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Research review paper

Zika virus structural biology and progress in vaccine development

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ABSTRACT

The growing number of zika virus (ZIKV) infections plus a 20-fold increase in neonatal microcephaly in newborns in Brazil have raised alarms in many countries regarding the threat to pregnant women. Instances of microcephaly and central nervous system malformations continue to increase in ZIKV outbreak regions. ZIKV is a small enveloped positive-strand RNA virus belonging to the *Flavivirus* genus of the Flaviviridae family. High-resolution ZIKV structures recently identified by cryo-electron microscopy indicate that the overall ZIKV structure is similar to those of other flaviviruses. With its compact surface, ZIKV is more thermally stable than the dengue virus (DENV). ZIKV E proteins have a characteristic "herringbone" structure with a single glycosylation site. The ZIKV E protein, the major protein involved in receptor binding and fusion, is formed as a head-to-tail dimer on the surfaces of viral particles. The E monomer consists of three distinct domains: DI, DII, and DIII. The finger-like DII contains a fusion loop (FL) that is inserted into the host cell endosomal membrane during pH-dependent conformational changes that drive fusion. Quaternary E:E dimer epitopes located at the interaction site of prM and E dimers can be further divided into two dimer epitopes. To date, more than 50 ZIKV vaccine candidates are now in various stages of research and development. Candidate ZIKV vaccines that are currently in phase I/II clinical trials include inactivated whole viruses, recombinant measles viral vector-based vaccines, DNA and mRNA vaccines, and a mosquito salivary peptide vaccine. Stabilized forms of ZIKV E:E dimer proteins have been successfully obtained either by introducing additional inter-subunit disulfide bond(s) in DII or via the direct assembly of E:E dimer proteins by immobilization with monomeric E proteins. The VLP-based approach is another alternative method for presenting native E:E dimer antigens among the vaccine components. Several forms of ZIKV VLPs have been reported featuring the co-expression of the prM-E, prM-E-NS1, C-prM-E, and NS2B/NS3 viral genes in human cells. To minimize the effect of the cross-reactive ADE-facilitating antibodies between ZIKV and DENV, several novel mutations have been reported either in or near the FL of DII or DIII to dampen the production of cross-reactive antibodies. Future ZIKV vaccine design efforts should be focused on eliciting improved neutralizing antibodies with a reduced level of cross-reactivity to confer sterilizing immunity.

1. Introduction

The Zika virus (ZIKV) was first identified in a blood sample taken from a rhesus monkey captured in the Zika forest of Uganda in 1947. The first human infection was reported in 1952 in Nigeria (Macnamara, 1954), and one year later the virus was isolated from *Aedes* mosquitoes (Dick et al., 1952). The first isolation of ZIKV in Asia was from *Aedes aegypti* mosquitoes in Malaysia in 1969 (Marchette et al., 1969). ZIKV infections in humans have been confirmed by sero-surveillance in Africa, the Indian subcontinent, and Southeast Asia, but clinical cases have been limited (Plourde and Bloch, 2016). The first major outbreak occurred on Yap Island in the Federated States of Micronesia in 2007, with 73% of the population (approximately 8000 individuals) being infected, but with only mild and short-lived symptoms reported (Duffy et al., 2009). The second significant outbreak occurred in French Polynesia in 2013–2014, with a 66% overall infection rate (approximately 183,000 individuals) (Cao-Lormeau et al., 2014). Some of these infections were associated with neurological abnormalities such as Guillain-Barre syndrome (Cao-Lormeau et al., 2016). ZIKV infections in Brazil in 2014 (Campos et al., 2015) were blamed for a 20-fold increase in neonatal microcephaly (Schuler-Faccini et al., 2016). The ZIKV epidemic has continued to spread throughout South and Central America (Fig. 1) (Fauci and Morens, 2016; Ferguson et al., 2016), and in February of 2016 the World Health Organization (WHO) declared a "Public Health Emergency of International Concern" (WHO, 2016).

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Fig. 1. World map of area with risk of zika, according to the Centers for Disease Control and Prevention (CDC, 2017)

Although the post-ZIKV infection hospitalization rate is low, and ZIKV-related fatalities are rare, recent reports indicate a possible correlation between ZIKV infection and Guillain-Barré syndrome (GBS), an autoimmune disorder of the peripheral nervous system leading to muscle weakness, numbness, paralysis, and occasional deaths (van den Berg et al., 2013). Increases in GBS incidence were reported during the 2013–2014 ZIKV outbreak in French Polynesia (Cao-Lormeau et al., 2016; Oehler et al., 2014) and by the Brazilian Ministry of Health during the 2015 outbreak in that country (de Araújo et al., 2016). To date, 23 countries and territories have reported increases in GBS that may be related to ZIKV infections (WHO, 2017a). Outbreaks during the past five years have also done much to confirm the relationship between ZIKV infection during pregnancy and microcephaly and neurological disorders (Schuler-Faccini et al., 2016). A total of 31 countries and territories have reported increases in microcephaly and other central nervous system malformations that can be associated with ZIKV infections (WHO, 2017a). The strongest evidence of a link is from French Polynesia and Brazil (Cauchemez et al., 2016; Franca et al., 2016). Persistent ZIKV replication and viremia in fetal brains and placentas have been identified as the most likely causes of microcephaly (Bhatnagar et al., 2017; Suy et al., 2016). There is evidence indicating that ZIKV can be sexually transmitted (D'Ortenzio et al., 2016; Nutt and Adams, 2017).

2. Structural biology

ZIKV is a small enveloped positive-strand RNA virus belonging to the *Flavivirus* genus of the Flaviviridae family (Lindenbach and Rice, 2003). In all flaviviruses, the RNA genome encodes three structural (core C, membrane precursor prM, and envelope E) and seven non-structural genes (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5), with untranslated region (UTR) genes flanking the 5′ and 3′ ends (Fig. 2A) (Lindenbach and Rice, 2003). The flavivirus particle assembly process entails (i) the interaction of prM and E proteins leading to heterodimer formation in the endoplasmic reticulum, (ii) genomic RNA encapsulation by the C protein and enclosure by cell membrane-derived lipid bilayers containing prM and E proteins to form immature virions, and (iii) the cleaving of prM proteins to M proteins via furin or a furin-like protease in the *trans*-Golgi network, thereby triggering viral particle release (Lindenbach and Rice, 2003) (Fig. 2B). The flavivirus E protein, the major protein involved in receptor binding and fusion, is

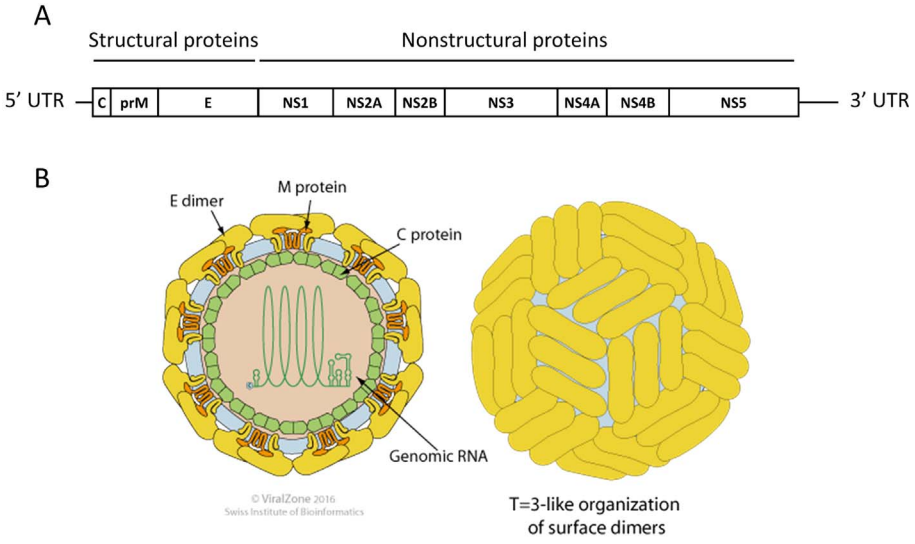

Fig. 2. (A) The ZIKV RNA containing a single open reading frame that encodes three structural and seven nonstructural proteins flanked by two untranslated regions (UTR). (B) The ZIKV virions are enveloped, spherical, about 50 nm in diameter and the surface prM/M and E proteins are arranged in an icosahedral-like T = 3 symmetry. Pictures are modified from ViralZone, SIB Swiss Institute of Bioinformatics (http://viralzone.expasy.org/6756?outline=all_by_protein).