGN have been suggested to be attachment receptors for Sindbis virus via envelope glycan moieties [14]. Infection with Sindbis was greatly enhanced when virus was produced in either mosquito cells or in mammalian cells under conditions that prevented glycan remodeling and eliminated complex glycans. Studies with other alphaviruses including eastern equine encephalitis, western equine encephalitis, RRV, and Venezuelan equine encephalitis virus produced in mammalian versus mosquito cells indicated that multiple alphaviruses can use CLRs as attachment receptors when complex glycan processing is limited [24].

Though CLRs such as DC-SIGN can clearly mediate or enhance viral entry, there is also evidence which suggests that pathogens manipulate DC function through distinct mechanisms that abrogate antigen processing or alter TLR-mediated signaling. This implies that adaptation of a pathogen to allow interaction with DC-SIGN might support pathogen survival [25–27]. The best studied example of DC-SIGN subversion by a virus comes not from an arbovirus but from HIV-1. Although the infection of DCs by HIV-1 remains somewhat controversial, the HIV-1 gp120 protein binds with high affinity to DC-SIGN [28, 29]. However, binding of HIV-1 by DC-SIGN does not result in internalization for antigen presentation, but rather DC-SIGN acts as a *trans*-receptor that efficiently transmits the attached HIV-1 particle to target T cells [30]. Furthermore, HIV gp120 interactions with mannose binding lectins on dendritic cells have been shown to suppress DC function and enhance IL10 production, thereby modifying DC functional activity and also potentially shaping the host cytokine response within the infected host [8].

Taken together the data reviewed above depict critical roles for N-linked glycans in early steps of the viral infection cycle including cell tropism, cell attachment and entry. Further, the importance of contributions by viral glycan interactions to pathogenesis and virulence is becoming increasingly clear. Therefore although glycan-mediated interactions are not unique to arboviruses, differences in glycosylation processes between mosquito and mammalian cells make the study of arbovirus glycan-mediated interactions especially intriguing.

Viral Glycan-Mediated Modulation of Host Innate Immunity

IFN and Cytokine Response

A growing body of evidence suggests that CLRs can modulate the host cytokine response. Several CLRs have been shown to down-regulate pro-inflammatory cytokine responses, suggesting that viruses interface with these receptors to avoid or suppress the host antiviral response and enhance viral replication. For example, activated DCs undergo a process of maturation which includes down-regulation of cell surface CLRs to render DCs less permissive to infection [31]. A number of DC-targeting pathogens have evolved mechanisms to block DC maturation mediated by inflammatory cytokines such as TNF-α and IFN-α [reviewed in 32]. Although DEN induces TNF-α production and maturation of bystander cells, it blocks DC maturation and subsequent antigen presentation by rendering infected DCs refractory to TNF-α stimulation [33]. Though the viral mediators of this process are not known, it is interesting to note that the HIV gp120 protein has been shown to exhibit a similar effect upon DC maturation through interactions with CLRs [8]. In contrast, interaction between DEN and another CLR, CLEC5A, stimulates pro-inflammatory cytokine production without affecting IFN-α production such that binding of DEN virus to CLEC5A initiates a signaling cascade leading specifically to pro-inflammatory cytokine release [34].

Another Flavivirus, WNV, has also been shown to modify host responses through glycan dependent interactions with the host. In vitro macrophage studies designed to elucidate mechanisms by which glycosylation of the WNV E protein facilitates neuroinvasion showed that the pro-inflammatory cytokines IL-1β and TNF-α were up-regulated only by glycosylated virus [35]. WNV E protein was shown to block both type I IFN production and pro-inflammatory cytokine production induced by viral double-stranded RNA [36]. Most interestingly, this effect was induced by WNV derived from mosquito cells, but not mammalian cells, suggesting that high mannose N-linked glycans on the mosquito-derived virus might actively inhibit type I IFN induction and thereby promote viral infection. Similarly, infection of myeloid DCs or plasmacytoid DCs (pDCs) with mosquito- vs. mammalian-derived WNV resulted in comparable levels of IFN-α induction in myeloid DCs but IFN-α expression was abolished in pDCs stimulated with mosquito-derived virus [37]. These same experiments demonstrated that mosquito cell virus did not interfere with the ability of Sendai virus to induce IFN synthesis in pDC cultures, suggesting that the mosquito cell-derived WNV was not actively inhibiting pDC function.

Studies with the alphaviruses, RRV, Barmah Forest virus (BFV) and Venezuelan equine encephalitis virus also demonstrated that mosquito cell-derived viruses were poor inducers of type I IFN in myeloid dendritic cells, further suggesting that several arboviruses might be capable of suppressing, avoiding, or modifying host antiviral responses through the actions of high mannose N-linked glycans [38]. Further analysis of RRV demonstrated that, unlike WNV, where the viral E protein suppressed type I IFN induction, lack of a type I IFN response by mosquito-derived RRV did not appear to be due to an active suppressive effect [39]. Instead, induction of high levels of type I IFN by RRV in myeloid DCs required the presence of complex N-linked glycans on the virus, suggesting that the mosquito-derived virus might avoid induction of high type I IFN levels simply due to its lack of complex N-linked glycans. This finding is supported by studies with several other viruses that suggest that the presence of complex N-linked glycans promotes induction of the type I IFN response [40]. However, the mechanisms underlying this process are poorly understood and require further investigation.

Viral Glycan Interactions with Host Complement System

Though the role of glycosylation in regulating type I IFN and inflammatory cytokine responses by mosquito-borne viruses is a relatively new area of research, N-linked glycans have long been known to regulate interactions with another arm of the innate immune system, the complement cascade. The complement cascade plays a central role in regulating viral infections, both by direct inhibition of the virus and through regulation of other arms of the innate and adaptive immune system [41]. Several studies have concluded that complement controls WNV infection, in part, by inducing a protective antibody response [42]. While mice deficient in key components of all 3 activation pathways showed increased susceptibility to severe WNV-mediated disease, loss of the lectin pathway resulted specifically in deficient B and T cell responses to WNV. Complement also plays a significant role in regulating alphavirus infection. In the case of Sindbis virus, complement controls peripheral replication, while potentially contributing to virus-induced disease in the CNS [43]. Complement does not appear to control Ross River virus infection, but instead is up-regulated in the joints of persons suffering from RRV-induced arthritis,

and complement activation is required for virus-induced inflammatory myositis in a mouse model of RRV-induced inflammation [44].

The complement cascade, like other pattern recognition systems, is activated by conserved, non-self, molecular patterns. Of particular importance for complement activation is the presence or absence of sialic acid. Sindbis virus derived from mosquito cells activates the complement cascade via the alternative pathway more efficiently than the same virus derived from mammalian cells, while neuraminidase treatment of mammalian-cell-derived virus also led to enhanced complement activation, suggesting that sialic acid content on the virion plays a major role in regulating complement activation [45]. This suggests that virus initially delivered by the mosquito vector may be more susceptible to complement-mediated clearance than the virus produced in subsequent rounds of replication within the host. Though previous studies with Sindbis virus focused on complement activation via the alternative pathway, there is some evidence to suggest that arbovirus N-linked glycans may interact with other arms of the complement cascade. Though mosquito-borne viruses have not been shown to directly activate the mannose-binding lectin pathway of complement activation, WNV-induced immune responses are partially regulated by the this pathway [46]. Polymorphisms in the mannose-binding lectin gene have also been associated with susceptibility to DEN virus-induced thrombocytopenia [47]. Furthermore, SIGN-R1, a CLR with homology to DC-SIGN, has been shown to directly initiate activation of the classical pathway of complement activation during bacterial infection [48]. Given the role of SIGN-R1 and other CLRs in recognition of mosquito-borne viruses, it is certainly possible that this pathway will be involved in complement activation during arbovirus infection. Therefore, additional studies are required to more fully dissect the interactions between N-linked glycans on mosquito-borne viruses and the host complement cascade that determine the outcome of virus-induced disease.

Additional Viral Glycan-Mediated Interactions with Host Innate Immunity

Pattern recognition receptors such as TLRs or cytosolic helicases like retinoic-acid-inducible-gene I (RIG-1) and melanoma differentiation-associated gene (Mda5) couple pathogen recognition to downstream induction of genes involved in the innate immune response [49]. The evidence for direct CLR signaling and downstream immune activation is far less abundant, but is beginning to emerge. In addition, crosstalk between CLRs and TLRs has been described. In the presence of TLR signaling, myeloid DC co-stimulatory molecules are up-regulated, and antigen uptake and presentation through CLRs can initiate immunity through T cell stimulation [50]. CLR modulation of TLR signaling was recently demonstrated for several pathogens that bind DC-SIGN [27]. The evidence for CLR-TLR crosstalk on plasmacytoid DCs, the major IFN-α-producing cell type, is also just beginning to emerge. An interesting connection was recently demonstrated for DC immunoreceptor, a CLR with putative immune-inhibitory function, and TLR9 on human plasmacytoid DCs [51]. While triggering of TLR9 leads to pDC maturation and reduced DC immunoreceptor cell surface expression, DC immunoreceptor triggering inhibits TLR9-induced IFN-α production without changing the maturation state of the pDC. Although characterization of CLR-TLR crosstalk is only beginning to occur, several viruses have already been shown to exploit the system by targeting CLRs to subvert CLR-TLR communication and escape detection [50].

Concluding Remarks

Glycosylation is clearly important to both the pathogen and the host. While mammalian lectins recognize pathogens and alert the immune system, some lectins recognize pathogens but a lack of direct signaling capacity or negative signaling capacity leads to exploitation of these receptors for undetected pathogen entry. Understanding how viruses interface with and/or exploit host glycan binding proteins will not only enhance our general understanding of arbovirus-induced disease, but may ultimately lead to the development of new vaccines or therapies that take advantage of our ability to modify or inhibit viral interactions with glycan binding proteins.

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