T Cell Immunity to Zika and Dengue Viral Infections

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The 2015–2016 Zika virus (ZIKV) outbreak in the Americas has brought this relatively unknown flavivirus into the spotlight as the etiologic agent of congenital Zika syndrome in newborns and, potentially, of Guillain-Barré syndrome in adults. ZIKV shares a high degree of amino acid sequence homology with the closely related flavivirus Dengue virus (DENV). Due to the paucity of research investigating T cell-mediated immune responses to ZIKV, the precise roles of T cells in protection against and pathogenesis of ZIKV infection are unclear. Nevertheless, knowledge gained from studies of T cell responses to other flaviviruses, such as the four DENV serotypes, provides a framework for investigating and understanding anti-ZIKV T cell responses. In this review, we summarize our current understanding of how T cells respond to primary and secondary infections with DENV and ZIKV. A thorough understanding of the role of ZIKV-specific and DENV cross-reactive T cells in controlling infection is essential for developing ZIKV- and DENV-specific vaccines with maximal safety and efficacy.

Keywords: Zika, dengue, mouse models, T cell immunity

Introduction

VIRUSES IN THE GENUS *FLAVIVIRUS* of the family *Flaviviridae* have a positive-sense RNA genome that encodes three structural proteins (Capsid [C], premembrane/membrane [prM/M], and envelope [E]) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Dengue virus (DENV), Yellow fever (YFV), West Nile (WNV), Japanese encephalitis (JEV), and Zika virus (ZIKV) are well-known flaviviruses that circulate in many countries and cause significant disease in humans (Blitvich and Firth 2017). The most recent outbreaks of ZIKV, which was first isolated in 1947 from a sentinel monkey in Zika Forest (Dick 1953), started in the Yap islands in 2007 followed by French Polynesia in 2013 and Brazil/Americas in 2015–2016 (Lazear and Diamond 2016).

Humans can be infected with ZIKV through multiple routes, including mosquito bites (*Aedes aegypti*) (Ferreira-de-Brito and others 2016), as well as sexual (Moreira and others 2017) and vertical (Besnard and others 2014) transmission. ZIKV is now known to be the etiologic agent of congenital Zika syndrome and is associated with Guillain-Barré syndrome in adults. ZIKV shares a high degree of homology with DENV, WNV, JEV, and YFV, with more than 50% amino acid sequence identity in E, NS1, NS3, NS4B, and NS5 proteins (Fig. 1). E is the major target of the anti-flaviviral humoral response, while NS3, NS4B, and NS5 are the major targets of the anti-DENV T cell response.

Although our understanding of the adaptive T cell response to ZIKV is currently poor, it is likely to share many features with responses to other flaviviruses, including the four serotypes of DENV, suggesting that published studies on DENV immunity can guide investigations of the T cell response to ZIKV. Moreover, the response against DENV is the most extensively investigated T cell response to flaviviruses, and increasing evidence supports a protective rather than a pathogenic role for DENV-reactive T cells. Interestingly, recent studies suggest a similarly protective role for T cells during infection with ZIKV, the most closely related flavivirus to DENV (Elong Ngono and others 2017; Wen and others 2017). These findings imply that a ZIKV vaccine should elicit robust responses by both T cells and antibodies (Abs). In this study, we summarize our current understanding of the protective functions of T cells during infection with DENV and ZIKV.

The Protective Role of T Cells in DENV Infection

The four serotypes of DENV (DENV1–4) cause a spectrum of diseases, ranging from an acute, self-limited febrile illness to the life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), that affect 3.9 billion people in 128 countries (Bhatt and others 2013). Dengue fever is an acute illness with symptoms that include fever, headache, myalgia, and retro-orbital pain. DHF and DSS are characterized by thrombocytopenia, elevated hematocrit and cytokine levels, vascular leakage, hemorrhage, and in DSS,

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FIG. 1. Amino acid homology between ZIKV and DENV, WNV, JEV, and YFV. C, Capsid; DENV, Dengue virus; E, envelope; JEV, Japanese encephalitis; NS, nonstructural; prM/M, premembrane/membrane; WNV, West Nile; YFV, Yellow fever; ZIKV, Zika virus.

shock, which can be fatal if untreated. There is an urgent need for a widely available DENV vaccine that is safe and effective in multiple age groups and in both naive and DENV-immune backgrounds.

One challenge in the development of a DENV vaccine is that it must protect against all four DENV serotypes, since DHF/DSS most often occurs in individuals experiencing a secondary infection with a heterologous DENV serotype in addition to infants born to DENV-immune mothers (Sangkawibha and others 1984; Guzman and others 2000; Halstead and others 2002). The antibody-dependent enhancement (ADE) model is now the most widely accepted explanation for these epidemiological observations. According to this model, preexisting, cross-reactive anti-DENV Abs facilitate DENV entry into Fcγ receptor-bearing cells, thereby inducing a higher viral load that triggers an excessive cytokine response and vascular leakage syndrome. In 2010, almost four decades after the hypothesis was first proposed, two research groups simultaneously provided direct evidence for ADE by developing mouse models of ADE-mediated severe dengue (Balsitis and others 2010; Zellweger and others 2010).

In addition to Abs, the T cell response to DENV has been postulated to play a dual role in protection and pathogenesis. However, there is currently no direct evidence to support a pathogenic role for T cells during DENV infection. In fact, accumulating evidence from human studies suggests that T cells actually play a protective role. Similarly, studies in mouse models have demonstrated a critical contribution of T cells to protection against DENV in a variety of infection and vaccination settings.

Early studies reported that DENV-specific human CD8⁺ and CD4⁺ T cells can produce interferon-γ (IFN-γ) upon DENV stimulation and lyse DENV-infected target cells (Kurane and others 1989; Mathew and others 1996; Gagnon and others 1999). Prompted by these observations, Yauch and others (2009) investigated the protective role of T cells using a model of primary DENV infection in type I IFN receptor-deficient (IFNAR^{-/-}) mice. These mice develop a disease with the key features of severe dengue in humans, including viral tropism/viremia, thrombocytopenia, vascular leakage, and cytokine storm. The authors found that IFNAR^{-/-} mice depleted of CD8⁺ T cells before infection had significantly higher DENV burden in tissues compared with nondepleted animals. In addition, immunization with immunodominant CD8⁺ T cell DENV peptide epitopes enhanced viral clearance from the mice, confirming a protective role for CD8⁺ T cells during primary DENV infection (Yauch and others 2009).

Similar loss- and gain-of-function studies of CD4⁺ T cells in this mouse model did not find a similar reduction in tissue viral burden when CD4⁺ T cells were depleted before DENV infection; however, immunization with CD4⁺ T cell peptide epitopes significantly lowered the tissue viral burden compared with mock-immunized animals (Yauch and others 2010). Therefore, although CD4⁺ T cells are not required for controlling primary DENV infection in this mouse model, their induction by epitope immunization can contribute to viral clearance (Yauch and others 2010). Of note, the viral dose used in this study was high, and the role of CD4⁺ T cells in primary infections with low DENV doses is yet to be evaluated. Since low doses may limit induction of inflammatory signals, CD4⁺ T cells may be required to help both Ab and CD8⁺ T cell responses under such conditions.

Investigations of secondary DENV infections often exploit not only IFNAR^{-/-} mice but also double-deficient mice lacking both type I and II IFN receptors (known as AG129 mice). Several studies have investigated the contribution of T cells to protection against heterotypic secondary infection with one of the four DENV serotypes. Using AG129 mice, Zompi and others (2012) first showed that naive mice transferred with B and T cells from DENV1-immunized mice exhibited delayed mortality upon challenge with DENV2 (Zompi and others 2012). Moreover, this protective effect was partially abrogated when only B cells were transferred, suggesting that cross-reactive T cells primed by one serotype provided significant protection against infection with a second serotype (Zompi and others 2012).

Subsequently, Zellweger and others (2015) used IFNAR^{-/-} mice to directly compare the protective roles of T cells during homotypic versus heterotypic DENV secondary infection. Using adoptive transfer and T cell depletion approaches, they demonstrated that CD8⁺ T cells mediated cross-protection against heterotypic DENV reinfection (Zellweger and others 2015). Interestingly, cross-protection was observed in mice with subneutralizing levels of Abs specific for the heterotypic DENV, suggesting that CD8⁺ T cells may confer protection even against ADE. In fact, the same group had previously shown that CD8⁺ T cells could prevent Ab-enhanced infection and provide protection against ADE-mediated lethal dengue disease in mice (Zellweger and others 2014).

The relevance of DENV-specific CD8⁺ T cells to human DENV infections was demonstrated in studies using HLA-transgenic mice, which displayed the same T cell epitope specificity and immunodominance patterns as DENV-infected humans (Weiskopf and others 2011, 2014). Specifically, immunization of IFNAR^{-/-} HLA-B*0702 transgenic mice with

DENV peptides induced a T cell response that was both serotype specific and cross-reactive and could contribute to protection against DENV infection (Elong Ngono and others 2016). Collectively, the studies described here in IFN receptor-deficient mice, that can recapitulate key features of severe dengue disease in humans, have demonstrated a critical role for CD8⁺ T cells in protection against DENV infection *in vivo*.

IFN receptor-deficient mice have also emerged as highly stringent platforms for evaluating T cell responses to DENV vaccines. In particular, Zellweger and others (2013) explored the immunological basis of protective immunity elicited by DENV replicon particles expressing the E protein. In this study, serum and T cells were isolated from vaccinated wild-type mice and transferred into naive AG129 mice, which were then challenged with DENV. This approach has several advantages in that it allows the vaccine-induced immune response to be generated in a normal (wild-type) environment and allows the efficacy and mechanism of vaccine-induced protection to be examined in a highly susceptible challenge model. By isolating the cellular component of the vaccine-induced immune response, this study showed that the vaccine-induced CD8⁺ T cells protected against infection in the DENV-challenged AG129 mice, whereas the vaccine-induced serum Abs exhibited neutralizing activity against DENV *in vitro* but not in mice. These findings revealed that a DENV vaccine can confer protection against infection in a CD8⁺ T cell-dependent manner (Zellweger and others 2013).

Several studies have investigated the association between DENV-reactive T cells and dengue disease in humans. Hatch and others (2011) observed higher frequencies of DENV-specific IFN- γ -producing T cells in children who subsequently develop subclinical infection than in those developing symptomatic secondary DENV infections. Weiskopf and others (2013, 2015b) generated a comprehensive map of the human CD8⁺ T cell response to DENV, and their results using blood samples from donors in two different DENV endemic countries (Sri Lanka and Nicaragua) provided support for an HLA-linked protective role for CD8⁺ T cells (de Alwis and others 2016). In particular, low magnitude and breadth T cell responses were associated with dengue disease susceptibility and certain HLA alleles (HLA-A*0101 and HLA-A*2402), whereas high magnitude and breadth T cell responses were associated with disease resistance and a distinct set of HLA molecules (B*0702, B*3501).

The same investigators also defined HLA-DRB1-restricted DENV epitopes that are recognized by CD4⁺ T cells from individuals previously exposed to DENV, and they identified a correlation between the magnitude of the CD4⁺ T cell response as a function of the restricting DRB1 allele and disease susceptibility (Weiskopf and others 2016). A prior study from this group had revealed that a protective DRB1*0401-associated DENV epitope-specific CD4⁺ T cell response was strongly biased toward a CX3CR1⁺, eomesodermin⁺, perforin⁺, granzyme B⁺, and CD45RA⁺ cytotoxic T cell phenotype (Weiskopf and others 2015a). Accordingly, these DRB1*0401-restricted DENV epitope-specific CD4⁺ T cells displayed DENV-specific cytolytic activity *ex vivo*. These findings suggest that cytotoxic DENV-specific CD4⁺ T cells may play a protective role.

A recent study used an integrated approach of transcriptional profiling and immunological analysis to compare

populations of Cambodian DENV-exposed children who had asymptomatic viremia versus clinical disease (Simon-Loriere and others 2017). Although expression of proteins associated with inflammatory and innate immune response pathways was similar in both cohorts, expression of proteins related to antigen presentation and T and B cell activation pathways differed in a manner independent of viral load and DENV infection history, and symptom-free DENV infection in children was associated with increased activation of the T cell compartment. Thus, these recent studies using human samples, in combination with those described above using mouse models, strongly suggest that both serotype-specific and cross-reactive CD4⁺ and CD8⁺ T cells play critical roles in protection against DENV infection.

The Protective Role of T Cells in ZIKV Infection

Although research investigating T cell immunity to ZIKV is currently in its infancy, mice with genetic or acquired deficiency of IFNAR signaling are now being used to explore the role of CD8⁺ T cells in protection against ZIKV. Similar to DENV, ZIKV can disrupt IFNAR signaling in human but not murine cells (Grant and others 2016); thus, wild-type mice are resistant to ZIKV infection while mice lacking IFNAR signaling are highly susceptible to ZIKV infection and exhibit key features of the human disease (Lazear and Diamond 2016; Lazear and others 2016).

A study using *LysMCre⁺IFNAR^{fl/fl}* mice, which lack the IFNAR in macrophages, neutrophils, and some dendritic cell subsets, demonstrated that primary ZIKV-specific CD8⁺ T cells were polyfunctional and cytotoxic, and depletion of these cells led to higher viral burdens in multiple tissues such as the serum, spleen, brain, and sciatic nerve. Conversely, adoptive transfer of CD8⁺ T cells from the ZIKV-infected mice into naive mice resulted in decreased tissue viral burden (Elong Ngono and others 2017). Also in this study, ZIKV infection was lethal to *Cd8^{-/-}* mice, but not wild-type control animals, treated with a blocking anti-IFNAR Ab. The results from this study, based on multiple approaches and mouse models, demonstrated an important contribution of CD8⁺ T cells to protection against primary ZIKV infection.

In another primary ZIKV infection study, anti-IFNAR Ab-treated *Rag1^{-/-}* mice (which are deficient in mature T and B cells) but not similarly treated wild-type mice exhibited severe weight loss and high levels of infection in the brain and testes (Winkler and others 2017). ZIKV-infected neonatal wild-type mice displayed signs of neurological disease that subsided gradually over 2 weeks and exhibited a cellular infiltration in the CNS composed predominantly of T cells, particularly CD8⁺ T cells (Manangeeswaran and others 2016). Taken together, this study (Manangeeswaran and others 2016) and those described above using *LysM-Cre⁺IFNAR^{fl/fl}* and anti-IFNAR Ab-treated *Cd8^{-/-}* mice (Elong Ngono and others 2017) indicate that CD8⁺ T cells are likely to have played an important role in protection against ZIKV in the anti-IFNAR Ab-treated *Rag1^{-/-}* mouse model (Winkler and others 2017).

In another study, CD8⁺ T cell responses were examined in ZIKV-infected adult wild-type mice. In this study, adoptively transferred ZIKV-experienced CD8⁺ T cells were shown to traffic to the brain, where they protected against not only ZIKV infection but also DENV infection (Huang and others 2017). Indeed, a study using two different HLA

transgenic mouse strains and peptide immunization revealed that both ZIKV-specific and DENV cross-reactive CD8⁺ T cells could protect against ZIKV infection (Wen and others 2017). In addition, this HLA transgenic mouse study showed that prior DENV immunity alters the immunodominance pattern of the subsequent ZIKV infection and that the CD8⁺ T cell response to ZIKV may be HLA-linked and targeting mainly NS proteins.

This is an important observation in the context of individuals previously exposed to DENV. Collectively, the reports to date indicate that the CD8⁺ T cell response to ZIKV shares many similarities with DENV in both primary and heterotypic secondary infection settings.

Conclusion

Increasing evidence implicates a critical role for T cell responses in protection against DENV in various scenarios, including primary and secondary heterotypic infection, vaccination, and ADE, suggesting that a DENV vaccine that does not elicit a robust T cell response, in addition to Abs, may be dangerous. Accordingly, phase III data on Dengvaxia, the DENV vaccine that does not contain the major targets of the T cell response (ie, the DENV NS proteins) and has been licensed for use in a few countries, suggest that vaccination-elicited sensitizing Abs changed an expected mild/subclinical initial DENV infection into an infection resulting in hospitalization/vascular leakage syndrome in the 2- to 5-year-old group (Hadinegoro and others 2015).

Recent studies investigating the protective role of T cells against ZIKV are beginning to reveal similarities to the anti-DENV T cell response. Moreover, ADE of ZIKV infection has now been demonstrated *in vivo* (Bardina and others 2017). Thus, ZIKV vaccines may also need to be optimized to induce both T cell and Ab responses to achieve maximal efficacy and safety.

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