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Review

Recent advances in understanding the adaptive immune response to Zika virus and the effect of previous flavivirus exposure

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ABSTRACT

Zika virus (ZIKV) caused explosive epidemics across the Americas, starting in Brazil in 2015, and has been associated with severe manifestations such as microcephaly in babies born to infected mothers and Guillain-Barré syndrome in adults. As the underlying mechanisms of pathogenesis remain largely unknown, diverse investigations have focused on a potential role for flavivirus cross-reactive antibodies in enhancing ZIKV infection. Antibody-dependent enhancement is especially concerning due to structural similarities between ZIKV and other flaviviruses, especially dengue virus (DENV), that co-circulate in areas affected by ZIKV. Conversely, investigating cross-neutralizing antibodies is important for understanding protection among flaviviruses, including ZIKV. In this review, we discuss the latest findings regarding ZIKV-induced adaptive immunity, such as monoclonal and polyclonal antibody responses, structural immunology, and T cell-mediated responses. Much progress has been made in a short amount of time, but many questions remain. Fully understanding the specificity, magnitude, and kinetics of B cell/antibody and T cell responses in ZIKV-infected individuals with or without prior exposure to flaviviruses is of great relevance for diagnostics and vaccine development.

1. Introduction

Zika, declared "a public health emergency of international concern" by the World Health Organization (WHO) in 2016 (WHO, 2016), spread rapidly across the Americas (ECDC, 2016) after being introduced into Brazil in 2014 (Faria et al., 2017; Zanluca et al., 2015). Recently, Zika virus (ZIKV) circulation has been reported throughout Latin America, the Caribbean, the Pacific Islands, and to some extent in Southeast Asia (ECDC, 2016). The major ZIKV genetic lineages, namely African and Asian (Haddow et al., 2012; Metsky et al., 2017), are carried by *Aedes* mosquitoes as well as other species (Musso and Gubler, 2016). In addition to possibly via blood transfusion (Herriman, 2015), non-vector forms of transmission of ZIKV include sexual (Hills et al., 2016), congenital (de Oliveira et al., 2016), and perinatal (Besnard et al., 2014) routes, making it unique from other flaviviruses affecting humans. ZIKV has been detected in the blood (Waggoner et al., 2016), urine (Gourinat et al., 2015; Abd El Wahed et al., 2017), saliva (Barzon et al., 2016), semen (Mansuy et al., 2016), cerebrospinal fluid (Rozé et al., 2016), vaginal or cervical secretions (Nicastri et al., 2016; Prisant et al., 2016), and other human body fluids by reverse transcriptase-polymerase chain reaction (RT-PCR). ZIKV remained detectable for up to 29 days after onset of symptoms in saliva (Barzon et al., 2016) and up to 80 days in semen (Paz-Bailey et al., 2017), and prolonged viremia has been reported in pregnant women (Driggers et al., 2016; Suy et al., 2016). Studies in mice have shown that ZIKV is able to replicate in immune-privileged sites, such as the eyes (Jampol and Goldstein, 2016) and testes (Govero et al., 2017; Ma et al., 2017), which could complicate the control and treatment of infection. Investigation of the timeline of ZIKV persistence in immune-privileged sites and body fluids has important implications for diagnostic recommendations and prevention of transmission.

ZIKV infection has been historically associated with a mild, self-limiting acute febrile illness (Duffy et al., 2009; Simpson, 1964). However, the massive epidemic that emerged in the Americas in 2015 and previous outbreaks in French Polynesia (Nishiura et al., 2016) have elicited major concerns due to the association of ZIKV infection with microcephaly, congenital malformations, and fetal demise (van der Eijk et al., 2016). The neurodevelopmental pathogenesis may be explained by the tropism of ZIKV to neural progenitor cells (Tang et al., 2016), with apoptosis triggered following infection (Dang et al., 2016; Onorati et al., 2016). In addition to the fetal human brain, ZIKV has been found in cord blood, several types of placental cells, and amniotic fluid (Bhatnagar et al., 2017). *Ex vivo* studies have documented ZIKV infection of primary human placental cells and explants of the human placenta, including cytotrophoblasts, endothelial cells, fibroblasts, and Hofbauer cells in chorionic villi, and amniotic epithelial cells and

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trophoblast progenitors in amniochorionic membranes (Tabata et al., 2016). In adults, Guillain-Barré Syndrome (GBS) has been associated with ZIKV infection. GBS is characterized by ascending paralysis and polyneuropathy (Oehler et al., 2014), which can occur after and even during ZIKV infection (do Rosário et al., 2016; Siu et al., 2016). Autoimmune responses triggered by pathogen exposure have been implicated in the pathogenesis of GBS (Shoenfeld et al., 1996), though the short time window between ZIKV infection and GBS onset has raised questions of potential direct viral pathogenesis (PAHO, 2015).

The underlying mechanisms that drive severe outcomes of ZIKV infection remain unknown. ZIKV is a member of the flavivirus genus of the *Flaviviridae* family, along with the four serotypes of dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV) and yellow fever virus (YFV). In dengue, it has been well established that secondary infection with a heterologous serotype is the main risk factor for severe disease (Halstead et al., 1970; Halstead and Yamarata, 1965), due in part to a hypothesized role of poorly neutralizing cross-reactive antibodies that can enhance DENV infection (Halstead et al., 1970). This phenomenon, demonstrated *in vitro* and in animal models, is referred to as antibody-dependent enhancement (ADE). ADE is triggered by cross-reactive antibodies targeting envelope (E) and pre-membrane/membrane (prM/M) proteins that fail to neutralize the infecting virus while facilitating virus entry into Fcγ receptor-bearing immune cells, resulting in immune activation and a "cytokine storm" that eventually leads to endothelial permeability and vascular leak (Guzman et al., 2003). Phylogenetic analysis using the amino acid sequences of the E protein indicates that ZIKV is more closely related to the DENV serotypes than other flaviviruses (Barba-Spaeth et al., 2016). Given this degree of similarity between ZIKV and DENV, it is hypothesized that the shared epitope repertoire could potentially enable pre-existing cross-reactive antibodies to enhance ZIKV infection and possibly lead to severe clinical manifestations. However, whether individuals with previous DENV immunity develop a more severe ZIKV infection or have a higher risk of fetal transmission of the virus is unknown. Similarly, it is not established whether anti-ZIKV antibodies impact subsequent DENV infection. Therefore, studies that dissect the level of cross-reactive immunity at the B- and T-cell level in response to ZIKV infection are urgently needed.

In the current review, we address the most relevant findings regarding adaptive immunity of ZIKV and its interplay with other flavivirus infections, focusing on the impact of prior DENV exposure. Dissecting the host immune response to ZIKV infection is critical for development of novel therapeutics and a safe and effective ZIKV vaccine.

1.1. Structural immunology of ZIKV: findings from studies with monoclonal antibodies

In addition to the three structural proteins, capsid (C), prM/M, and E, the ZIKV 11-kb positive-stranded RNA genome also encodes for 7 non-structural proteins (Klema et al., 2015). Similarly to DENV, the mature ZIKV virion is comprised of 180 copies of the E protein, which are arranged as 90 anti-parallel homodimers configured in icosahedral symmetry (Zhang et al., 2013). The E protein is the main target of flavivirus neutralizing antibodies and is composed of three domains, EDI, EDII and EDIII (Roehrig, 2003). EDI contains the N terminus, EDII contains the fusion loop that mediates viral fusion in the endosome, and EDIII is an immunoglobulin-like domain that is involved in attachment to host cells (Modis et al., 2003; Zhang et al., 2004). Analysis of ZIKV E protein sequence in comparison to other flaviviruses shows homology ranging from 40 to 58% (Kostyuchenko et al., 2016), with the highest similarity found with DENV serotypes (55–56%) (Xu et al., 2016). Similarly, superposition of the ZIKV cryo-EM structure with the DENV2 cryo-EM structure (Zhang et al., 2013) and crystal structures of WNV (Nybakken et al., 2006) and JEV (Luca et al., 2012) E proteins shows a high level of structural homology between ZIKV and other flaviviruses

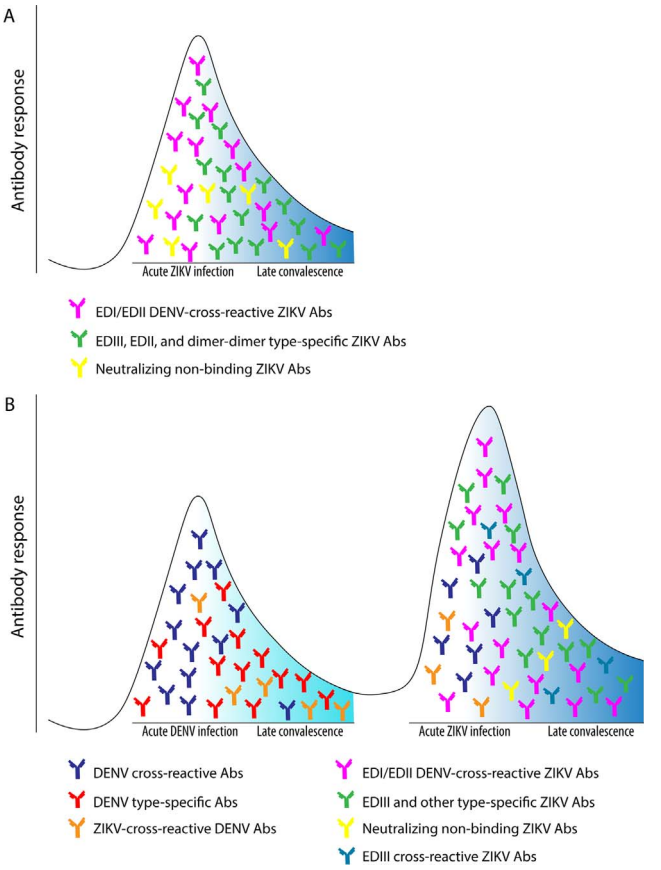

Fig. 1. Polyclonal antibody responses in DENV-naïve and DENV-immune ZIKV infections. **A**. The polyclonal sera of ZIKV-infected individuals without previous DENV immunity include ZIKV-specific, as well as some DENV-ZIKV cross-reactive antibodies. **B**. In polyclonal sera from DENV-immune ZIKV-infected individuals, ZIKV cross-reactive DENV antibodies from the previous infection, as well as ZIKV-specific antibodies and ZIKV-DENV cross-reactive ZIKV antibodies are generated. See references (Robbiani et al., 2017; Sapparapu et al., 2016; Stettler et al., 2016; Wang et al., 2016).

(Sirohi et al., 2016). However, ZIKV appears to be more thermally stable than DENV and presents a more compact surface (Kostyuchenko et al., 2016).

Monoclonal antibodies (mAbs) isolated from ZIKV-infected patients, either DENV-immune or DENV-naïve, have been fundamental in dissecting the antibody responses that are specific to ZIKV or cross-reactive with DENV; current understanding based on these studies is summarized in Fig. 1. As shown by sequence alignment studies, EDII contains highly conserved residues between ZIKV and DENV (Sirohi et al., 2016). The ability of the majority of ZIKV EDI/II-specific mAbs isolated from ZIKV donors to cross-neutralize DENV serotypes reflects the high degree of conserved epitopes between ZIKV and DENV. Conversely, DENV EDI/II-targeted mAbs from DENV donors also cross-reacted with ZIKV E protein (Stettler et al., 2016). In contrast, most of the ZIKV and DENV EDIII-reactive mAbs isolated from either ZIKV or DENV donors were specific to ZIKV or DENV E protein, respectively (Stettler et al., 2016). In addition, the neutralization potential of EDIII-specific mAbs was higher compared to EDI/II specific mAbs. The EDIII-specific ZKA64 mAb, containing mutations engineered in the Fc region that eliminate binding to FcγR and complement (LALA mutant), protected A129 mice from a lethal dose of ZIKV in a prophylactic setting (Stettler et al., 2016). Another highly neutralizing ZIKV-specific mAb targeting the domain I–III linker and the lateral ridge (LR) region of EDIII is therapeutic in mouse models (Davide Corti, personal communication). While mouse mAbs targeting EDIII play an important role in DENV neutralization, EDIII-specific antibodies do not constitute a large percentage of the human anti-DENV antibody repertoire (Beltramello