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Host-Virus Interaction of ZIKA Virus in Modulating Disease Pathogenesis

Nanda Kishore Routhu¹ and Siddappa N. Byrareddy^{1,2,*}

¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198

²Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198

Abstract

The Zika virus (ZIKV) is a newly emerging pathogen that has resulted in a worldwide epidemic. It primarily spreads either through infected *Aedes aegypti* or *Aedes albopictus* mosquitos leading to severe neurological disorders such as microcephaly and Guillain-Barré syndrome in susceptible individuals. The mode of ZIKV entry into specific cell types such as: epidermal keratinocytes, fibroblasts, immature dendritic cells (iDCs), and stem-cell-derived human neural progenitors has been determined through its major surface envelope glycoprotein. It has been known that oligosaccharides that are covalently linked to viral envelope proteins are crucial in defining host-virus interactions. However, the role of sugars/glycans in exploiting host-immune mechanisms and aiding receptor-mediated virus entry is not well defined. Therefore, this review focuses on host-pathogen-interactions to better understand ZIKV pathogenesis.

Keywords

Host-virus interactions; Zika virus; virus glycosylation; immunity; neuropathogenesis

Introduction

The Zika virus (ZIKV) is a major emerging pathogen (Jamil et al., 2016) that has recently caused sporadic but large epidemics world-wide. This includes countries in South and Southeast Asia, Oceania, the Americas, and Africa. ZIKV belongs to a member of the genus *Flavivirus* in the *Flaviviridae* family. These are single-stranded, positive sense RNA viruses typically transmitted by arthropod vectors (Malone et al., 2016). This genus also includes dengue virus (DENV) 1–4, West Nile virus (WNV) 1 and 2, Yellow Fever virus (YFV), and Japanese encephalitis virus (JEV) I–V (Lindenbach and Rice, 2003). The flaviviruses infect a wide range of hosts such as rodents, pigs, birds, non-human primates, and humans (Daep et al., 2014).

*Corresponding Author: Siddappa N. Byrareddy, Ph.D., Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, 985880 Nebraska Medical Center, Omaha, NE 68198-5880, sid.byrareddy@unmc.edu, 402-559-5416.

ZIKV was first isolated from a rhesus monkey in Zika forest, Uganda in 1947 (Dick et al., 1952). Thereafter it was isolated from the mosquito (*Aedes africanus*) in 1948 (Dick et al., 1952), and humans in 1952 (Macnamara, 1954). ZIKV is mainly spread through *Aedes aegypti* and *Aedes albopictus* mosquitoes which comprise two major lineages: African and Asian (Ladner et al., 2016; Weaver et al., 2016). Due to the increase in ZIKV infections in recent years, in May 2015 the Pan American Health Organization issued an epidemiological alert about Zika virus infection. In February 2016, the World Health Organization stated “Zika virus is a public health emergency of international concern” due to extensive travel of infected humans across the world (Lupton, 2016) (Table 1).

Zika virus genome organization and polyprotein processing

ZIKV contains a single-stranded positive sense RNA genome that is approximately 10 kb in size with a single open reading frame (ORF). The ORF encodes a large polyprotein that is processed to produce the structural proteins (capsid [C], precursor membrane [prM], envelope [E]), and non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) that assist in virus replication and in pathogenesis (Fig. 1). The non-structural proteins are important for entry, translation, replication, morphogenesis, and pathogenesis (Lindenbach and Rice, 2003; Olagnier et al., 2016; Sirohi et al., 2016). The ORF is flanked by highly conserved secondary structures at the 5′ and 3′ untranslated regions that help in translation and replication of the genome (Zhu et al., 2016). ZIKV replicates in the virus-induced membranous vesicles that are derived from the endoplasmic reticulum (ER) and Golgi complex by exploiting membrane trafficking (Miller and Krijnse-Locker, 2008). After release of the genome into the cytoplasm, ZIKV replicates through a negative strand intermediate (Lindenbach and Rice, 2003). Due to its limited coding capacity, ZIKV largely depends on host machinery for virus replication, translation, and viral morphogenesis (Gale et al., 2000).

The non-structural protein NS1 is a 46 kDa glycoprotein containing 2–3 glycosylation sites and 12 conserved cysteine residues that can form disulphide bonds (Muller and Young, 2013; Edeling et al., 2014). Mutations in glycosylation sites Asn130 and Asn207 drastically affect virus replication and virus production (Pryor et al., 1998; Crabtree et al., 2005). Although the NS1 protein has no hydrophobic transmembrane domain, it associates with the membrane through a glycosylphosphatidylinositol (GPI) anchor (Jacobs et al., 2000). It is located inside the cell and is also found in foci on the cell surface. Upon proteolytic separation from the envelope protein, it is secreted out of the cell (Alcon-LePoder et al., 2005). Cell surface expression of NS1 could elicit a strong humoral immune response, which further aids in antibody-mediated killing of virus infected cells (Crook et al., 2014). The NS1 and NS4 non-structural proteins interact with each other and co-localize in the viral replicase complex to help negative strand synthesis during the viral genome replication (Lindenbach and Rice, 1999).

The NS2A is a 22 kDa hydrophobic protein and plays an important role in infectious viral particle production (Xie et al., 2013). Cleavage of NS2A upstream of the C-terminus by viral serine protease produces a smaller 20 kDa protein, which is essential in viral replication (Leung et al., 2008). The NS2A non-structural protein interacts with the NS3, NS5, and

Kunjin virus (KUN) 3' Non-coding-region and co-localizes with the replication complex (Liu et al., 2002). These interactions are essential in managing the shift between RNA packaging and RNA replication in virus particle production (Mackenzie et al., 1998; Yu et al., 2013). The NS2B is a 14 kDa membrane-associated protein that mediates membrane interaction by its conserved central hydrophobic region (Bollati et al., 2009). These interactions increase the host cell membrane permeability (Chang et al., 1999). Furthermore, NS2B interacts with and acts like a cofactor for NS3 trypsin-like serine protease function (Luo et al., 2008).

NS3 is a multifunctional 70 kDa protein that has multiple enzymatic activities, such as trypsin-like serine protease activity, (Chambers et al., 1990; Brinkworth et al., 1999; Droll et al., 2000; Tautz et al., 2000; Yusof et al., 2000; Luo et al., 2008), Mg²⁺ inhibited and poly(A)-stimulated NTPase activity (Feito et al., 2008). In addition, it also has Mg²⁺ stimulated, poly(A)-inhibited RNA triphosphatase (RTPase) activity and ATP-driven RNA duplex unwinding activity at its C-terminus. Therefore, mutations in the putative NTP-binding site (Lys199) destroy the NTPase and RTPase activities (Gebhard et al., 2012). The NS3 localizes to the membranes by interacting with the membrane-localizing transmembrane viral protein NS2B and helps in viral polyprotein processing and viral replication (Falgout et al., 1991; Markoff et al., 1997; Yu et al., 2013). The trypsin-like serine protease activity has been mapped to His53, Asp77, and Ser138 (catalytic triad) (Chambers et al., 1990; Gupta et al., 2015).

NS4A is a 16 kDa multifunctional, hydrophobic protein. It regulates membrane proliferation, localizes the replication complex to the membrane, aids in polyprotein processing, and is crucial for immune evasion (Mackenzie et al., 1998; Miller et al., 2007). The NS4A C-terminus interacts and translocates NS4B to the ER lumen (Miller et al., 2007). The NS4B is a 27 kDa transmembrane protein present at the replication site with double-stranded RNA, an intermediate of viral replication and NS3 protein (Miller et al., 2007).

NS5 is a highly conserved 103 kDa protein and plays a crucial role in viral replication (Lu and Gong, 2013; Li et al., 2014; Klema et al., 2016). It has N-terminal RNA cap-processing activity (homology with the S-adenosyl-methionine (SAM)-dependent methyltransferases) (Klema et al., 2016), GTP-binding activity (Benarroch et al., 2004), and C-terminal RdRP activity (Malet et al., 2007; Zhang et al., 2008). The interaction of NS5 protein stimulates NS3 NTPase activity (Feito et al., 2008) and creates an importin binding site. As a result, this helps in nuclear localization (Vasudevan et al., 2001; Klema et al., 2016).

Zika virus cellular entry

Compared to other flaviviruses, little is known about the morphology, replication, and pathogenesis of ZIKV. Recently, the structure of the thermo-stable French Polynesian ZIKV strain H/PF/2013 was determined by cryoelectronmicroscopy. The overall structural architecture of ZIKV is similar to that of DENV and WNV, except for approximately 10 amino acids. These amino acids surround the Asn154 glycosylation site in each of the 180 envelope glycoproteins that make up the icosahedral shell (Kostyuchenko et al., 2016; Sirohi et al., 2016). Sequence comparisons of the glycoprotein (E) of ZIKV with the other

members of the flaviviruses family indicating unusual degree of variability within the ZIKV strains and between the family (Fig. 2 and Table 2).

ZIKV is an enveloped, icosahedral virus approximately 40 nm in diameter. The capsid of ZIKV is made up of three structural proteins: the capsid protein, prM, and the envelope protein (Zhang et al., 2012; Kostyuchenko et al., 2016; Sirohi et al., 2016). The outer shell is composed of 180 copies of both the envelope (E) glycoprotein (53 kDa) and the membrane (M) protein (21 kDa), and is surrounded by a lipid envelope (Kostyuchenko et al., 2016). The capsid protein is attached to the viral RNA genome. The envelope protein is a surface protein that is involved in viral attachment, fusion, penetration, hemagglutination, host range, and cell tropism.

The envelope protein contains three domains (I, II, III) and two transmembrane helices. Domain I contains a β -barrel central structure. Domain II comprises the fusion loop, the segment directly involved in the fusion of viral and cellular membranes during virus entry. Domain III is located on the surface of the virion. It has an immunoglobulin-like β -barrel structure that helps in cellular-binding (Modis et al., 2005). The Zika virus envelope protein EDI, EDII, and EDIII domains share 35%, 51%, and 29% amino acid identity with the DENV ED I-III domains, respectively (Stettler et al., 2016). Maturation of the virion requires cleavage of the membrane protein by the trans-Golgi network and cellular protein (furin) to pr and M protein. This is followed by the release of the newly formed viral particles from the cell (Zhang et al., 2012; Sirohi et al., 2016)

The ZIKV envelope protein has one N-linked glycosylation site (N154). Whereas, the DENV envelope protein contains two glycosylation sites (N67 and N153) that are involved in the interaction with attachment factors on the cell surface (Hacker et al., 2009; Dai et al., 2016; Zhu et al., 2016) such as receptor tyrosine kinases like TYRO3 and AXL (Hamel et al., 2015; Dang et al., 2016; Hanners et al., 2016; Tang et al., 2016) (Fig. 3). In addition, other *flaviviridae* members (DENV and WNV) use dendritic cell-specific intercellular adhesion molecule 3-grabbing non integrin (DC-SIGN) (Tassaneetrithep et al., 2003; Wang et al., 2015), liver/lymph node-specific ICAM-3 grabbing non integrin (L-SIGN) (Perera-Lecoin et al., 2014; Wang et al., 2015), proteins of the transmembrane immunoglobulin and mucin domain 1 (TIM1) (Jemielity et al., 2013), and claudin-1 (Che et al., 2013) for cell entry (Fig. 3). However, it is unclear whether ZIKV uses such cell-specific adhesion molecules and whether they play critical roles in virus budding and transmission, thereby warranting further studies.

Role of glycosylation in Zika virus-host interactions

Recently, Zhu et al (2016) analysed potential O-glycosylation and N-glycosylation sites in 24 representative pre-epidemic and epidemic ZIKV using NetOGlyc 4.0 (<http://www.cbs.dtu.dk/services/NetOGlyc/>) and NetNGlyc 1.0(<http://www.cbs.dtu.dk/services/NetNGlyc/>) and found that the O-glycosylation and N-glycosylation sites are highly conserved (Zhu et al., 2016). Virus particles contain one strand of RNA genome, surrounded by a lipid bilayer and three distinct types of viral structural proteins (E, M/prM, and C). The glycoprotein E is responsible for targeting virus to host cell receptors, and it facilitates virus

entry. As such, it is a major target for antibodies (Dai et al., 2016; Kostyuchenko et al., 2016; Sirohi et al., 2016)(Fig. 4). However, ZIKV is able to mediate antibody escape due to highly variable amino acids (approximately 10) that surround the Asn154 glycosylation site in its envelope protein (Kostyuchenko et al., 2016; Sirohi et al., 2016). Sequence analysis (Fig. 2) and glycosylation site prediction (Table 3) studies indicate that DENV has two (Asn67 and Asn153) glycosylation motifs; whereas, ZIKV has one (Asn154) glycosylation site (Zhu et al., 2016). The connection between glycosylation, structure, tropism, immune recognition/ evasion mechanisms, and pathogenesis of ZIKV remains largely unknown and addressing these will aid in the rapid development of vaccines and therapeutics.

As described above, the first ZIKV was isolated in the Zika forest in 1947. Later strains with Asian and African lineages did not cause significant numbers of human infections until 2007 (Dick et al., 1952). The Yap Island epidemic in 2007 in Micronesia brought ZIKV into notice, along with the alarming increase from 2007 to 2016 in epidemics in New Caledonia, French Polynesia, Brazil, and South and Central America, and its association with the severe birth defects (microcephaly) and neurological diseases Guillain-Barré syndrome (GBS) (Haddow et al., 2012; Al-Qahtani et al., 2016; Nishiura et al., 2016). ZIKV attained many sequence and structural changes from 1947 to 2016, which increased virulence and became responsible for the many epidemics across the globe (Haddow et al., 2012; Zhu et al., 2016). These studies suggested that ZIKV pathogenicity has gradually evolved during inter-species transmission via the intra-cerebral route making a relatively non-pathogenic viral strain to a “pathogenic” strain. The ZIKV glycoprotein E is the major surface protein that makes initial contact with the cell-surface receptor to gain entry into the host cell. However, the glycoprotein E-linked glycan structure and composition have not yet been examined. Therefore, analysing the structure and composition of the glycans at each site across the family will aid in developing a signature that would further assists in elucidating emerging ZIKV pathogenesis and developing drug targets.

ZIKV infection is associated with rashes, headache, nausea, diarrhea and fever, including serious neuroimmunological diseases such as microcephaly in neonates and GBS in adults (Oehler et al., 2014; Araujo et al., 2016b; Cao-Lormeau et al., 2016; Watrin et al., 2016). The association between GBS is clearly revealed in French Polynesia in a case study between October 2013 and April 2014 (Kindhauser et al., 2016). The presence of Zika viral RNA and antigens in two congenitally infected new-borns’ placenta and brain tissues from Brazil strengthen the connection between Zika virus infections in infants (2015) (de Oliveira Poersch et al., 2005; Oehler et al., 2014; Sarno et al., 2016).

Several modes of ZIKV infection has been studied such as antibody-dependent enhancement of infection and receptor mediated entry (DC-SIGN, Tyro3, and AXL and TIM-1) to facilitate the virus entry to macrophages, monocytes, neuronal progenitor cells, and fetal cells (Hamel et al., 2015; Li et al., 2016b; Tang et al., 2016). However, the correlation between the increased number of ZIKV infections and microcephaly cases remains to be defined. Furthermore, the analysis of tissue samples for viral RNA and proteins during the different stages of gestation from expectant mothers with ZIKV infection indicate the virus can cross the placenta and cause chronic placentitis and microcalcifications (Noronha et al., 2016). This observation was further supported by viral RNA and protein detection in

amniotic fluids, placental tissues, and fetal brain tissues (Martines et al., 2016; Mlakar et al., 2016). Recent studies have demonstrated that the receptors (Tyro3, Axl, and TIM1) expressed on primary human placental cells, endothelial cells, fibroblast cells, amniotic epithelial cells, trophoblast progenitors, and macrophages (Hofbauer cells) facilitate ZIKV (PRVABC59 and MR766) maternal-fetal transmission (Bayer et al., 2016; Noronha et al., 2016; Tabata et al., 2016). The ZIKV infects the primary human placental macrophages and placental cytotrophoblasts, thereby induces the production of type I interferon (IFN) IFN- α , pro-inflammatory cytokines IL-6, chemokine MCP-1, and antiviral genes such as RIG-I, MDA5, and LGP2 (Quicke et al., 2016). During infection, ZIKV stimulates cell death, induces type I interferon (IFN) response and pro-inflammatory cytokines that disrupt the placental barrier leading to neurological disorders such as microcephaly (Miner et al., 2016). However, another study shows that trophoblast-derived type III IFN encodes resistance to ZIKV infections in barrier cells of the placenta and primary human trophoblasts (PHTs). The IFN λ 1 protects placental cells and non-placental cells from ZIKV infection (Bayer et al., 2016). The ZIKV^{BR} infected mice, human cortical progenitor cells, and brain organoids presented microcephaly related symptoms, like cell death, disrupted cortical layers, and reduced proliferative zones (Wu et al., 2016). Furthermore, it has been demonstrated that Asian ZIKV strain SZ01 has shown that ZIKV replicate efficiently in mouse neural precursor cells (NPCs). Next, SZ01 replication was associated with differential expression of genes related to the cell-cycle, immune responses and apoptosis (Li et al., 2016a).

Zika virus association with the neurological abnormalities

ZIKV infections are linked to the GBS and newborn microcephaly (Oehler et al., 2014; Araujo et al., 2016a; Cugola et al., 2016). GBS is a rare neurological syndrome caused by immune system failures wherein the immune system targets myelinating Schwann cells of the peripheral nervous system and leads to a rapid-onset of muscle weakness (Hughes and Cornblath, 2005). Microcephaly is caused by embryonic and fetal developmental failures that lead to reduced cerebral cortex size. The normal development of the cerebral cortex requires an adequate proliferation, differentiation, and migration of neural stem cells. Failure in any of these processes can result in microcephaly (Jackson et al., 2002). Recent reports imply that these neurological abnormalities are also observed in congenital infections (Wakerley and Yuki, 2013). Increase in the number of GBS cases during French Polynesia outbreak and newborn microcephaly cases during Brazil outbreak were observed (Oehler et al., 2014; Araujo et al., 2016a; Cugola et al., 2016). In addition to GBS and microcephaly, infected persons also experienced paralysis of four limbs (tetraparesis) in adults (Broutet et al., 2016). Severe ocular abnormalities were observed in infants with microcephaly (Vasconcelos-Santos et al., 2016). ZIKV positive antibodies have been found in patients with tetraparesis, and the presence of ZIKV RNA has been reported in the brain and tissues of a neonate who died of microcephaly (Araujo et al., 2016a).

The exact mechanism behind ZIKV induced neurological disorders is not clearly known. However, several recent studies have aimed at understanding the link between ZIKV infections and microcephaly (Bayer et al., 2016; Bayless et al., 2016; Brault et al., 2016; Broutet et al., 2016). A recent comprehensive study involving ZIKV (Pf13 strain), DENV-4, and WNV strongly supports the neurotropic nature of ZIKV; wherein, the embryonic brain

cells were able to support virus replication, but not closely related DENV-4 replication (Brault et al., 2016) in mice. Cranial neural crest cells (CNCCs) are very crucial in development of facial bones of the skull and play important role in the formation of bones, cartilage, and nerves. ZIKV, but not DENV, efficiently infect CNCCs, promote increased production of neurodevelopmental cytokines such as Vascular endothelial growth factor (VEGF) and leukemia inhibitory factor (LIF) that initiate premature neuronal differentiation (Bayless et al., 2016). At the molecular level, ZIKV NS4A and NS4B inhibit the Akt-mTOR pathway in infected human fetal neural stem cells (fNSCs) leading to dysregulation of autophagy pathways and faulty neurogenesis (Liang et al., 2016). However, the closely related DENV NS4A and NS4B do not demonstrate the same effect on neurogenesis (Liang et al., 2016).

Due to high recombination frequencies, ZIKV has potentially evolved faster and attained the ability to exploit multiple cell surface receptors and cellular factors to facilitate infection in a variety of cells types, including AXL-independent entry into cortical progenitor cells, neuronal progenitor cells, and cerebral organoids in mice and some human ex vivo studies (Hamel et al., 2015; Garcez et al., 2016; Shao et al., 2016; Wells et al., 2016). Also, mice pups delivered from SJL and C57BL/6 mice infected intravenously with a ZIKV Brazil (ZIKV^{BR}) isolate, exhibited delay in whole-body growth and intrauterine restrictions, compared to control mice pups (Cugola et al., 2016). In addition, significant viral RNA and proteins were detected in the brain (Cugola et al., 2016). The extensive microglial activation, massive neuronal death, axonal rarefaction, and blood-brain barrier (BBB) leakage indicate the ability of the virus to cross placenta and cause congenital malformation (Shao et al., 2016). Correspondingly, a quantitative study using three-dimensional forebrain organoids demonstrates that ZIKV infections decrease the neuronal cell-layer volumes by increasing cell-death and decreasing cell proliferation (Qian et al., 2016). This correlates with cortical defects associated with reduced human cortical progenitor cell numbers and cortical thickness due to alteration in the expression of genes involved in immune response, cell-cycle, autophagy and apoptosis (Cugola et al., 2016; Qian et al., 2016; Tang et al., 2016).

Zika virus-host immune system interactions

A contemporary circulating PRVABC59, a ZIKV strain isolated from Puerto Rico in the Western Hemisphere during the 2015 outbreak, was characterized for its cytopathic effects (cell rounding, pyknosis, and caspase 3 activation) in primary human neural progenitors (hNPs), THP-1, Huh7.5, BHK-J, and Vero E6 cells (Hanners et al., 2016). An antibody-based array to detect 102 human inflammatory cytokines in ZIKV-infected hNPs and THP-1 cells showed that multiple cytokines and growth factors were secreted by hNPs cells. However, there was no significant difference in secreted proinflammatory cytokines TNF α , CCL2, and the neuroprotective chemokine CX3CL1 between ZIKV-infected and mock-infected cultures. Thus, confirming that ZIKV can replicate in hNPs without inducing the production of neuroinflammatory or neuroprotective cytokines (Hanners et al., 2016).

Infection of DENV results in a characteristic phenomenon known as a ‘cytokine storm’; wherein, the infected cells produce high levels of circulating pro-inflammatory cytokines, such as IFN- α , IFN- γ , tumour necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-2,

IL-6, IL-8, IL-10, CCL2, CCL3, CCL4, CCL5, CXCL-8, CXCL-10, CXCL-11, macrophage migration inhibitory factor, and vascular endothelial growth factor (Mangada and Rothman, 2005; Palmer et al., 2005; Amaral et al., 2011). However, such symptoms are not observed in ZIKV-infected individuals even though there is a high degree of similarities in the viral proteins. The multiplex cytokine serum analyses (MCSA) of acute and convalescent phase of ZIKV infections indicate that the elevation of chemokines was more noticeable than elevation of cytokines (Tappe et al., 2015). In acute phase, substantial increases in concentration of IL-1 β , IL-2, IL-4, IL-6, IL-9, IL-10, IL-13, IL-17, interferon- γ -induced protein 10 (IP-10), normal T cell expressed and secreted (RANTES), macrophage inflammatory protein 1 alpha (MIP-1 α), and VEGF are observed as compared to normal controls (Tappe et al., 2015). In the convalescent phase, noticeable increase in IL-1 β , IL-6, IL-8, IL-10, IL-13, IP-10, RANTES, MIP-1 α , MIP-1 β , VEGF, fibroblast growth factor (FGF) and granulocyte-macrophage colony stimulating factor (GM-CSF) levels compared to MCSA of uninfected persons (Tappe et al., 2015). A circulating strain, PF-13 was isolated during the French Polynesia outbreak in 2013/2014. It is closely related to strains isolated from Cambodia in 2010 and Yap State in 2007, and it infects permissive cells like human dermal fibroblasts, epidermal keratinocytes, and immature dendritic cells (Musso et al., 2014a; Hamel et al., 2015).

The pathogen recognition receptors (PRR), such as toll-like receptor 3 (TLR-3), retinoic acid-inducible gene I (RIG-I), and melanoma differentiation-associated gene 5 (MDA5) bind to pathogen associated molecular patterns (PAMP) (Hamel et al., 2015). This leads to restriction of the pathogen through the stimulation of a cascade of signalling events resulting in the activation of the antiviral pathway. The antiviral pathway is comprised of IRF-7-dependent antiviral gene expression that includes: the interferon-stimulated genes OAS2, ISG15, and MX1; type I IFNs; and the inflammatory chemokines CXCL10, CCL5, and CXCL11 (Chen et al., 2013; Hamel et al., 2015). The interferon system is known to play a key role DENV and WNV infections (Umareddy et al., 2008; Munoz-Jordan, 2009; Nasirudeen et al., 2011; Bayer et al., 2016). Similarly, A129 immunocompromised mice lacking the type I interferon receptor and AG129 mice lacking types I and II IFN receptors infected with the Asian lineage FSS13025 strain exhibited severe neurological symptoms and death. In wild-type immunocompetent mice, this was not observed. In spite of all this, these neurological infections have not been reported in infected rhesus macaques (Dudley et al., 2016). Taken together, this data suggests that the interferon system plays a crucial role in controlling ZIKV pathogenesis (Aliota et al., 2016; Rossi et al., 2016). Interestingly, the antigen presenting system and interferon system is not developed to control these infections and associated diseases in the neonates (Jaspan et al., 2006). Furthermore, pre-treatment of primary skin fibroblasts with human recombinant type I and type II interferon, such as IFN- α , IFN- β , or IFN- γ strongly restrict ZIKV infections in a dose-dependent manner supporting the importance of the interferon system in controlling ZIKV infection and pathogenesis (Navarro-Sanchez et al., 2005; Hamel et al., 2015).

Cross-reactivity of other flavivirus antibodies with ZIKV

It has been shown that DENV and ZIKV are antigenically related with the severity of ZIKV infection significantly increased when antibodies against DENV are present and vice versa

(Lanciotti et al., 2008; Dejnirattisai et al., 2016; Priyamvada et al., 2016; Zhu et al., 2016). The DENV has four human serotypes, DENV-1, DENV-2, DENV-3, and DENV-4, which share 65% of genome homology (Holmes, 1998; Mustafa et al., 2015). Each of the serotypes of DENV produce unique humoral immune responses in infected hosts (Holmes, 1998). Infection of humans with a single serotype is typically self-resolving; but when the same individual is infected with a different DENV serotype, it results in higher levels of infectivity. Antibodies generated against the first serotype can bind to other serotypes resulting in enhanced antibody-Fc receptor-mediated entry into the macrophages (Peiris and Porterfield, 1979; Boonnak et al., 2010; Chotiwan et al., 2014; Dejnirattisai et al., 2016). Serum collected from four people who had recovered from DENV showed significant enhancement of ZIKV infection indicating that the DENV neutralizing sera can enhance ZIKV infections in an antibody-Fc receptor dependent pathway. In presence of anti-Fc receptor antibody, the infectivity of ZIKV was significantly reduced (Priyamvada et al., 2016). Similarly, the cross-reactivity of acute and convalescent serum of nine Thai DENV-infected individuals to ZIKV was tested and showed cross-reactivity in the binding as well as in the neutralization of ZIKV (Priyamvada et al., 2016). This is further supported by findings that demonstrated DENV-specific monoclonal antibodies (mAbs) bound to both whole ZIKV virions and ZIKV lysate (Priyamvada et al., 2016). Only a subset of these antibodies could neutralize ZIKV infection, but all the nine sera and 47 mAbs specific to DENV enhanced *in vitro* ZIKV infection of Fc gamma receptor (Fc γ R)-bearing cells.

A set of 119 monoclonal antibody-producing B-cells were isolated from the four patients (two had ZIKV-infection and two had serological records of DENV infection) from contemporary epidemics. Due to the high degree of structural similarities between the EDI and EDII loops, 67% (31 of 46) of the monoclonal antibodies were cross-reactive with each other and exhibited low neutralizing activity with antibody-mediated enhancement of Fc-receptors (Stettler et al., 2016). However, 90% (27 of 30) of the EDIII-specific monoclonal antibodies isolated from ZIKV or DENV donors were specific for either ZIKV or DENV envelope proteins and exhibited high neutralizing ability (Stettler et al., 2016). Serological examination of German travellers who developed signs of dengue showed ZIKV-specific neutralizing antibodies, as well as anti-ZIKV-IgM and -IgG antibodies (Tappe et al., 2014). However, pre-existing immunity does not confer resistance to other flaviviruses (Murphy and Whitehead, 2011). The ZIKV-immunized rhesus monkeys developed partial resistance to challenge with YFV (Henderson et al., 1970). Neutralizing antibodies developed after ZIKV and YFV infection has shown haemagglutination inhibition that could also partially protect mice from subsequent lethal ZIKV infection (Monath et al., 1973; Musso and Gubler, 2016). It has been shown that 47% (8 of 17 mAbs) of DENV EDE2-specific mAbs and 81% (27 of 33) of DENV EDE1-specific mAbs could cross-react with ZIKV (Dejnirattisai et al., 2016).

Concluding remarks and future perspectives

The global spread of ZIKV infections and pathological mechanisms behind the emergence of Zika virus are not clearly known. The factors influencing the rate of Zika infections include, but are not limited to, viral envelope protein glycosylation and its parallel connection with the evolution in the receptors utilization for cellular entry. Glycosylation

plays an important role in both virus entry into host cells and in modulating the host-response (innate and adaptive immune response) towards ZIKV virus. Understanding the unique Zika viral and host factors that determine the tissue tropism and thereby disease severity could delineate the mechanism of Zika viral infections. Therefore, elucidating the ZIKV-host interactions could possibly aid in the development of diagnostic tools and effective therapeutics to prevent Zika viral infections and associated diseases in future outbreaks.

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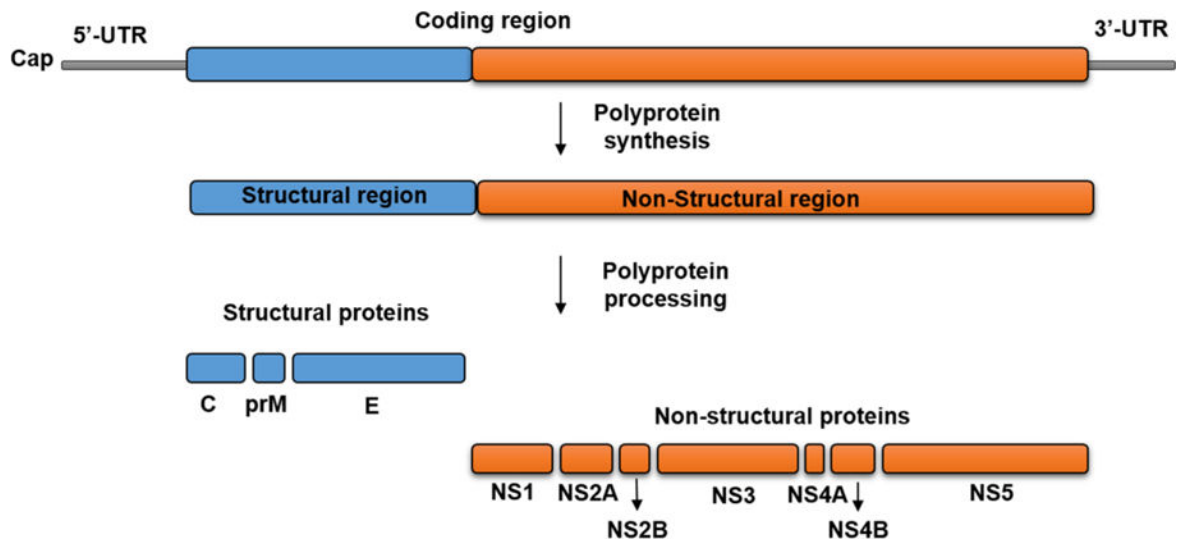


Figure 1. Schematic representation of Flaviviruses genome organization, and polyprotein processing. The genome of flaviviruses contains single open reading frame, flanked by 5'- and 3'-UTRs. The ORF translated into a polyprotein is cleaved into three structural proteins -C, prM and E (blue color) and seven non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (orange color).

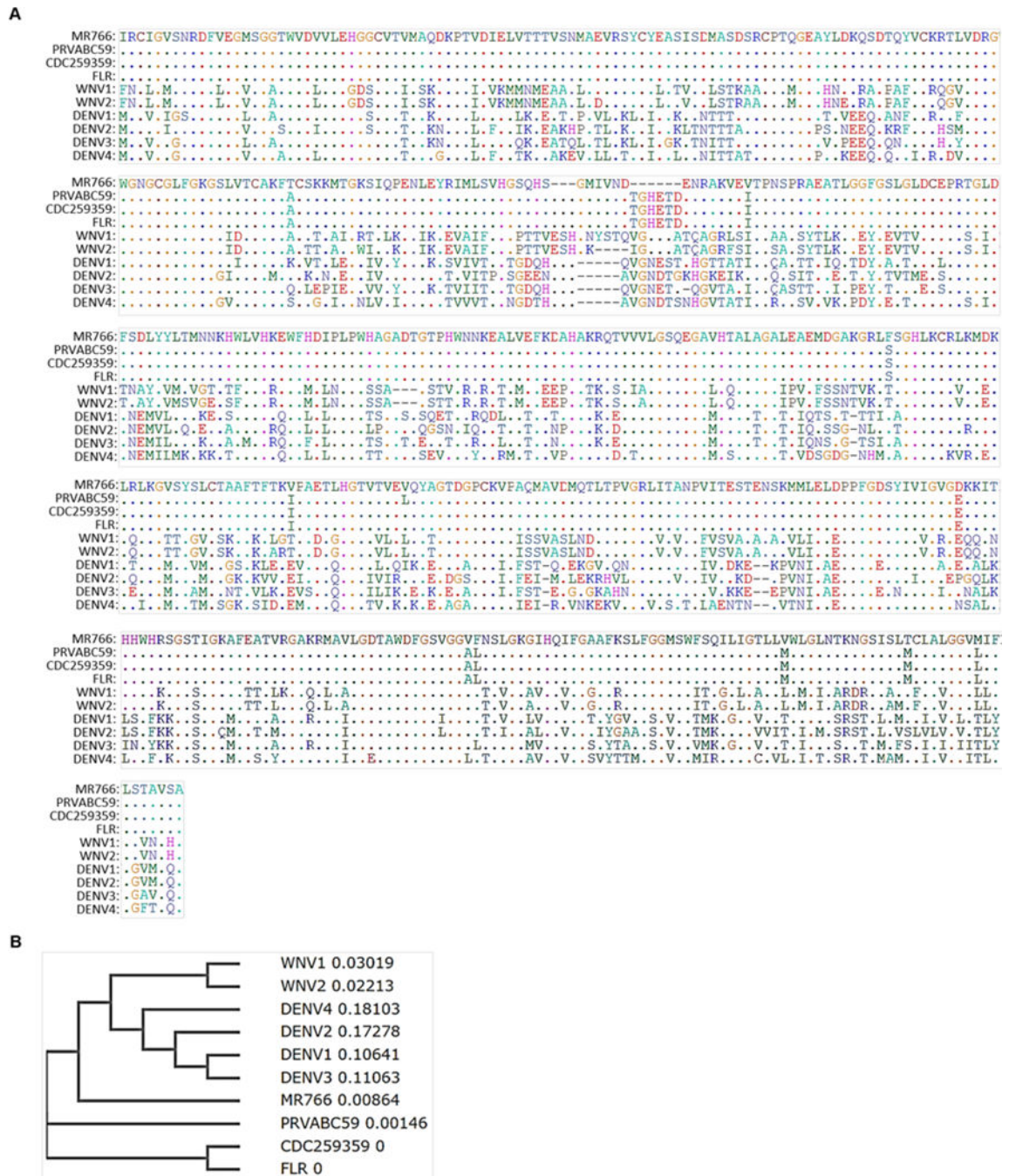


Figure 2. Multiple sequence alignment of glycoprotein E of Flaviviruses. The multiple sequence alignment of flaviviruses glycoprotein E sequences from Zika virus (MR766, PRVABC59, CDC259359, and FLR), WNV (lineage 1 and 2), and Dengue (Serotype 1, 2, 3 and 4) were analyzed. and The common residues between sequences were represented as dot and was analysed using bioEdit tool.

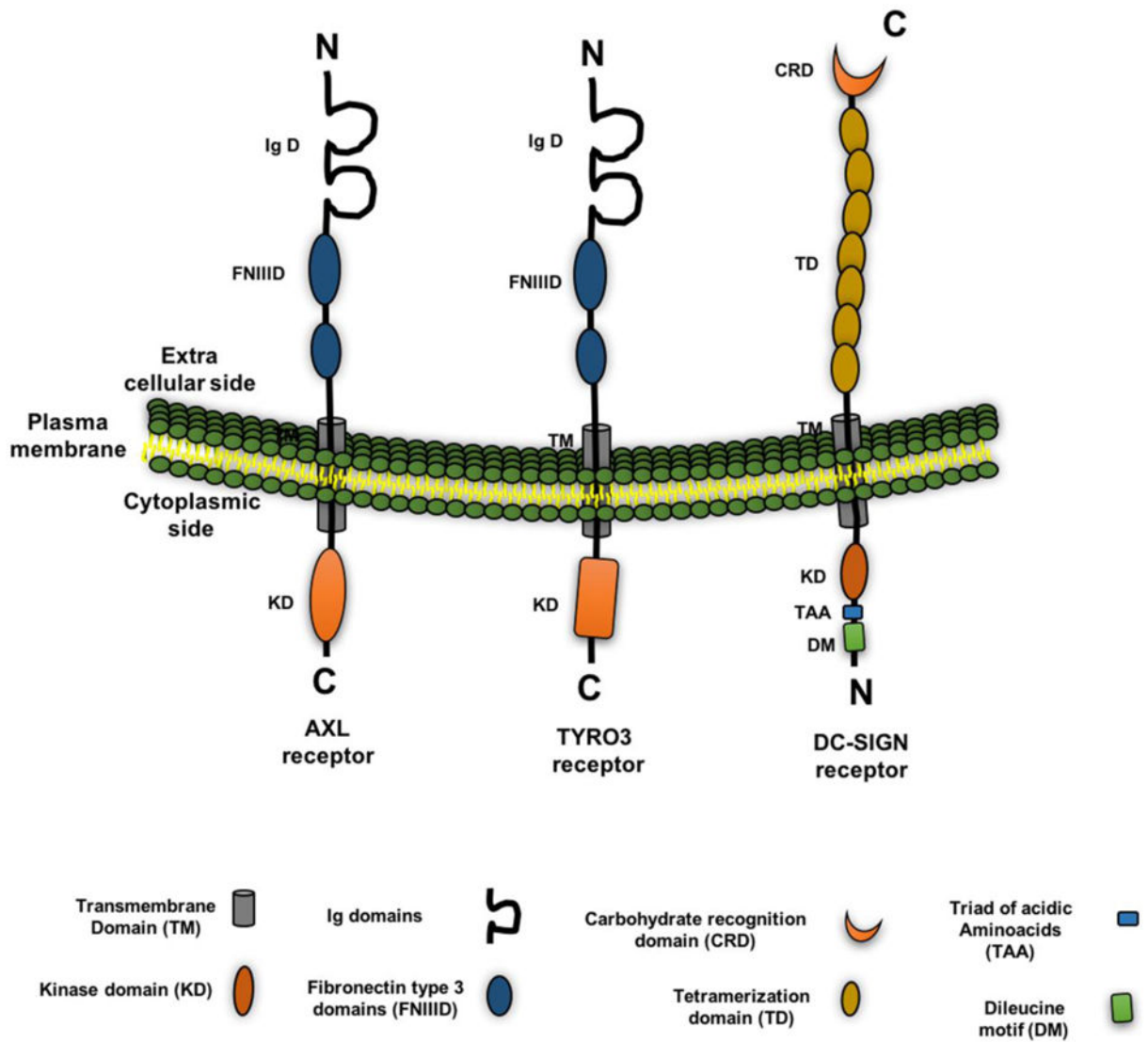


Figure 3.

Flavivirus receptors and their domain organization. Figure represents various receptor(s) expressed on the cell such as TIM (T-cell immunoglobulin and mucin domain), TAM (TYRO3, and AXL), and C-type lectin (DC-SIGN) that are known to interact with the Zika virus and other flaviviruses and to facilitate their entry. The domain organization of each receptor(s) such as transmembrane domain (TM), kinase domain (KD), Ig domain, Fibronectin type 3 domains (FNIID), carbohydrate recognition domain (CRD), triad of acidic aminoacids (TAA), tetramerization domain (TD), and dileucine motif (DM) are represented in the figure with color and a particular shape.

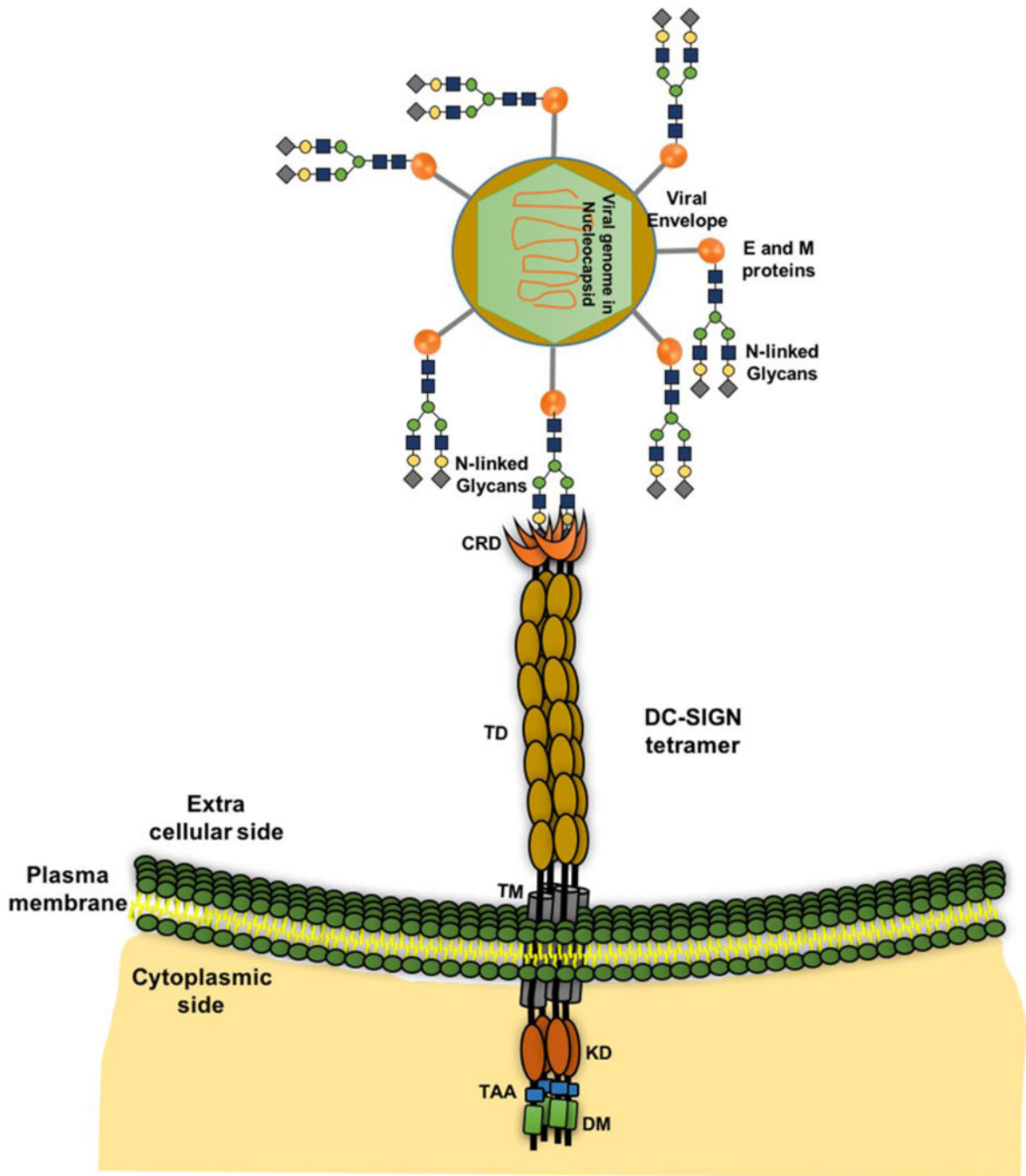


Figure 4. Interaction between glycan (glycoprotein E) and receptor (DC-SIGN). Representation of the host-virus interactions through DC-SIGN tetramer on cell surface and N-linked glycans on glycoprotein of flavivirus are shown.

Table 1

Key events in Zika virus disease. Table representing crucial events that are taken place from the Zika virus (ZIKV) identification to the therapeutics development.

| Year | Incident | References |
|------|---|---|
| 1947 | Identified in the Zika forest of Uganda in a rhesus monkey. | (Dick, Kitchen et al. 1952) |
| 1948 | Isolated from infected <i>Aedes africanus</i> , in the Zika forest. | (Dick 1952) |
| 1952 | Serum analysis revealed presence of neutralizing antibodies in infected individuals in East Africa. | (Smithburn 1952) |
| 1964 | A researcher infected with Zika, presented mild illness. | (Macnamara 1954) |
| 2007 | Zika first large human infection outbreak in the Pacific Island of Yap. Three quarters of yap residents got infected with Zika virus during the outbreak. | (Duffy, Chen et al. 2009) |
| 2008 | First documented case of sexual transmission. | (Foy, Kobylinski et al. 2011) |
| 2012 | Phylogenetic analysis indicating the geographic expansion of African MR766 to Asian lineage EC Yap (Yap State, Micronesia, 2007). | (Haddow, Schuh et al. 2012) |
| 2014 | Outbreaks on Easter Island, French Polynesia (FP), the Cook Islands, and New Caledonia. | (Cao-Lormeau and Musso 2014; Musso 2015) |
| | 1,505 asymptomatic blood samples were analyzed during FP outbreak and 42 donors have shown Zika virus infection. | (Musso, Nhan et al. 2014; Musso, Roche et al. 2015) |
| | Zika virus can be transmitted sexually and through blood transfusions. | |
| 2015 | Many places including Brazil, Cabo Verde, Colombia, Suriname, El Salvador, French Polynesia, Mexico, Paraguay and Panama reported the Zika viral infections. | (Kindhauser, Allen et al. 2016) |
| | Brazil's National Reference Laboratory confirmed that Zika virus is circulating in the country and is correlating with the increase in neurological disorders (Guillain-Barre syndrome and microcephaly). | |
| 2016 | Brazil reports 3,893 suspected microcephaly cases, and 1,708 cases of Guillain-Barre syndrome cases, including 49 deaths. | (Kindhauser, Allen et al. 2016) |
| | The Vector Control Advisory Group recommend two new tools to control the Zika virus transmitting <i>Aedes</i> mosquitoes. | (WHO 2016) |
| | Revised Zika Strategic Response Plan launched by WHO/PAHO and partners, with major importance given to preventing Zika disease and managing Zika associated medical complications. | (WHO 2016) |
| | WHO generates a Target Product Profile for Zika vaccines to use in emergency conditions against congenital Zika syndrome and more than 55 diagnostic kits have been developed. | (Hombach, Friede et al. 2016) |

Table 2

Percentage of sequence similarities between Zika virus (Asian and African lineages) and other flaviviruses glycoprotein E sequences were calculated using BioEdit tool.

| Strain | MR766 | PRVABC59 | CDC259359 | FLR | WNV1 | WNV2 | DENV1 | DENV2 | DENV3 | DENV4 |
|-----------|-------|----------|-----------|------|------|------|-------|-------|-------|-------|
| MR766 | 100 | | | | | | | | | |
| PRVABC59 | 96.6 | 100 | | | | | | | | |
| CDC259359 | 96.8 | 99.8 | 100 | | | | | | | |
| FLR | 96.8 | 99.8 | 100 | 100 | | | | | | |
| WNV1 | 53.7 | 54 | 53.8 | 53.8 | 100 | | | | | |
| WNV2 | 54.5 | 54.8 | 54.6 | 54.6 | 94 | 100 | | | | |
| DENV1 | 56.5 | 56.5 | 56.5 | 56.5 | 49.1 | 49.8 | 100 | | | |
| DENV2 | 53.3 | 52.5 | 52.7 | 52.7 | 46.3 | 47.4 | 68.4 | 100 | | |
| DENV3 | 56.6 | 56.7 | 56.9 | 56.9 | 45.3 | 45.6 | 77.9 | 67.6 | 100 | |
| DENV4 | 54.7 | 54.5 | 54.7 | 54.7 | 48.3 | 48.4 | 63.8 | 63.6 | 63.2 | 100 |

Table 3

Predicted potential glycosylation sites in the glycoprotein E of Flaviviruses. Table represents the potential glycosylation sites in the glycoprotein E sequences of -ZIKV strains (MR766, PRVABC59, CDC259359, and FLR), WNV (lineage 1 and 2), and Dengue (Serotype 1, 2, 3 and 4) and predicted using NetNGlyc tool. The default threshold of 0.5, represents a predicted glycosylated site in Asn-X-Ser/Thr, X is any amino acid except proline. The 'potential' score is the averaged output of nine neural networks. The jury agreement column indicates how many of the nine networks support the prediction. The N-Glyc Result column shows one of the following outputs for predictions indicating glycosylated sites: + Potential > 0.5; ++ Potential > 0.5 and Jury agreement (9/9).

| S.no | Strain | Position | Potential | Jury agreement | N-Glyc results |
|------|----------------|----------|-----------|----------------|----------------|
| 1 | Zika MR766 | - | - | - | - |
| 2 | Zika PRVABC59 | 154 NDTG | 0.63 | (6/9) | + |
| | | 481 NGSi | 0.36 | (8/9) | - |
| 3 | Zika CDC259359 | 154 NDTG | 0.63 | (6/9) | + |
| | | 481 NGSi | 0.36 | (8/9) | - |
| 4 | Zika FLR | 154 NDTG | 0.63 | (6/9) | + |
| | | 481 NGSi | 0.36 | (8/9) | - |
| 5 | WNV1 | 154 NYST | 0.68 | (9/9) | ++ |
| 6 | WNV2 | - | - | - | - |
| 7 | DENV1 | 67 NTTT | 0.66 | (7/9) | + |
| | | 153 NEST | 0.54 | (6/9) | + |
| 8 | DENV2 | 67 NTTT | 0.65 | (9/9) | ++ |
| | | 153 NDTG | 0.55 | (6/9) | + |
| 9 | DENV3 | 67 NITT | 0.69 | (8/9) | + |
| | | 153 NETQ | 0.63 | (9/9) | ++ |
| 10 | DENV4 | 470 NTSM | 0.26 | (8/9) | -- |
| | | 67 NITT | 0.7 | (8/9) | + |
| | | 153 NDTS | 0.65 | (8/9) | + |
| | | 472 NTSM | 0.32 | (8/9) | - |