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## 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.

### Introduction

Zika, declared “a public health emergency of international concern” by the World Health Organization (WHO) in 2016 (2016), spread rapidly across the Americas (2016) after being introduced into Brazil in 2014 (Faria, 2017; Zanoluca, 2015). Recently, Zika virus (ZIKV) circulation has been reported throughout Latin America, the Caribbean, the Pacific Islands, and to some extent in Southeast Asia (1997). The major ZIKV genetic lineages, namely African and Asian (Haddow, 2012; Metsky, 2017), is carried by *Aedes* mosquitoes as well as other species (Musso, 2016). In addition to possibly via blood transfusion (Herriman, 2015), non-vector forms of transmission of ZIKV include sexual (Hills, 2016), congenital (de Oliveira, 2016), and perinatal (Besnard, 2014) routes, making it unique from other flaviviruses affecting humans. ZIKV has been detected in the blood, urine (Gourinat, 2015),

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saliva (Barzon, 2016), semen (Mansuy, 2016), cerebrospinal fluid (Rozé, 2016), vaginal or cervical secretions (Nicastri, 2016; Prisant, 2016), and other human body fluids by reverse transcriptase-polymerase chain reaction (RT-PCR) (Abd El Wahed, 2017). ZIKV remained detectable for up to 29 days after onset of symptoms in saliva (Barzon, 2016) and up to 80 days in semen (Paz-Bailey, 2017), and prolonged viremia has been reported in pregnant women (Driggers, 2016; Suy, 2016). Studies in mice have shown that ZIKV is able to replicate in immune-privileged sites, such as the eyes (Jampol, 2016) and testes (Ma, 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, 2009; Simpson, 1964). However, the massive epidemic that emerged in the Americas in 2015 and previous outbreaks in Micronesia (Duffy, 2009) and French Polynesia (Nishiura, 2016) have elicited major concerns due to the association of ZIKV infection with microcephaly, congenital malformations, and fetal demise (van der Eijk, 2016). The neurodevelopmental pathogenesis may be explained by the tropism of ZIKV to neural progenitor cells (Tang, 2016), with apoptosis triggered following infection (Dang, 2016; Onorati, 2016). In addition to the fetal human brain, ZIKV has been found in cord blood, several types of placental cells, and amniotic fluid (Bhatnagar, 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 trophoblast progenitors in amniochorionic membranes (Tabata, 2016). In adults, Guillain-Barré Syndrome (GBS) has been associated with ZIKV infection. GBS is characterized by ascending paralysis and polyneuropathy (Oehler, 2014), which can occur after ZIKV infection (do Rosário, 2016; Siu, 2016). Auto-immune responses triggered by pathogen exposure have been implicated in the pathogenesis of GBS (Shoenfeld, 1996), though the short time window between ZIKV infection and GBS onset has raised questions of potential direct viral pathogenesis (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, 1970; Halstead, 1965), due in part to a hypothesized role of poorly neutralizing cross-reactive antibodies that can enhance DENV infection (Halstead, 1970; Halstead, 1965). 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 Fc $\gamma$  receptor-bearing immune cells, resulting in immune activation and a “cytokine storm” that eventually leads to endothelial permeability and vascular leak (Guzman, 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, 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 potentially 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.

### **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, 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, 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, 2003; Zhang, 2004). Analysis of ZIKV E protein sequence in comparison to other flaviviruses shows homology ranging from 40 to 58% (Kostyuchenko, 2016), with the highest similarity found with DENV serotypes (55–56%) (Xu, 2016). Similarly, superposition of the ZIKV cryo-EM structure with the DENV2 cryo-EM structure (Zhang, 2013) and crystal structures of WNV (Nybakken, 2006) and JEV E proteins (Luca, 2012) shows a high level of structural homology between ZIKV and other flaviviruses (Sirohi, 2016). However, ZIKV appears to be more thermally stable than DENV and presents a more compact surface (Kostyuchenko, 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. As shown by sequence alignment studies, EDII contains highly conserved residues between ZIKV and DENV (Sirohi, 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, 2016). In contrast, most of the EDIII-reactive mAbs isolated from either ZIKV or DENV donors were specific to ZIKV or DENV E protein, respectively (Stettler, 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 Fc $\gamma$ R and complement (LALA mutant), protected A129 mice from a lethal dose of ZIKV in a prophylactic setting (Stettler, 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, 2010). In another study, sera of ZIKV-infected individuals displaying high anti-ZIKV antibody titers were shown to bind ZIKV EDIII (ZEDIII) (Robbiani, 2017). Memory B cells (MBCs) were sorted by ZEDIII binding, and the frequency of ZEDIII-specific MBCs was found to range from 0-13-1.98% of the total circulating IgG<sup>+</sup> MBCs. ZEDIII-binding mAbs were derived from 6 individuals, and expanded clones sharing the same IGH and IGL variable gene segments were found across individuals from two different locations, in Mexico and Brazil. Further characterization of these mAbs demonstrated cross-reactivity to DENV1 EDIII but not to other DENV serotypes, WNV or YFV. Binding to ZEDIII and DENV1 EDIII were shown to be associated with somatic mutation of MBCs, as reversion of the mutations to the germ line sequence impaired binding to ZIKV and DENV1 antigen. The observation that antibodies derived from expanded clones that neutralize DENV1 and ZIKV suggest that prior exposure to DENV1 may prime the development of ZIKV immunity (Robbiani, 2017). Further studies are needed to determine the kinetics and durability of ZIKV-specific EDIII neutralizing antibodies and how previous DENV infection shapes the MBC repertoire.

A significant portion of the antibody response to flaviviruses targets the highly conserved fusion loop epitope (FLE) in EDII (Beltramello, 2010), but this antibody subpopulation is weakly neutralizing (Deng, 2011). Notably, however, a broadly cross-reactive mouse mAb mapped to the FLE binds and neutralizes DENV1-4, YFV, JEV, and TBEV (Deng, 2011). This mAb, designated 2A10G6, has been previously shown to confer protection from lethal challenge with DENV1-4 and WNV in a mouse model (Deng, 2011), as well as against lethal ZIKV infection (Dai, 2016).

Importantly, a subset of potent neutralizing antibodies against ZIKV recognize quaternary epitopes that are displayed on the intact virion but not on the recombinant E protein, as recently demonstrated for DENV (Dejnirattisai, 2015; Fibriansah, 2014; Gallichotte, 2015). Among this class of antibodies is ZIKV-117, isolated from a subject previously infected with ZIKV, which binds specifically to the EDII domain across two adjacent dimers, defined as the “dimer-dimer” interface (Hasan, 2017). This potently neutralizing mAb was able to neutralize all ZIKV strains tested, including African, Asian, and American strains. ZIKV-117 did not bind to WNV E protein or cells infected with DENV1-DENV4, confirming its type-specificity. To evaluate a potential protective effect of ZIKV-117 *in vivo*, immunocompetent C57BL/6 mice were treated with an anti-interferon- $\alpha/\beta$  receptor (Ifnar1)-blocking mAb, making them susceptible to ZIKV infection. Administration of ZIKV-117 on days 1 and 5 post-inoculation with a mouse-adapted ZIKV strain was shown to be protective in a mouse model (Sapparapu, 2016). Further, administration of ZIKV-117 to Ifnar1<sup>-/-</sup> pregnant mice on embryo day 5.5, followed by ZIKV challenge, resulted in improved fetal outcome in pregnant female mice (Sapparapu, 2016). While these findings are promising, the extent to which these results translate from mice to humans remains unclear. ZIKV-specific MAbs that bind across EDI and EDII of different E monomers were isolated from a ZIKV-infected traveler and shown to protect against ZIKV challenge therapeutically in a mouse model (Wang et al., 2016). In another study, highly neutralizing mAbs that failed to

bind to ZIKV E and EDIII proteins were isolated from ZIKV-infected donors. These mAbs, called "neutralizing non-E-binding" (NNB), are thought to only recognize ZIKV quaternary epitopes that are displayed on the infectious virions but not on soluble proteins (Stettler, 2016).

Another example of antibodies that target quaternary epitopes on the surface of the intact virion is antibodies to the envelope dimer epitope (EDE) (Dejnirattisai, 2015). Isolated from DENV-immune individuals, EDE1 antibodies neutralize ZIKV *in vitro* (Barba-Spaeth, 2016; Dejnirattisai, 2016). When administered *in vivo*, the EDE1 mAb C10 was shown to protect AG129 mice, which are deficient in both type I and II interferon receptors, against lethal ZIKV infection (Swanstrom, 2016). This cross-neutralization phenotype may be due to the considerable conservation of the EDE1 contact residues between all four DENV serotypes and ZIKV (Rouvinski, 2015). Although these data suggest the EDE epitope may be critical in eliciting antibodies that cross-neutralize DENV and ZIKV, it should be noted that these antibodies were isolated from circulating plasmablasts in the two weeks following DENV infection. It is yet to be determined whether these plasmablasts mature into the MBC pool and if so, what the frequency of antibodies directed to this epitope is in populations living in dengue-endemic regions. Therefore, studies focused on describing EDE antibodies in human populations living in Zika- and dengue-endemic areas are needed.

Non-structural protein 1 (NS1) plays a role in viral replication (Suthar, 2013) and has been implicated in immune evasion and pathogenesis (Avirutnan et al., 2011; Beatty, 2015; Chung, 2006; Modhiran, 2015). Similarly to E protein, ZIKV NS1 structure has conserved features with other flaviviruses (Song, 2016). mAbs isolated from ZIKV-infected individuals without prior DENV infection history displayed limited cross-reactivity against DENV NS1, while mAbs isolated from ZIKV-infected patients with previous DENV exposure displayed a high degree of cross-reactivity. Reciprocally, NS1-reactive mAbs isolated from DENV-immune, ZIKV-naïve donors showed variable patterns of cross-reactivity between DENV serotypes, but only one mAb was found to be cross-reactive with ZIKV NS1 (Stettler, 2016). ZIKV-specific NS1 mAbs have been exploited to develop sensitive and specific serological assays, such as the NS1 blockade-of-binding ELISA that shows high sensitivity and specificity for ZIKV infection versus DENV infections, including secondary DENV infections (Angel, 2017).

### The human polyclonal response to ZIKV infection

The antigenic similarity between ZIKV and DENV, combined with early serological studies (Fagbami, 1987), indicate that cross-reactivity between ZIKV and DENV exists at the binding and neutralization levels. During the 2007 Yap Island epidemic, serum samples were tested by ELISA for anti-ZIKV IgM antibodies, and neutralizing titers to ZIKV were determined by plaque reduction neutralization titer (PRNT) assays (Duffy, 2009). However, diagnosis using acute and early convalescent samples was particularly challenging given the cross-reactivity between ZIKV and other flaviviruses and the number of patients with previous DENV exposure or yellow fever vaccination (Lanciotti, 2008).

To understand the immunological relation between ZIKV and DENV, it is useful to first review the current state of knowledge of the immune response to primary and secondary



DENV infections. Upon a first DENV infection, subpopulations of type-specific and cross-reactive antibodies are found in polyclonal human sera (de Alwis, 2012). Serotype-specific antibodies are strongly neutralizing to the homologous infecting serotype and are postulated to confer lifelong protection against disease caused by the same serotype. On the other hand, after primary DENV infection, cross-reactive antibodies are weakly neutralizing and are thought to potentially contribute to ADE of secondary DENV infections (Guzman, 2013; Halstead, 2003). In dengue, the quality of the neutralizing antibody response varies according to the number of sequential infections with different DENV serotypes. As shown by antibody depletion studies, in contrast to a predominantly type-specific neutralizing antibody response in individuals who experience primary DENV infection (de Alwis, 2012), in secondary infection, some individuals display a predominantly cross-reactive neutralizing response, whereas in others, both type-specific and cross-reactive antibodies contribute to neutralization (Patel, 2017). In individuals who experience two DENV infections, type-specific as well as cross-reactive neutralizing antibodies to the two infecting serotypes and cross-reactive antibodies to the other serotypes are elicited (Patel, 2017). It is presumed that the profile of only cross-reactive antibodies is characteristic of third or fourth DENV infections. In a context where ZIKV infection occurs in individuals with previous DENV immunity, the different number of prior DENV infections may influence the levels of antibody cross-reactivity between ZIKV and DENV.

To investigate cross-reactivity between DENV and ZIKV antibodies, acute sera from nine patients with RT-PCR-confirmed DENV infection were analyzed and found to bind to and neutralize ZIKV (Priyamvada, 2016). Given the abundance of cross-reactive antibodies at early time-points after infection, this finding is consistent with studies identifying a transient capacity to protect against heterologous infection immediately after flavivirus infection (Sabin, 1952). However, late convalescent DENV-immune sera collected years after primary DENV infection from returned travelers showed that most sera have limited cross-neutralization against ZIKV (Collins, 2017; Swanstrom, 2016), which resembles the type-specific response that is observed in returned travelers years after primary DENV infection with one serotype (de Alwis, 2012). In addition, despite the abundance of cross-reactive antibodies following secondary DENV infection, late convalescent DENV-immune sera from most individuals who had experienced repeat DENV infection poorly neutralized ZIKV compared to cross-neutralization of DENV serotypes (Collins, 2017; Swanstrom, 2016).

To further investigate whether individuals with previous DENV immunity develop cross-reactive antibodies that recognize conserved epitopes across the four DENV serotypes and ZIKV, or whether distinct subpopulations of antibodies targeting type-specific epitopes on DENV and ZIKV are generated, depletion studies were undertaken. For reference, depletion of primary DENV-immune sera with the homologous serotype greatly reduces neutralizing activity from sera collected years after infection, whereas depletion with heterologous serotypes does not reduce neutralizing antibody titers (de Alwis, 2012). The same technique was employed to determine the antibody subpopulations that neutralize DENV and ZIKV in late convalescent (>6 months post-infection) serum samples from individuals who were exposed to DENV prior to their ZIKV infection (Collins, 2017). As expected, in a primary ZIKV infection, depletion of DENV-cross-reactive antibodies did not affect ZIKV

neutralizing antibody titers. However, unlike secondary DENV infections, where neutralizing antibodies to the infecting serotype are composed of both type-specific and cross-reactive subpopulations (Patel, 2017), it was found that depletion of DENV cross-reactive antibodies did not affect anti-ZIKV neutralizing antibody titers, indicating that the cross-reactivity is limited to the DENV serocomplex (Collins, 2017). Altogether, this study suggests that a population of ZIKV-specific neutralizing antibodies is developed regardless of previous DENV immunity (Collins, 2017).

Cross-neutralization was also investigated in dengue-endemic areas. Well-characterized DENV-immune sera (primary and secondary) collected at 2–3 weeks, 3, 6, 12 and 18 months and 1, 2, and 3 years post-infection in Nicaragua were analyzed for neutralization to all four DENV serotypes and ZIKV. In accordance with previous studies (Collins, 2017; Swanstrom, 2016), long-lasting cross-reactive antibodies from DENV infection neutralized ZIKV, but to a much lower extent than they neutralized DENV. Conversely, convalescent and 3-month post-infection samples from ZIKV-infected individuals neutralized ZIKV to a much greater degree than they neutralized DENV1–4, regardless of previous DENV exposure (M. Montoya, H. Puerta-Guardo, L. Katzelnick, and E. Harris, unpublished). At the MBC level, children with RT-PCR-confirmed ZIKV infection with either no prior exposure to DENV or documented prior infection with DENV showed a strong type-specific response to ZIKV regardless of DENV immune history (P. Andrade, J. Coloma, and E. Harris, unpublished).

In summary, the high level of cross-reactivity between ZIKV and other flaviviruses at the binding level is consistent with the sequence homology and structural similarities among flaviviruses. However, neutralization studies demonstrated that the cross-neutralization between DENV and ZIKV is much lower in magnitude than titers raised to the homologous and even heterologous DENV serotypes, especially at late convalescent time-points. Depletion studies indicate that neutralizing antibodies in secondary ZIKV infections in returned travelers are specific to the ZIKV serocomplex and comprise a different population than DENV-cross-reactive antibodies. In terms of MBCs, a strong ZIKV-specific response has been described at the single-cell level in ZIKV-infected individuals regardless of prior DENV exposure. These data argue against the concept of ZIKV behaving immunologically as a “fifth DENV serotype”. However, the issue of enhancement has less to do with neutralizing antibodies and more to do with non-neutralizing, binding antibodies, and these antibodies have not been adequately studied in human populations in endemic regions. These issues have profound implications for Zika epidemiology and the design of vaccines for Zika and dengue.

### **Previous immunity to flavivirus and ADE**

Increased viremia and disease severity observed in secondary DENV infection is often attributed to ADE of infection with heterologous DENV serotypes (Halstead, 1988). As the serological cross-reactivity between ZIKV and DENV is influenced by timing and infection history, understanding the binding and neutralization properties against ZIKV of antibodies in polyclonal sera of individuals with prior flavivirus exposure is critical in relation to ADE. Beyond natural infection, ADE could potentially occur in DENV vaccine recipients infected with ZIKV and conversely, in ZIKV vaccine recipients experiencing a subsequent DENV



infection. While a human vaccine against ZIKV is still not available, studies with non-human primates are ongoing. Passive transfer of ZIKV-specific purified IgG from the plasma of ZIKV-vaccinated animals into two naïve rhesus monkeys, followed by challenge with ZIKV, did not cause enhancement of viral replication even when subtherapeutic IgG concentrations were used (Abbink, 2016). Further studies with larger sample sizes and that explore the potential of ADE in the context of vaccination in recipients with previous flavivirus immunity of varying intervals of time are of great relevance for a safe and effective ZIKV vaccine development.

*In vitro*, cross-reactive anti-DENV antibodies enhance ZIKV infection of non-permissive K562 cells (Priyamvada, 2016). Conversely, mAbs isolated from ZIKV-infected individuals were shown to enhance DENV infection of K562 cells as well (Stettler, 2016). Additionally, convalescent ZIKV-immune plasma enhanced ZIKV infection of K562 cells (Stettler, 2016). Serum enhancement was completely blocked by the ZIKV-specific EDIII mAb, ZAK64-LALA, but the LALA version of the EDI/EDII cross-reactive mAb DV82, isolated from a DENV-infected individual, only partially blocked serum enhancement (Stettler, 2016). ZIKV-immune plasma also significantly enhanced DENV infection, an effect that was blocked by DENV EDI/II specific mAb DV82-LALA, but not by EDIII-specific ZIKV mAb ZKA64-LALA (Stettler, 2016). A different study demonstrated that the enhancement of ZIKV infection of K562 cells by DENV- or WNV-immune plasma is mediated by IgG (Bardina, 2017).

To evaluate the potential for ADE *in vivo*, two EDI/II cross-reactive mAbs isolated from either a DENV-infected (DV82) or ZIKV-infected (ZKA78) individual were administered to AG129 mice prior to DENV infection. Both mAbs lethally enhanced DENV infection in a mouse model of DENV-induced vascular leak (Stettler, 2016). Conversely, to investigate potential enhancement of ZIKV infection by DENV and other flavivirus antibodies *in vivo*, a model in *Stat2*<sup>-/-</sup>C57BL/6 mice was used. *Stat2*<sup>-/-</sup> mice, which display morbidity and mortality to ZIKV infection (Tripathi, 2017), were treated with DENV-immune and WNV-immune plasma and subsequently infected with ZIKV. The majority of the animals treated with a pool of DENV-immune plasma presented significant weight loss and an enhanced clinical symptom score, including severe neurological symptoms. Animals pre-treated with WNV-immune plasma also presented symptoms but to a lesser extent. Further, staining with antibodies to ZIKV NS3 indicated the presence of ZIKV in spinal cord and testes in animals treated with DENV-immune plasma (Bardina, 2017). Thus, ZIKV antibodies can enhance DENV infection and vice versa *in vitro* and in mouse models, but whether this occurs in human population is unknown, and epidemiological studies addressing these questions are urgently needed.

### T cell responses

The role of T cells in protection and pathogenesis of DENV infection has been extensively investigated (Duangchinda et al., 2010; Dung et al., 2010; Rothman, 2011; Weiskopf, 2013; Weiskopf, 2016; Weiskopf, 2014). However, very few studies exist to date on the role of T cells in ZIKV infection. One study showed that CD8<sup>+</sup> T cells are important for control of ZIKV, as depletion of CD8<sup>+</sup> T cells in mice resulted in higher viremia (Elong, 2017). This is

consistent with reduction of viral burden observed when memory CD8<sup>+</sup> T cells were adoptively transferred into naïve mice that were subsequently challenged with ZIKV. Further, at the peak of the T cell response to ZIKV infection, CD4<sup>+</sup> T cells polarized to a Th1 profile, with production of IFN- $\gamma$ , TNF- $\alpha$ , and IL-2 (Elong, 2017).

Another recent study examined T cell responses against ZIKV in samples from ZIKV-infected donors derived from a variety of different geographical locations, including mainland U.S. (travelers returning from affected areas), Puerto Rico, Brazil, Nicaragua, and Mexico. This enabled characterization of both ZIKV-specific and ZIKV/DENV cross-reactive T cell responses and the influence of DENV serostatus on T cell immunity to ZIKV. Pre-existing T cell responses against DENV were found to recognize peptide sequences encoded in the ZIKV proteome and thus are cross-reactive between the two flaviviruses (D. Weiskopf and A. Sette, personal communication). In addition, this cross-reactivity is immunologically consequential, as individuals previously exposed to DENV displayed CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses to ZIKV more rapidly and of greater magnitude than DENV-naïve ZIKV-infected individuals. Patterns of immunodominance were found to be different in the case of DENV and ZIKV infection, with ZIKV-specific CD8<sup>+</sup> T cell responses predominantly targeting structural proteins such as E, prM, and C (D. Weiskopf and A. Sette, personal communication), in contrast to the CD8<sup>+</sup> T cell response against DENV, which mostly targets non-structural (NS) proteins such as NS3, NS4B and NS5 (Weiskopf, 2013; Weiskopf, 2016; Weiskopf, 2014). Both ZIKV and DENV CD4<sup>+</sup> T cell responses are directed in approximately equal proportions against structural and non-structural proteins (Weiskopf, 2013; Weiskopf, 2016; Weiskopf, 2014) (D. Weiskopf and A. Sette, personal communication). These findings highlight a critical difference in the immunodominant targets of T cells in ZIKV and DENV infections, which has implications for Zika vaccine design.

## Conclusions

The understanding of the immune response to ZIKV infection has dramatically increased in the last year through *in vitro* studies with mAbs and polyclonal antibodies derived from ZIKV-infected individuals, animal studies, and longitudinal analysis of well-characterized human immune sera from returned travelers and from dengue-endemic regions. However, many questions still need to be addressed, particularly by placing *in vitro* and *in vivo* findings in an epidemiological context, such as analyzing how pre-existing DENV immunity affects ZIKV transmission dynamics and infection outcome in endemic settings. Knowledge gained from these studies is fundamental for serodiagnosis and vaccine development.

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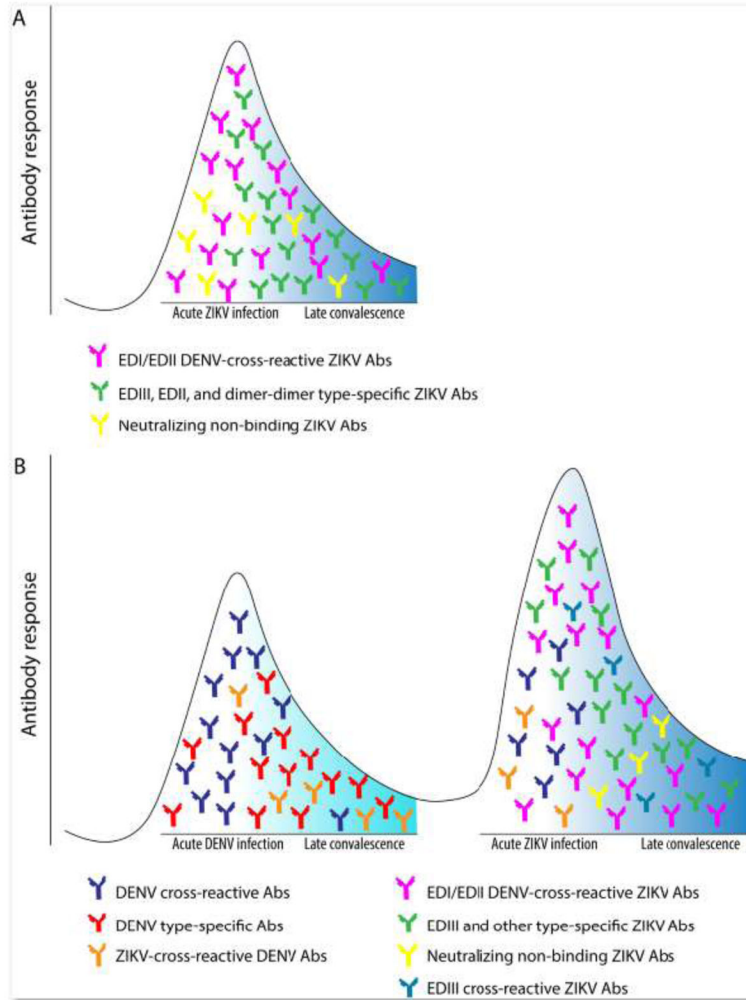


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### HIGHLIGHTS

- ZIKV shares structural similarities with other flaviviruses, especially DENV, and co-circulates in dengue-endemic regions.
- The understanding of the immune response to ZIKV infection has dramatically increased in the last year through *in vitro* studies with monoclonal and polyclonal antibodies derived from ZIKV-infected individuals, animal studies, and longitudinal analysis of human immune sera from returned travelers and from dengue-endemic regions.
- Both ZIKV type-specific antibodies and DENV cross-reactive are generated, which have been shown to be either protective or enhancing in animal models.
- The effect of prior DENV exposure on the adaptive immune response to ZIKV infection is an area of active investigation, and many questions still need to be addressed, particularly by placing *in vitro* and *in vivo* findings in an epidemiological context.



**Figure 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, 2017; Sapparapu, 2016; Stettler, 2016; Wang et al., 2016).