HHS Public Access

Author manuscript

Cell Rep. Author manuscript; available in PMC 2020 May 30.

Published in final edited form as:

Cell Rep. 2020 April 28; 31(4): 107566. doi:10.1016/j.celrep.2020.107566.

CD4+ T Cells Cross-Reactive with Dengue and Zika Viruses Protect against Zika Virus Infection

Jinsheng Wen[1,2,4,*], **Ying-Ting Wang**[1,4], **Kristen M. Valentine**[1], **Rúbens Prince dos Santos Alves**[1,3], **Zhigang Xu**[1], **Jose Angel Regla-Nava**[1], **Annie Elong Ngono**[1], **Matthew P. Young**[1], **Luís C.S. Ferreira**[3], **Sujan Shresta**[1,5,*]

[1]Division of Inflammation Biology, La Jolla Institute for Immunology, La Jolla, CA 92037, USA

[2]Immunology Innovation Team, Ningbo University School of Medicine, Ningbo, Zhejiang 315211, China

[3]Vaccine Development Laboratory, Institute of Biomedical Sciences, University of São Paulo, São Paulo 14040-901, Brazil

[4]These authors contributed equally

[5]Lead Contact

SUMMARY

The underlying mechanisms by which prior immunity to dengue virus (DENV) affords cross-protection against the related flavivirus Zika virus (ZIKV) are poorly understood. Here, we examine the ability of DENV/ZIKV-cross-reactive $CD4^+$ T cells to protect against versus exacerbate ZIKV infection by using a histocompatibility leukocyte antigen (HLA)-DRB1*0101 transgenic, interferon α/β receptor-deficient mouse model that supports robust DENV and ZIKV replication. By mapping the HLA-DRB1*0101-restricted T cell response, we identify DENV/ZIKV-cross-reactive $CD4^+$ T cell epitopes that stimulate interferon gamma (IFNγ) and/or tumor necrosis factor (TNF) production. Vaccination of naive HLA-DRB1*0101 transgenic mice with these peptides induces a $CD4^+$ T cell response sufficient to reduce tissue viral burden following ZIKV infection. Notably, this protective response requires IFNγ and/or TNF secretion but not anti-ZIKV immunoglobulin G (IgG) production. Thus, DENV/ZIKV-cross-reactive $CD4^+$ T cells producing canonical Th1 cytokines can suppress ZIKV replication in an antibody-independent manner. These results may have important implications for increasing the efficacy and safety of DENV/ZIKV vaccines and for developing pan-flavivirus vaccines.

*Correspondence: wenjinsheng@nbu.edu.cn (J.W.), sujan@lji.org (S.S.).

AUTHOR CONTRIBUTIONS

J.W., Y.-T.W., and S.S. designed the experiments. J.W., Y.-T.W., R.P.d.S.A., Z.X., K.M.V., J.A.R.-N., A.E.N., and M.P.Y. performed the experiments. J.W., Y.T.W., K.M.V., R.P.d.S.A., L.C.S.F., and S.S. interpreted the data. J.W. Y.T.W., K.M.V., and S.S. wrote the manuscript. S.S. edited the manuscript and supervised the project.

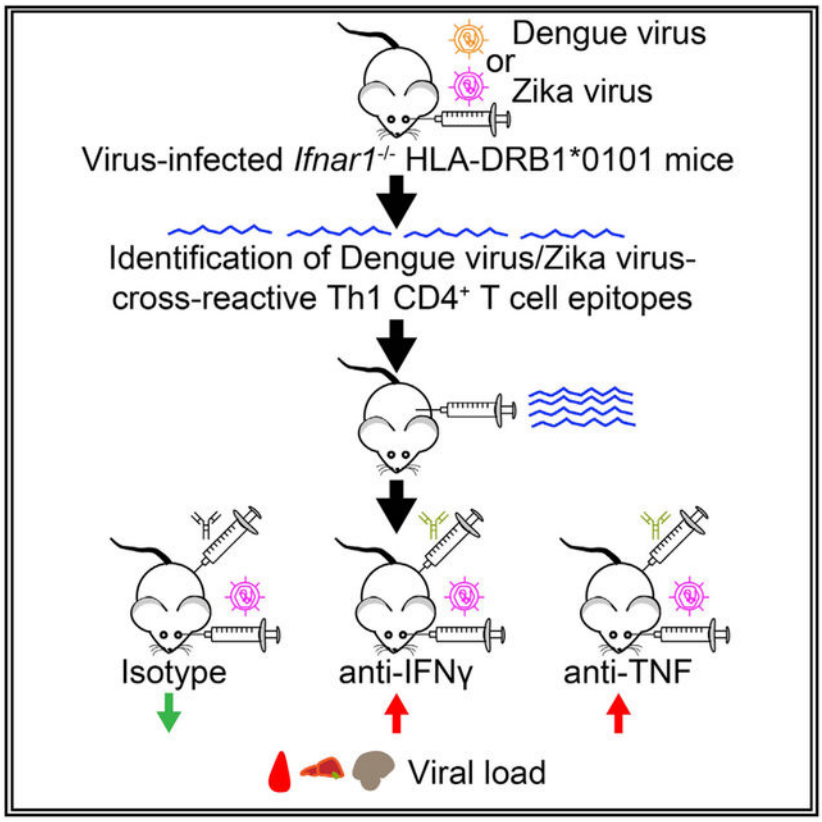
SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2020.107566.

DECLARATION OF INTERESTS

The La Jolla Institute for Immunology has a pending USA patent application, 16/537,447, covering methods and compositions utilizing the epitopes described in this manuscript for the treatment and prevention of flaviviruses, with S.S. listed as the inventor.

Graphical Abstract



In Brief

Wen et al. show that dengue and Zika virus cross-reactive $CD4^+$ T cells reduce Zika viral burden in interferon α/β receptor-deficient HLA-DRB1*0101 transgenic mice in an IFNγ- or TNF-dependent, antibody-independent manner.

INTRODUCTION

Zika virus (ZIKV) is a positive-sense, single-stranded, enveloped RNA virus of the *Flavivirus* genus, which includes the closely related dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), and yellow fever virus (YFV) (Choumet and Despres, 2015; Lazear and Diamond, 2016; Ngono and Shresta, 2018). ZIKV and DENV share similar amino acid sequences, with 43% overall homology and up to 68% identity for the non-structural proteins (Lazear and Diamond, 2016; Wen and Shresta, 2019). Additionally, ZIKV and DENV use the same vectors for transmission and have overlapping geographical ranges. Anti-DENV and anti-ZIKV immune responses have been shown to cross-react at the antibody (Ab) level (Castanha et al., 2017; Charles and Christofferson, 2016; Dejnirattisai et al., 2016; Kawiecki and Christofferson, 2016; Paul et al., 2016; Priyamvada et al., 2016; Swanstrom et al., 2016) and $CD4^+$ and $CD8^+$ T cell levels (Grifoni et al., 2017; Lim et al., 2018; Paquin-Proulx et al., 2017). These cross-reactive immune responses may contribute to both protection and pathogenesis during ZIKV and DENV

infections (Ngono and Shresta, 2018). In particular, cross-reactive Abs produced during a primary infection with one DENV serotype can exacerbate, rather than protect, against secondary infection with a different DENV serotype (Katzelnick et al., 2017; Salje et al., 2018). This occurs through a process known as Ab-dependent enhancement (ADE) of infection and can lead to a potentially life-threatening infection with hemorrhagic fever and shock (known as severe dengue) (Halstead, 2007). Accordingly, studies using mouse models have shown that DENV/ZIKV-cross-reactive Abs play a dual role in mediating protection and pathogenesis during infection with DENV or ZIKV (Bardina et al., 2017; Fernandez et al., 2017; Fowler et al., 2018; Kam et al., 2017; Slon Campos et al., 2017). Although there is limited epidemiologic evidence demonstrating ZIKV-ADE in humans (Robbiani et al., 2019), three recent epidemiologic studies have demonstrated that prior DENV exposure provides cross-protection against ZIKV infection in humans (Gordon et al., 2019; Pedroso et al., 2019; Rodriguez-Barraquer et al., 2019). At present, the mechanisms responsible for the cross-protection in humans is poorly understood. Because natural infection and/or vaccination against these viruses could have either beneficial or disastrous consequences, it is crucial that we deepen our understanding of the mechanisms by which DENV/ZIKV-cross-reactive immunity can mediate these distinct outcomes.

A variety of mouse models have been used to investigate anti-DENV and anti-ZIKV T cell responses, including wild-type (WT) mice, mice deficient in the type I interferon (IFN) receptor on macrophages (*LysMCre*$^{+}$*Ifnar1*$^{-/-}$mice) or all cells (*Ifnar1*$^{-/-}$mice), WT mice with Ab-mediated blockade of the type I IFN receptor, and—of particular importance for the human response—*Ifnar1*$^{-/-}$histocompatibility leukocyte antigen (HLA) transgenic mice (Wen and Shresta, 2017; Wen and Shresta, 2019). Studies using these mouse models have demonstrated a critical role for CD8^{+} T cells in protecting against primary ZIKV infection (Elong Ngono et al., 2017; Huang et al., 2017) and for DENV-elicited CD8^{+} T cells in mediating cross-protection against subsequent ZIKV infection (Huang et al., 2017; Regla-Nava et al., 2018; Wen et al., 2017a, 2017b). Additionally, studies with *Ifnar1*$^{-/-}$HLA-B*0702 and HLA-A*0101 transgenic mice, which express two of the most common HLA class I alleles in countries where ZIKV is endemic, showed that ZIKV-specific and DENV/ZIKV-cross-reactive CD8^{+} T cells from mice vaccinated with peptides can control the viral burden during subsequent ZIKV infection (Wen et al., 2017b). These findings suggest an important role for DENV-elicited CD8^{+} T cells in cross-protecting against ZIKV infection in humans.

More recent studies using mouse models have also identified a protective role for ZIKV-specific CD4^{+} T cells. In particular, ZIKV-specific CD4^{+} T cells are required for generation of a protective anti-ZIKV Ab response (Elong Ngono et al., 2019; Hassert et al., 2018; Lucas et al., 2018). A comparison of unmanipulated WT mice and anti-Ifnar1 Ab-treated WT mice demonstrated an important role for CD4^{+} T cell responses in controlling ZIKV infection in both mouse models (Liang et al., 2019). DENV/ ZIKV-cross-reactive CD4^{+} T cells have been described in HLA transgenic mice and in humans (Grifoni et al., 2017; Lim et al., 2018; Paquin-Proulx et al., 2017; Reynolds et al., 2018). Thus, CD4^{+} T cells primed by vaccination of HLA class II transgenic mice with purified ZIKV proteins show reactivity *in vitro* not only to the priming ZIKV peptides but also to variants of the same peptides present in the four DENV serotypes (DENV1–4), WNV, and YFV (Reynolds et al., 2018).

Conversely, $CD4^+$ T cells isolated from DENV-vaccinated individuals display cross-reactivity to ZIKV peptides *in vitro* (Grifoni et al., 2017; Lim et al., 2018; Paquin-Proulx et al., 2017). However, at present, the protective versus potentially pathogenic roles of DENV/ZIKV-cross-reactive $CD4^+$ T cells are unknown.

In the present study, we investigated whether $CD4^+$ T cells with cross-reactivity to HLA-class-II-restricted DENV2/ZIKV epitopes are protective versus pathogenic during ZIKV infection in the *Ifnar1*$^{-/-}$HLA-DRB1*0101 transgenic mouse model. We identified a panel of ZIKV peptides predicted to bind to HLA-DRB1*0101 and then characterized the *in vitro* response to the peptides of $CD4^+$ T cells from ZIKV- or DENV-infected HLA-DRB1*0101 mice. Of the 30 ZIKV peptides screened, 7 induced interferon gamma (IFNγ) and/or TNF production by ZIKV-primed $CD4^+$ T cells and 4 induced IFNγ and/or TNF production by cross-reactive DENV2-primed $CD4^+$ T cells. Vaccination of HLA-DRB1*0101 mice with DENV2/ZIKV-cross-reactive $CD4^+$ T cell epitopes induced a protective response upon ZIKV infection, and viral control required IFNγ and/or TNF secretion but not anti-ZIKV immunoglobulin G (IgG). These findings suggest a mechanism by which prior DENV exposure cross-protects against ZIKV infection in humans and may, therefore, have important implications for vaccine development.

RESULTS

Identification of ZIKV-Derived HLA-DRB1*0101-Restricted $CD4^+$ T Cell Epitopes

WT mice are highly resistant to DENV and ZIKV infection due to the inability of the viruses to inhibit the host IFN system (Aguirre et al., 2012; Ding et al., 2018; Grant et al., 2016; Yu et al., 2012). Because the antigenic load dictates the magnitude and quality of antiviral T cell responses (Earl et al., 2004; Panagioti et al., 2018; Salek-Ardakani et al., 2011), *Ifnar1*$^{-/-}$mice have been widely used to investigate T cell responses to DENV and ZIKV (Ngono and Shresta, 2018; Tang et al., 2015). We previously showed that vaccination of *Ifnar1*$^{-/-}$mice with DENV2 peptides elicited $CD4^+$-T-cell-mediated protective immunity against reinfection with the DENV2 homotype (Yauch et al., 2010). HLA-DRB1*0101 is a commonly expressed HLA molecule in humans (Marsh et al., 1999), and human studies have confirmed the relevance of DENV-reactive $CD4^+$ T cells identified using the HLA-DRB1*0101 transgenic mouse model (Ngono and Shresta, 2018; Weiskopf et al., 2011, 2014). Therefore, for the present study, we used the *Ifnar1*$^{-/-}$HLA-DRB1*0101 mouse model. We selected a total of 30 ZIKV peptides from the top 2% of epitopes predicted to bind HLA-DRB1*0101 by the predictive database Immune Epitope Database and Analysis Resource (IEDB-AR) (Table 1). The 30 peptides were distributed in 9 ZIKV proteins: 3 in C, 2 in M, 4 in E, 1 in NS1, 6 in NS2A, 3 in NS3, 2 in NS4A, 7 in NS4B, and 2 in NS5. To test the reactivity of ZIKV-primed $CD4^+$ T cells, *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice were infected with ZIKV SD001 strain for 7 days, and splenocytes were isolated, stimulated with the candidate epitopes for 6 h, and analyzed for production of canonical Th1 (IFNγ, TNF, and interleukin-2 [IL-2]), Th17 (IL-17), and Th2 (IL-4, IL-5) cytokines by intracellular staining and flow cytometry (ICS assay). This analysis identified eight peptides ($C_{27–41}$, $C_{53–67}$, $C_{81–95}$, $E_{134–148}$, $E_{450–464}$, $NS2A_{66–80}$, $NS3_{601}$-$NS4A_{12}$, and $NS5_{222–236}$) as Th1 epitopes (Figure 1), of which $NS5_{222–236}$ stimulated $CD4^+$ T cells to produce IFNγ, TNF,

and IL-2; and E134–148 stimulated $CD4^+$ T cells to produce only IL-2; and the other six epitopes stimulated $CD4^+$ T cells to produce IFNγ and/or TNF (Figure 1; Table 1). Notably, none of the 30 peptides tested induced $CD4^+$ T cells to produce IL-17, IL-4, or IL-5 (Figure S1). These data indicate that the primary response to ZIKV infection in *Ifnar1*$^{-/-}$HLA-DRB1*0101 transgenic mice is dominated by Th1 cytokine-secreting $CD4^+$ T cells responding to eight peptides in ZIKV structural and non-structural proteins.

Identification of ZIKV-Derived $CD4^+$ T Cell Epitopes That Stimulate DENV-Primed $CD4^+$ T Cells

To determine the suitability of *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice for investigating the DENV/ZIKV-cross-reactive $CD4^+$ T cell response, we assessed whether DENV-primed $CD4^+$ T cells respond to cross-reactive ZIKV peptides *in vitro*. Splenocytes were isolated from *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice on day 7 after infection with the DENV2 S221 strain, stimulated *in vitro* for 6 h with the 30 ZIKV-derived candidate epitopes, and then analyzed for cytokine production by the ICS assay. Five ZIKV-derived $CD4^+$ T cell epitopes elicited cross-reactive responses by DENV2-primed $CD4^+$ T cells: $NS2A_{184–198}$ and $NS4B_{40–54}$ induced only TNF-producing cells; $NS2A_{66–80}$ and $NS5_{222–236}$ induced cells producing IFNγ plus TNF; and $E_{134–148}$ and $NS5_{222–236}$ induced cells producing IL-2, although their Benjamini-Hochberg adjusted p values (0.41 and 0.14, respectively) were higher than our target false discovery rate (FDR) of 10% (Figure 2; Table 1). Thus, in this mouse model, the non-structural proteins NS2A, NS4B, and NS5 in ZIKV contain epitopes that stimulate cross-reactive DENV-primed $CD4^+$ T cells to produce the canonical Th1 cytokines IFNγ and TNF.

Influence of DENV2 Immunity on the $CD4^+$ T Cell Response to ZIKV Infection

Based on our previous finding that prior DENV immunity affords cross-protection against ZIKV (Regla-Nava et al., 2018; Wen et al., 2017a), we assessed the effect of pre-existing DENV2 immunity on the outcome of ZIKV infection in this mouse model. Naive *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice were inoculated with DENV2, followed by infection of the DENV2-immune or naive *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice with ZIKV 4 weeks later. ZIKV viral loads in the serum, liver, and brain were examined at 3 and 7 days post-infection (dpi). As expected, these experiments revealed near-complete suppression of ZIKV replication in DENV2-immune mice compared with naive mice (Figures 3A–3C and 3F). To investigate the influence of DENV2 immunity on the $CD4^+$ T cell response, *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice or naive mice were primed with DENV2 and infected with ZIKV 4 weeks later, and splenocytes were isolated 3 or 7 dpi. Splenic $CD4^+$ T cells were then evaluated for expression of IFNγ, TNF, or IFNγ plus TNF after *in vitro* stimulation with DENV2/ZIKV-cross-reactive peptides ($NS2A_{66–80}$, $NS4B_{40-54}$, and $NS5_{222–236}$) and ZIKV-specific peptides ($C_{27–41}$, $C_{53–67}$, $C_{81–95}$, $E_{450–464}$, and $NS3_{601}$-$NS4A_{12}$). $CD4^+$ T cells from naive mice harvested at 3 dpi showed no response to the peptides (Figure 3G), whereas cells harvested from DENV2-primed mice displayed a strong response to the DENV2/ZIKV-cross-reactive peptide $NS5_{222–236}$, with expansion of $CD4^+$ T cells producing IFNγ, TNF, and IFNγ plus TNF (Figure 3H). By 7 dpi, $CD4^+$ T cells from both naive mice and DENV-immune mice showed responses to ZIKV-specific and DENV2/ZIKV-cross-reactive peptides

(Figures 3I and 3J). These results show that the memory $CD4^+$ T cell response elicited by prior DENV infection recognizes ZIKV in *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice.

Protective Effect of Vaccination with ZIKV-Specific and DENV2/ZIKV-Cross-Reactive Epitopes in ZIKV-Infected Mice

Next, we asked whether the ZIKV-derived $CD4^+$ T cell epitopes elicited a protective or pathogenic response during ZIKV infection. *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice were injected subcutaneously (s.c.) with adjuvant alone (mock vaccinated) or with a mixture of five ZIKV-derived peptides: two ZIKV specific ($C_{27–41}$ and $NS3_{601}$-$NS4A_{12}$), and three DENV2/ZIKV cross-reactive ($E_{134–148}$, $NS2A_{66–80}$, and $NS5_{222–236}$). Four weeks later, all mice were infected with ZIKV. At 3 dpi, blood, spleen, liver, and brain were harvested to determine viral titers by using a focus-forming assay (FFA). We found that infectious ZIKV titers were significantly lower in the serum, spleen, and brain of peptide-vaccinated mice than in mock-vaccinated mice (12-, 17-, and 12-fold, respectively), whereas no significant differences were observed between the liver viral titers in mock- and peptide-vaccinated mice (Figure S2). Thus, ZIKV-derived peptides (ZIKV specific and DENV2/ZIKV cross-reactive) elicit a protective response against subsequent ZIKV infection.

We then examined whether vaccination with DENV2/ZIKV-cross-reactive peptides alone could elicit a protective $CD4^+$ T cell response upon ZIKV viral infection. We vaccinated *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice with the DENV2/ZIKV cross-reactive peptides $E_{134–148}$, $NS2A_{66–80}$, $NS4B_{40–54}$, and $NS5_{222–236}$ (targeting four different ZIKV proteins) or with four irrelevant (influenza hem-agglutinin derived) peptides and infected the mice with ZIKV 4 weeks later. At 3 or 7 dpi, the frequency of peptide-specific $CD4^+$ T cells in the spleen was determined using the ICS assay, and ZIKV titers in the serum, liver, and brain were measured using the FFA. We found that the frequencies of peptide-specific IFNγ-, TNF-, and IFNγ plus TNF-producing $CD4^+$ T cells were significantly higher in the DENV2/ZIKV-cross-reactive peptide-vaccinated mice than in the irrelevant-peptide-vaccinated mice at 3 dpi, whereas the response had waned by 7 dpi, at which time the frequencies of DENV2/ZIKV-cross-reactive $CD4^+$ T cells were similar in the mice vaccinated with DENV2/ZIKV-cross-reactive peptides and irrelevant peptides (Figures 4A–4C). Because we previously showed that the development of a ZIKV-specific $CD4^+$ T cell response develops later than 3 dpi (Elong Ngono etal., 2019), these results suggest that vaccination with DENV2/ZIKV-crossreactive peptides induced a memory $CD4^+$ T cell response that expanded rapidly (within 3 days) upon ZIKV infection. Consistent with this finding, the level of infectious ZIKV was significantly lower in the serum, liver, and brain of mice vaccinated with DENV2/ZIKV-cross-reactive peptides than that of the mice vaccinated with irrelevant peptides at 3 dpi (7.6-, 3.6-, and 4.5-fold, respectively; Figures 4D–4F). At 7 dpi, the viral burden in the serum and liver of both mouse groups was completely cleared, likely because the primary T cell response to ZIKV developed by 7 dpi and contributed to ZIKVclearance;however, although the ZIKV burden in the brain of irrelevant-peptide-vaccinated mice remained high, it was significantly lower (21.7-fold) in DENV2/ZIKV-cross-reactive peptide-vaccinated mice (Figures 4D–4F).

To determine whether the ZIKV-protective response could be elicited in mice vaccinated for a longer period than 4 weeks, we also examined the $CD4^+$ T cell response and viral burden in mice infected with ZIKV at 6 weeks after peptide vaccination. Notably, the results were comparable to those obtained at 4 weeks post-vaccination. Thus, significantly higher frequencies of cytokine-producing $CD4^+$ T cells were detected in the spleens from mice vaccinated with DENV2/ZIKV-cross-reactive peptides than from mice vaccinated with irrelevant peptides at 3 dpi (Figures S3A–S3C), and ZIKV infection was effectively controlled, with viral titers in the liver reduced by 4.8-fold in the DENV2/ZIKV-cross-reactive peptide-vaccinated compared with the irrelevant-peptide-vaccinated mice (Figures S3D–S3F). We note that ZIKV was not detectable in the serum samples from either mouse group at 3 or 7 dpi. This may be because the 11-week-old (6-week-immunized) mice are more resistant to ZIKV infection than 9-week-old (4-week-immunized) mice.

Collectively, these results demonstrate that vaccination with DENV2/ZIKV-cross-reactive $CD4^+$ T cell epitopes can induce a memory $CD4^+$ T cell response that expands after ZIKV infection and contribute to the reduction of viral load at 4 and 6 weeks post-vaccination.

Protection Induced by Vaccination with DENV2/ZIKV-Cross-Reactive $CD4^+$ T Cell Epitopes Is Mediated by IFNγ and/or TNF in the Absence of Antibodies

To confirm that protection conferred by 4-week vaccination with DENV2/ZIKV-cross-reactive epitopes was mediated by $CD4^+$ T cells and the canonical Th1 cytokines, we depleted the vaccinated mice of $CD4^+$ T cells, IFNγ, or TNF. *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice were vaccinated with four DENV2/ZIKV-cross-reactive peptides (as described above), and 4 weeks later, they were treated with a $CD4^+$-T-cell-depleting Ab or isotype control Ab before (days −3 and −1) and after (day +1) ZIKV infection. Blood and tissues were then harvested and evaluated for infectious ZIKV titers at 3 dpi. Although the serum ZIKV titers were not significantly different between the two mouse groups (Figure 5A), ZIKV titers were markedly higher in the spleen, liver, and brain of $CD4^+$-T-cell-depleted mice than isotype control Ab-treated mice (5.7-, 12.2-, and 1.5-fold, respectively; Figures 5B–5D), confirming that $CD4^+$ T cells elicited by the DENV2/ZIKV-cross-reactive peptides contributed to protection against ZIKV infection.

To examine the contribution of IFNγ and/or TNF to the protection against ZIKV infection, we performed the same experiments as for $CD4^+$ T cell depletion, except that mice were instead treated with neutralizing Abs against either IFNγ or TNF before (days 3 and 1) and after (day +1) ZIKV infection. DENV2/ZIKV-cross-reactive peptide immunization elicited IFNγ- and/ or TNF-producing $CD4^+$ T cell response to individual peptide stimulation (Figures 6A–6C). Compared with the mice treated with the isotype control Ab, infectious ZIKV titers were significantly higher in the serum and brain of mice treated with the neutralizing anti-TNF Ab (6.6- and 35-fold, respectively) or anti-IFNγ Ab (59.2- and 26.4-fold, respectively) (Figures 6D and 6F), whereas ZIKV titers in the liver were significantly higher in mice depleted of IFNγ (82.1-fold) but not of TNF activity (Figure 6E). The same experiment performed in mice vaccinated with DENV2/ZIKV-cross-reactive peptides for 6 weeks before ZIKV infection confirmed that IFNγ was required for the reduction of ZIKV viral load in serum, liver, and brain (Figures S3G–S3I). No significant role for TNF was

observed in this experiment, although the levels of infectious ZIKV trended to be higher in the liver and brain, but not the serum, of mice with TNF blockade than mice treated with the isotype control mAb. These results indicate that both IFNγ and TNF contribute to the cross-reactive peptide vaccine-induced protection against ZIKV depending on the tissue and vaccination regimen.

Finally, we assessed whether an anti-ZIKV Ab response contributed to the protection against ZIKV infection in mice vaccinated with DENV2/ZIKV-cross-reactive peptides. *Ifnar1*$^{-/-}$HLA-DRB1*0101 mice were vaccinated with four DENV2/ZIKV-cross-reactive peptides, infected with ZIKV, and bled at 3 and 7 dpi for analysis of serum ZIKV E-reactive IgG titers. At 3 dpi, anti-ZIKV IgG titers were undetectable in mice vaccinated with either DENV2/ZIKV-cross-reactive peptides or irrelevant control peptides, whereas at 7 dpi, a low titer of anti-ZIKV IgG was detected in both mouse groups. Notably, however, there was no significant difference between the IgG titers in irrelevant-peptide-vaccinated and DENV2/ZIKV-cross-reactive peptide-vaccinated mice (Figure 6G). Thus, an anti-ZIKV IgG response had not developed in the vaccinated mice at 3 dpi, indicating that Abs do not contribute to the cross-reactive peptide vaccine-induced protection seen at 3 dpi.

Taken together, the results of this study demonstrate that protection against ZIKV after vaccination with DENV2/ZIKV-crossreactive epitopes is mediated by CD4$^+$ T cells and IFNγ- and/or TNF in an Ab-independent manner.

DISCUSSION

Recent epidemiologic studies have shown that prior DENV exposure provides protection against subsequent ZIKV infection in humans (Gordon et al., 2019; Pedroso et al., 2019; Rodriguez-Barraquer et al., 2019), but the mechanisms responsible for this cross-protection have yet to be defined. Evidence from mouse models supports roles for both Abs and CD8$^+$ T cells as mediators of cross-protective immunity (Fernandez et al., 2017; Huang et al., 2017; Kam et al., 2017; Regla-Nava et al., 2018; Wen et al., 2017a, 2017b), and more recent studies support a protective role for CD4$^+$ T cells in primary and secondary ZIKV infection (Elong Ngono et al., 2019; Hassert et al., 2018; Liang et al., 2019; Lucas et al., 2018; Pardy et al., 2017). However, the pathogenic versus protective functions of DENV-elicited CD4$^+$ T cells during ZIKV infection have not previously been investigated. Using *Ifnar1*$^{-/-}$HLA-DRB1*0101 transgenic mice, we identified DENV2/ZIKV cross-reactive CD4$^+$ T cell epitopes and showed that vaccination with the peptides induced a CD4$^+$ T cell response that protected against ZIKV infection in a manner dependent on CD4$^+$ T cells and IFNγ and/or TNF but not on anti-ZIKV Abs. Thus, in addition to Abs and CD8$^+$ T cells, the results of the present study establish that CD4$^+$ T cells and the canonical Th1 effector cytokines act in an Ab-independent manner to confer cross-protective immunity against ZIKV in mice. These results have important implications for ZIKV infections in humans previously exposed to DENV.

Our previous finding that the same epitopes and immunodominance patterns are detected in *Ifnar1*$^{-/-}$HLA transgenic mice and humans (Elong Ngono et al., 2016; Weiskopf et al., 2011, 2013, 2014, 2015b) validate the use of the *Ifnar1*$^{-/-}$HLA-DRB1*0101 transgenic mouse

model to evaluate human-relevant DENV/ZIKV-cross-reactive $CD4^+$ T cell responses against ZIKV infection. One of the ZIKV-specific epitopes identified here ($C_{27–41}$) shares a 13-amino acid sequence with a 15-mer (SPFGGLKRLPAGLLL) identified as a $CD4^+$ T cell epitope in humans by Koblischke et al. (2018), who examined the IL-2 response of $CD8^+$-T-cell-depleted peripheral blood mononuclear cells from ZIKV-infected individuals. The $E_{134–148}$ peptide, which we demonstrated here induces IL-2-producing $CD4^+$ T cells, shares a 14-amino acid sequence with another human $CD4^+$ T cell epitope (ENLEYRIMLSVHGSQ) reported in the same human study (Koblischke et al., 2018). Given that $C_{27–41}$ and $E_{134–148}$ were identified based on their predicted binding to HLA-DRB1*0101, it seems likely that the two epitopes identified by Koblischke et al. (2018) are also HLA-DRB1*0101 restricted.

Our results demonstrating that the DENV2/ZIKV-cross-reactive $CD4^+$ T cell response expanded early (day 3) after ZIKV infection and remained in the later phase of the response (day 7) is in agreement with data showing that ZIKV cross-reactive $CD4^+$ T cells are immunodominant and rapidly activated in DENV-immune individuals (Grifoni et al., 2017). This finding is also consistent with studies on the responses of cross-reactive $CD8^+$ T cells during sequential infections with DENV and ZIKV (Grifoni et al., 2017; Wen et al., 2017b) or with heterologous DENV serotypes (Weiskopf et al., 2014) in humans and *Ifnar1*$^{-/-}$HLA transgenic mice. Thus, prior DENV infection shapes both $CD4^+$ and $CD8^+$ T cell responses during subsequent ZIKV infection in humans and *Ifnar1*$^{-/-}$HLA transgenic mice. As DENV and ZIKV infection in *Ifnar1*$^{-/-}$HLA transgenic mice evoke T cell responses that are similar to those in humans with respect to timing, antigen specificity, and immunodominance patterns, *Ifnar1*$^{-/-}$HLA transgenic mouse models represent a valuable model for examining key features of human-relevant T cell responses in various DENV and ZIKV infection and vaccination settings.

Consistent with publications reporting cross-reactivity of pre-existing DENV-elicited $CD4^+$ T cell responses to ZIKV (Grifoni et al., 2017; Lim et al., 2018; Paquin-Proulx et al., 2017; Reynolds et al., 2018), we identified five HLA-DRB1*0101-restricted ZIKV epitopes ($E_{134–148}$, $NS2A_{66–80}$, $NS2A_{184–198}$, $NS4B_{40–54}$, and $NS5_{222–236}$) that were cross-reactive on DENV-primed $CD4^+$ T cells. The five DENV2/ZIKV-cross-reactive epitopes identified here share 33%–100% homology with the corresponding DENV2 sequences and 27%–100%, 33%– 93%, and 33%–100% homology with variants in DENV1, DENV3, and DENV4, respectively (Table S1). Notably, $NS5_{222–236}$ is a conserved T cell epitope among multiple flaviviruses; the identical sequence is present in DENV1, 2, and 4 and YFV, and the homologous sequences in DENV3 and JEV differ by one and two residues, respectively. Similarly, we previously identified an HLA-B*0702-restricted $CD8^+$ T cell epitope that is conserved among many flaviviruses, including ZIKV, DENV1–4, WNV, JEV, Usutu virus, Murray Valley encephalitis virus, and Kunjin virus (Wen et al., 2017b). The identification of conserved $CD4^+$ T cell and $CD8^+$ T cell epitopes among flaviviruses supports the feasibility of developing flavivirus vaccines that elicit both $CD4^+$- and $CD8^+$-T-cell-mediated cross-protective immunity.

In line with studies suggesting a protective role for serotype-cross-reactive $CD4^+$ T cells in DENV immunity (Weiskopf et al., 2015a, 2016), the present study provides evidence in

support of a role for $CD4^+$ T cells in conferring cross-protection against ZIKV in DENV2-immune mice. $CD4^+$ T cells from DENV2/ZIKV-cross-reactive peptide-vaccinated mice produced IFNγ and TNF upon cognate peptide restimulation *in vitro*, and depletion of $CD4^+$ T cells and neutralization of IFNγ and/or TNF disrupted the protection induced by peptide vaccination in mice. Thus, the protective roles of $CD4^+$ T cells elicited by peptide vaccination are likely to be mediated by ZIKV-induced secretion of IFNγ and/or TNF. Taken together with studies identifying important roles for $CD8^+$ T cells in cross-protecting against DENV (de Alwis et al., 2016; Elong Ngono et al., 2016; Weiskopf et al., 2013, 2015b; Zellweger et al., 2015) and ZIKV infections (Huang et al., 2017; Regla-Nava et al., 2018; Wen et al., 2017a, 2017b), our results suggest that a pan-flavivirus vaccine that induces $CD8^+$ T cell responses and canonical Th1-cytokine-producing $CD4^+$ T cell responses may not only be broadly effective against both DENV and ZIKV but also avoid ADE. This is a crucial point because the DENV- and ZIKV-specific vaccines that are currently licensed or in clinical trials aim to elicit Ab responses and may, at least in theory, cause ADE if the vaccine-induced Ab response is inefficient or wanes.

In summary, our results demonstrate that vaccination with DENV2/ZIKV-cross-reactive peptides elicits a Th1 $CD4^+$ T cell effector response that promotes protection against ZIKV infection in an IFNγ/TNF-dependent, Ab-independent manner. These findings suggest that the cross-reactive $CD4^+$ T cell response is one mechanism by which humans with prior DENV exposure are protected against ZIKV. Inclusion of cross-reactive $CD8^+$ T cell and $CD4^+$ Th1 cell epitopes could not only enhance the efficacy of ZIKV-specific vaccines but also pave the way for the development of a pan-flavivirus vaccine that simultaneously protects against both DENV and ZIKV and minimizes ADE.

STAR★METHODS

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Sujan Shresta (sujan@lji.org).

This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals—*Ifnar1*$^{-/-}$HLA-DRB1*0101 mice have been described in our previous study of anti-DENV responses (Weiskopf et al., 2011). Mice were bred under specific pathogen-free conditions with a 12 hour on/off light cycle at the La Jolla Institute for Immunology. Mouse experiments were approved by the Institutional Animal Care and Use Committee (protocol no. AP028-SS1–0615). Sample sizes were estimated based on experiments in similar studies, and the experiments were not randomized or blinded.

Cell Lines—Baby hamster kidney (BHK)-21 cells (ATCC, CCL-10) were grown in MEM-α medium containing 10% FBS and 1 3 penicillin/streptomycin. Growing cells were allowed to proliferate until 90% confluency and then split for propagation.

Virus Strains—ZIKV strain SD001 was isolated from the urine of a ZIKV-infected individual who traveled to Venezuela during the 2016 ZIKV epidemic. PCR sequencing showed that ZIKV SD001 belongs to the Asian lineage and is phylogenetically related to ZIKV isolates circulating in South American countries (Carlin et al., 2018). The mouse-adapted DENV2 strain S221 is a triple-plaque-purified clone derived from DENV2 D2S10 (Yauch et al., 2009). Both ZIKV and DENV2 were grown in C6/36 mosquito cells, and viral titers were measured using an FFA with the baby hamster kidney (BHK)-21 cell line as described below.

METHOD DETAILS

Peptide prediction and synthesis—The online software Immune Epitope Database and Analysis Resource (IEDB-AR) (http://www.iedb.org) (Vita et al., 2019) was used to predict HLA-DRB1*0101-binding peptides from ZIKV strain FSS13025 (Cambodia, 2010; Asian lineage). Thirty predicted epitope candidates were synthesized by Synthetic Biomolecules as crude peptides (> 75% purity) for use in *in vitro* experiments. Two ZIKV-specific ($C_{27–41}$ and $NS3_{601}$-$NS4A_{12}$), four DENV2/ZIKV-cross-reactive ($E_{134–148}$, $NS2A_{66–80}$, $NS4B_{40–54}$, and $NS5_{222–236}$) (ZIKV sequence numbering; Table 1), and four irrelevant peptides (influenza hemagglutinin-derived HLA-DRB1*0101-restricted epitopes: $H1_{221–235}$ [SRYSKKFKPEIAIRP], $H1_{225–239}$ [KKFKPEIAIRPKVRD], $H1_{305–319}$ [TSLPFQNIHPITIGK], and $H1_{441–455}$ [ELLVLLENERTLDYH]) (DiPiazza et al., 2017) were synthesized at high purity (> 99%) for use *in vitro* and in mouse vaccination experiments. All peptides were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 40 mg/ml and were stored at −20°C.

ZIKV infection of mice and peptide screening—For the 30-peptide epitope screening, 5-week-old female or male mice were infected retro-orbitally (r.o.) with either 1×10^2 focus-forming units (FFU) of ZIKV SD001 or 2×10^3 FFU of DENV2 S221 in 200 μL of 10% fetal bovine serum in phosphate-buffered saline (FBS/PBS). At 7 dpi, spleens were harvested and single-cell splenocyte suspensions were prepared. A total of 1×10^6 splenocytes was plated in each well of a 96-well U-bottom plate and stimulated with individual peptides (20 μg crude peptide/well) for 6 h, with brefeldin A added for the final 5 h (GolgiPlug, BD Biosciences). Positive and negative controls were splenocytes stimulated with phor-bol-12-myristate-13-acetate (PMA, 0.1 μg/ml) and ionomycin (1 μg/ml) (Thermo Fisher Scientific) or incubated with medium alone, respectively. Cells were harvested, washed, and processed for the ICS assay as described below.

ZIKV infection of DENV2-immune mice—Five-week-old female or male mice were inoculated intraperitoneally (i.p.) with 2×10^3 FFU of DENV2 S221. Four weeks later, the mice were infected r.o. with 1×10^4 FFU of ZIKV SD001, and on day 3 or 7 after infection, all mice were sacrificed and blood samples were collected for serum preparation. After cardiac perfusion with PBS, the spleen, liver, and brain were harvested. Splenocytes were stimulated *in vitro* with 20 μg crude peptide ($C_{27–41}$, $C_{53–67}$, $C_{81–95}$, $E_{450–464}$ or $NS3_{601}$-$NS4A_{12}$) or 1 μg purified peptide ($E_{134–148}$, $NS2A_{66–80}$, $NS4B_{40-54}$ or $NS5_{222–236}$) per well. Positive and negative controls were included in all experiments. Cells were processed for the

ICS assay as described below. ZIKV viral titers in serum and tissues were measured using the FFA as described below.

ZIKV infection of peptide-vaccinated mice—Several protocols were employed. Mixtures of five ZIKV-specific and DENV2/ZIKV-cross-reactive peptides ($C_{27–41}$, $E_{134–148}$, $NS2A_{66–80}$, $NS3_{601}$-$NS4A_{12}$, and $NS5_{222–236}$; 50 μg of each peptide/mouse) were emulsified in complete Freund's adjuvant and injected subcutaneously (s.c.) into 5-week-old female or male mice. Mock-vaccinated mice received the adjuvants in DMSO without peptides. Two weeks later, the mice were boosted by injection of the same peptides in incomplete Freund's adjuvant. Two weeks after the boost, mice were infected r.o. with 1×10^4 FFU of ZIKV SD001. Three days later, all mice were sacrificed and blood, spleen, liver, and brain were harvested. ZIKV viral titers in serum and tissues were measured using the FFA as described below.

The four DENV2/ZIKV-cross-reactive peptides ($E_{134–148}$, $NS2A_{66–80}$, $NS4B_{40–54}$, and $NS5_{222–236}$; 50 μg of each peptide/mouse) were emulsified in complete Freund's adjuvant and injected s.c. into 5-week-old female or male mice. Mock-vaccinated mice received the adjuvants in DMSO without peptides, and the irrelevant peptide-vaccinated mice received the adjuvants with four influenza-derived peptides ($H1_{221–235}$, $H1_{225–239}$, $H1_{305–319}$, and $H1_{441–455}$; 50 μg of each peptide/mouse). Two weeks later, the mice were boosted by injection of the same peptides in incomplete Freund's adjuvant. Two or 4 weeks after the boost, mice were treated by one of three protocols as follows. (i) Mice were infected r.o. with 1×10^4 FFU of ZIKV SD001. Three or 7 days later, all mice were sacrificed and blood, spleen, liver, and brain were harvested. Splenocytes were stimulated *in vitro* with individual or pooled DENV2/ZIKV-crossreactive peptides ($E_{134–148}$, $NS2A_{66–80}$, $NS4B_{40–54}$, and $NS5_{222–236}$) (1 μg purified peptide/well) and processed for the ICS assay as described below. ZIKV-reactive IgG in serum was measured using a capture ELISA assay, and ZIKV viral titers in serum and tissues were measured using the FFA as described below. (ii) Mice were infected r.o. with 1×10^4 FFU of ZIKV SD001 and injected i.p. with 250 μg of a $CD4^+$ T cell-depleting Ab (clone GK1.5, Bio X Cell) or isotype control Ab (LTF-2, Bio X Cell) on days 3 and 1 before and 1 day after ZIKV infection. Three days later, all mice were sacrificed and blood, spleen, liver, and brain were harvested. ZIKV viral titers in serum and tissues were measured using the FFA. (iii) Mice were infected r.o. with 1×10^4 FFU of ZIKV SD001 and injected i.p. with 100 μg of neutralizing anti-TNF monoclonal Ab (mAb; clone XT3.11, Bio X Cell), anti-IFNγ mAb (clone XMG1.2, Thermo Fisher Scientific), or isotype control mAb (clone HPRN, Bio X Cell) on days 3 and 1 before and 1 day after ZIKV infection. At 3 dpi, all mice were sacrificed and blood, spleen, liver, and brain were harvested. ZIKV viral titers in serum and tissues were measured using the FFA.

ICS assay—After incubation of splenocytes with peptides or PMA/ionomycin, cells were harvested, washed, and incubated with Fc Block (CD16/CD32 mAb 2.4G2; BD Biosciences), stained with fixable Live/Dead blue viability stain (Thermo Fisher Scientific), and then stained with the following Abs: PerCP-Cy5.5-conjugated anti-CD3 mAb (clone 145–2C11, Tonbo Biosciences), APC-eFluor780-conjugated anti-CD4 mAb (clone GK1.5, Thermo Fisher Scientific), Brilliant Violet (BV) 785-conjugated anti-CD44 mAb (clone

IM7, BioLegend), Alexa Fluor 700-conjugated anti-CD62L mAb (clone MEL-14, Thermo Fisher Scientific), PE-conjugated anti-CD11a (clone M17/4, BioLegend), BV605-conjugated anti-CD49d (clone R1–2, BD Biosciences), PE-conjugated anti-CD25 mAb (clone PC61, BioLegend), PE-conjugated or biotin-conjugated anti-CD185 mAb (CXCR5, clone SPRC15; Thermo Fisher Scientific), BV 605-conjugated anti-CD279 mAb (PD1, clone 29F.1A12; BioLegend), and/or BV 421-conjugated streptavidin (#405225, BioLegend). Cells were then fixed and permeabilized using Cytofix/Cytoperm solution (#554722, BD Biosciences), followed by staining with FITC-conjugated anti-IFNγ mAb (clone XMG1.2, Tonbo Biosciences), Alexa Fluor 700- or APC-conjugated anti-TNF mAb (clone MP6-XT22, Thermo Fisher Scientific), PE-, BV 421-, or BV 711-conjugated anti-IL-2 mAb (clone JES6–5H4, BioLegend), APC-conjugated anti-IL-4 mAb (clone 11B11, BioLegend), PE-conjugated anti-IL-5 mAb (clone TRFK5, Thermo Fisher Scientific), BV510-conjugated anti-IL-17A mAb (clone TC11–18H10.1, BioLegend), and/or Alexa Fluor 700-conjugated anti-FoxP3 mAb (clone FJK-16S, Thermo Fisher Scientific). Data were collected using an LSR Fortessa flow cytometer (BD Biosciences) and analyzed using FlowJo software X 10.0.7 (Tree Star).

ELISA—To quantify ZIKV-reactive IgG, 96-well high-affinity ELISA plates (Costar) were coated with ZIKV E protein (1 mg/ml ZIKVSU-ENV, The Native Antigen) in 100 μl coating buffer (0.1 M $NaHCO_3$) overnight at 4°C and then blocked for 1 h at room temperature (RT) with 5% Blocker Casein in PBS (Thermo Fisher Scientific). Mouse serum samples were serially diluted three-fold from 1:30 to 1:65,610 in 1% bovine serum albumin (BSA)/PBS and added to the coated wells. As a positive control, 10 μg of the pan-flavivirus envelope protein-specific mAb 4G2 (Absolute Antibody) was diluted in 1% BSA/PBS and titrated three-fold in the same manner as for the sera. After 1.5 h incubation at RT, the wells were washed with washing buffer (0.05% Tween 20 in PBS) and then incubated with HRP-conjugated goat anti-mouse IgG (1:5000 in 1% BSA/PBS) for 1.5 h at RT. TMB chromogen solution (Thermo Fisher Scientific) was added to the wells, the reaction was stopped by addition of 2N sulfuric acid, and the absorbance at 450 nm was read on a Spectramax M2E microplate reader (Molecular Devices). The ZIKV-specific IgG endpoint titers were calculated as the reciprocal of the highest serum dilution that gave a reading twice the cutoff absorbance of the negative control (1% BSA/PBS).

Focus-forming assay—BHK-21 cells (2×10^5/well) were plated in 24-well culture plates and incubated at 37°C in a CO_2 incubator overnight. Mouse spleen, liver, and brain were homogenized using TissueLyser II (QIAGEN) and centrifuged at 6000 rpm for 10 min. Aliquots of the supernatants (100 μl) were serially diluted 10-fold in medium, added to the BHK-21 cells, and incubated at 37°C for 1 h. The viral supernatant was aspirated, and a pre-warmed solution of 1% carboxymethyl cellulose medium was added to each well. After 2.5 days incubation, the cells were fixed with 4% paraformaldehyde solution for 30 min at RT, washed with PBS, permeabilized with 1% Triton X-100 for 20 min at RT, and washed again with PBS. Plates were blocked with 10% FBS/PBS for 40 min at RT and incubated with the pan-flavivirus envelope protein-specific mAb 4G2 (1 μg/ml) for 1 h at RT. Plates were washed with PBS and incubated with horseradish peroxidase-conjugated goat anti-mouse IgG mAb (1:1000 dilution) for 1.5 h at RT. Finally, the plates were washed with PBS and

developed with TrueBlue peroxidase substrate for 20 min at RT. Foci were counted and the viral titers were expressed as FFU/ml serum or FFU/g tissue.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistics and graphs—Statistics and graphs were prepared using Prism software version 6.0 (GraphPad Software) and are expressed as the mean ± standard error (s.e.m.). Statistically significant differences between two groups were determined using the non-parametric Mann– Whitney test followed by Benjamini-Hochberg (B-H) correction in R studio (v3.6.1), fixing the false discovery rate (FDR) at 10% or Kruskal–Wallis one-way ANOVA with Dunn's correction for multiple comparisons. $p < 0.05$ was considered significant. Further details on statistical analysis are listed in the figure legends.

DATA AND CODE AVAILABILITY

This study did not generate any unique datasets or code.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by the grants from the National Institutes of Health (AI116813, AI140063, and NS106387 to S.S.), the National Natural Science Foundation of China (31870159 to J.W.), and the Zhejiang Provincial Natural Science Foundation (LY17C010004 to J.W.).

REFERENCES

Aguirre S, Maestre AM, Pagni S, Patel JR, Savage T, Gutman D, Maringer K, Bernal-Rubio D, Shabman RS, Simon V, et al. (2012). DENV inhibits type I IFN production in infected cells by cleaving human STING. PLoS Pathog. 8, e1002934. [PubMed: 23055924]

Bardina SV, Bunduc P, Tripathi S, Duehr J, Frere JJ, Brown JA, Nachbagauer R, Foster GA, Krysztof D, Tortorella D, et al. (2017). Enhancement of Zika virus pathogenesis by preexisting antiflavivirus immunity. Science 356, 175–180. [PubMed: 28360135]

Carlin AF, Wen J, Vizcarra EA, McCauley M, Chaillon A, Akrami K, Kim C, Ngono AE, Lara-Marquez ML, Smith DM, et al. (2018). A longitudinal systems immunologic investigation of acute Zika virus infection in an individual infected while traveling to Caracas, Venezuela. PLoS Negl. Trop. Dis 12, e0007053. [PubMed: 30596671]

Castanha PMS, Nascimento EJM, Braga C, Cordeiro MT, de Carvalho OV, de Mendonça LR, Azevedo EAN, França RFO, Dhalia R, and Marques ETA (2017). Dengue Virus-Specific Antibodies Enhance Brazilian Zika Virus Infection. J. Infect. Dis 215, 781–785. [PubMed: 28039355]

Charles AS, and Christofferson RC (2016). Utility of a Dengue-Derived Monoclonal Antibody to Enhance Zika Infection In Vitro. PLoS Curr. 8, ecurrents.outbreaks.4ab8bc87c945eb41cd8a49e127082620.

Choumet V, and Despres P (2015). Dengue and other flavivirus infections. Rev. Sci. Tech 34, 473–478, 467–472. [PubMed: 26601449]

de Alwis R, Bangs DJ, Angelo MA, Cerpas C, Fernando A, Sidney J, Peters B, Gresh L, Balmaseda A, de Silva AD, et al. (2016). Immunodominant Dengue Virus-Specific CD8+ T Cell Responses Are Associated with a Memory PD-1+ Phenotype. J. Virol 90, 4771–4779. [PubMed: 26912627]

Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, Sakuntabhai A, Cao-Lormeau VM, Malasit P, Rey FA, et al. (2016). Dengue virus sero-cross-reactivity drives

antibody-dependent enhancement of infection with zika virus. Nat. Immunol 17, 1102–1108. [PubMed: 27339099]

Ding Q, Gaska JM, Douam F, Wei L, Kim D, Balev M, Heller B, and Ploss A (2018). Species-specific disruption of STING-dependent antiviral cellular defenses by the Zika virus NS2B3 protease. Proc. Natl. Acad. Sci. USA 115, E6310–E6318. [PubMed: 29915078]

DiPiazza A, Richards K, Poulton N, and Sant AJ (2017). Avian and Human Seasonal Influenza Hemagglutinin Proteins Elicit CD4 T Cell Responses That Are Comparable in Epitope Abundance and Diversity. Clin. Vaccine Immunol 24, e00548–16. [PubMed: 28100497]

Earl PL, Americo JL, Wyatt LS, Eller LA, Whitbeck JC, Cohen GH, Eisenberg RJ, Hartmann CJ, Jackson DL, Kulesh DA, et al. (2004). Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. Nature 428, 182–185. [PubMed: 15014500]

Elong Ngono A, Chen HW, Tang WW, Joo Y, King K, Weiskopf D, Sidney J, Sette A, and Shresta S (2016). Protective Role of Cross-Reactive CD8 T Cells Against Dengue Virus Infection. EBioMedicine 13, 284–293. [PubMed: 27746192]

Elong Ngono A, Vizcarra EA, Tang WW, Sheets N, Joo Y, Kim K, Gorman MJ, Diamond MS, and Shresta S (2017). Mapping and Role of the CD8+ T Cell Response During Primary Zika Virus Infection in Mice. Cell Host Microbe 21, 35–46. [PubMed: 28081442]

Elong Ngono A, Young MP, Bunz M, Xu Z, Hattakam S, Vizcarra E, Regla-Nava JA, Tang WW, Yamabhai M, Wen J, and Shresta S (2019). CD4+ T cells promote humoral immunity and viral control during Zika virus infection. PLoS Pathog. 15, e1007474. [PubMed: 30677097]

Fernandez E, Dejnirattisai W, Cao B, Scheaffer SM, Supasa P, Wongwiwat W, Esakky P, Drury A, Mongkolsapaya J, Moley KH, et al. (2017). Human antibodies to the dengue virus E-dimer epitope have therapeutic activity against Zika virus infection. Nat. Immunol 18, 1261–1269. [PubMed: 28945244]

Fowler AM, Tang WW, Young MP, Mamidi A, Viramontes KM, McCauley MD, Carlin AF, Schooley RT, Swanstrom J, Baric RS, et al. (2018). Maternally Acquired Zika Antibodies Enhance Dengue Disease Severity in Mice. Cell Host Microbe 24, 743–750.e745. [PubMed: 30439343]

Gordon A, Gresh L, Ojeda S, Katzelnick LC, Sanchez N, Mercado JC, Chowell G, Lopez B, Elizondo D, Coloma J, et al. (2019). Prior dengue virus infection and risk of Zika: A pediatric cohort in Nicaragua. PLoS Med. 16, e1002726. [PubMed: 30668565]

Grant A, Ponia SS, Tripathi S, Balasubramaniam V, Miorin L, Sourisseau M, Schwarz MC, Sánchez-Seco MP, Evans MJ, Best SM, and García-Sastre A (2016). Zika Virus Targets Human STAT2 to Inhibit Type I Interferon Signaling. Cell Host Microbe 19, 882–890. [PubMed: 27212660]

Grifoni A, Pham J, Sidney J, O'Rourke PH, Paul S, Peters B, Martini SR, de Silva AD, Ricciardi MJ, Magnani DM, et al. (2017). Prior Dengue virus exposure shapes T cell immunity to Zika virus in humans. J. Virol 91, e01469–17. [PubMed: 28978707]

Halstead SB (2007). Dengue. Lancet 370, 1644–1652. [PubMed: 17993365]

Hassert M, Wolf KJ, Schwetye KE, DiPaolo RJ, Brien JD, and Pinto AK (2018). CD4+T cells mediate protection against Zika associated severe disease in a mouse model of infection. PLoS Pathog. 14, e1007237. [PubMed: 30212537]

Huang H, Li S, Zhang Y, Han X, Jia B, Liu H, Liu D, Tan S, Wang Q, Bi Y, et al. (2017). $CD8^+$ T Cell Immune Response in Immunocompetent Mice during Zika Virus Infection. J. Virol 91, e00900–17. [PubMed: 28835502]

Kam YW, Lee CY, Teo TH, Howland SW, Amrun SN, Lum FM, See P, Kng NQ, Huber RG, Xu MH, et al. (2017). Cross-reactive dengue human monoclonal antibody prevents severe pathologies and death from Zika virus infections. JCI Insight 2, 92428. [PubMed: 28422757]

Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, Gordon A, Balmaseda A, and Harris E (2017). Antibody-dependent enhancement of severe dengue disease in humans. Science 358, 929–932. [PubMed: 29097492]

Kawiecki AB, and Christofferson RC (2016). Zika Virus-Induced Antibody Response Enhances Dengue Virus Serotype 2 Replication In Vitro. J. Infect. Dis 214, 1357–1360. [PubMed: 27521359]

Koblischke M, Stiasny K, Aberle SW, Malafa S, Tsouchnikas G, Schwaiger J, Kundi M, Heinz FX, and Aberle JH (2018). Structural Influence on the Dominance of Virus-Specific CD4 T Cell Epitopes in Zika Virus Infection. Front. Immunol 9, 1196. [PubMed: 29899743]

Lazear HM, and Diamond MS (2016). Zika Virus: New Clinical Syndromes and Its Emergence in the Western Hemisphere. J. Virol 90, 4864–4875. [PubMed: 26962217]

Liang H, Tang J, Liu Z, Liu Y, Huang Y, Xu Y, Hao P, Yin Z, Zhong J, Ye L, et al. (2019). ZIKV infection induces robust Th1-like Tfh cell and long-term protective antibody responses in immunocompetent mice. Nat. Commun 10, 3859. [PubMed: 31455769]

Lim MQ, Kumaran EAP, Tan HC, Lye DC, Leo YS, Ooi EE, MacAry PA, Bertoletti A, and Rivino L (2018). Cross-Reactivity and Anti-viral Function of Dengue Capsid and NS3-Specific Memory T Cells Toward Zika Virus. Front. Immunol 9, 2225. [PubMed: 30327651]

Lucas CGO, Kitoko JZ, Ferreira FM, Suzart VG, Papa MP, Coelho SVA, Cavazzoni CB, Paula-Neto HA, Olsen PC, Iwasaki A, et al. (2018). Critical role of CD4+ T cells and IFNγ signaling in antibody-mediated resistance to Zika virus infection. Nat. Commun 9, 3136. [PubMed: 30087337]

Marsh SGE, Parham P, and Barber LD (1999). The HLA FactsBook (Academic Press).

Ngono AE, and Shresta S (2018). Immune Response to Dengue and Zika. Annu. Rev. Immunol 36, 279–308. [PubMed: 29345964]

Panagioti E, Klenerman P, Lee LN, van der Burg SH, and Arens R (2018). Features of Effective T Cell-Inducing Vaccines against Chronic Viral Infections. Front. Immunol 9, 276. [PubMed: 29503649]

Paquin-Proulx D, Leal FE, Terrassani Silveira CG, Maestri A, Brockmeyer C, Kitchen SM, Cabido VD, Kallas EG, and Nixon DF (2017). T-cell Responses in Individuals Infected with Zika Virus and in Those Vaccinated Against Dengue Virus. Pathog. Immun 2, 274–292. [PubMed: 28835931]

Pardy RD, Rajah MM, Condotta SA, Taylor NG, Sagan SM, and Richer MJ (2017). Analysis of the T Cell Response to Zika Virus and Identification of a Novel CD8+ T Cell Epitope in Immunocompetent Mice. PLoS Pathog. 13, e1006184. [PubMed: 28231312]

Paul LM, Carlin ER, Jenkins MM, Tan AL, Barcellona CM, Nicholson CO, Michael SF, and Isern S (2016). Dengue virus antibodies enhance Zika virus infection. Clin. Transl. Immunology 5, e117.

Pedroso C, Fischer C, Feldmann M, Sarno M, Luz E, Moreira-Soto A, Cabral R, Netto EM, Brites C, Kummerer €BM, and Drexler JF (2019). Cross-Protection of Dengue Virus Infection against Congenital Zika Syndrome, Northeastern Brazil. Emerg. Infect. Dis 25, 1485–1493. [PubMed: 31075077]

Priyamvada L, Quicke KM, Hudson WH, Onlamoon N, Sewatanon J, Edupuganti S, Pattanapanyasat K, Chokephaibulkit K, Mulligan MJ, Wilson PC, et al. (2016). Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus. Proc. Natl. Acad. Sci. USA 113, 7852–7857. [PubMed: 27354515]

Regla-Nava JA, Elong Ngono A, Viramontes KM, Huynh AT, Wang YT, Nguyen AT, Salgado R, Mamidi A, Kim K, Diamond MS, and Shresta S (2018). Cross-reactive Dengue virus-specific $CD8^+$ T cells protect against Zika virus during pregnancy. Nat. Commun 9, 3042. [PubMed: 30072692]

Reynolds CJ, Suleyman OM, Ortega-Prieto AM, Skelton JK, Bonnesoeur P, Blohm A, Carregaro V, Silva JS, James EA, Maillère B, et al. (2018). T cell immunity to Zika virus targets immunodominant epitopes that show cross-reactivity with other Flaviviruses. Sci. Rep 8, 672. [PubMed: 29330423]

Robbiani DF, Olsen PC, Costa F, Wang Q, Oliveira TY, Nery N Jr., Aromolaran A, do Rosário MS, Sacramento GA, Cruz JS, et al. (2019). Risk of Zika microcephaly correlates with features of maternal antibodies. J. Exp. Med 216, 2302–2315. [PubMed: 31413072]

Rodriguez-Barraquer I, Costa F, Nascimento EJM, Nery N, Castanha PMS, Sacramento GA, Cruz J, Carvalho M, De Olivera D, Hagan JE, et al. (2019). Impact of preexisting dengue immunity on Zika virus emergence in a dengue endemic region. Science 363, 607–610. [PubMed: 30733412]

Salek-Ardakani S, Flynn R, Arens R, Yagita H, Smith GL, Borst J, Schoenberger SP, and Croft M (2011). The TNFR family members OX40 and CD27 link viral virulence to protective T cell vaccines in mice. J. Clin. Invest 121, 296–307. [PubMed: 21183789]

Salje H, Cummings DAT, Rodriguez-Barraquer I, Katzelnick LC, Lessler J, Klungthong C, Thaisomboonsuk B, Nisalak A, Weg A, Ellison D, et al. (2018). Reconstruction of antibody dynamics and infection histories to evaluate dengue risk. Nature 557, 719–723. [PubMed: 29795354]

Slon Campos JL, Poggianella M, Marchese S, Mossenta M, Rana J, Arnoldi F, Bestagno M, and Burrone OR (2017). DNA-immunisation with dengue virus E protein domains I/II, but not domain III, enhances Zika, West Nile and Yellow Fever virus infection. PLoS One 12, e0181734. [PubMed: 28742857]

Swanstrom JA, Plante JA, Plante KS, Young EF, McGowan E, Gallichotte EN, Widman DG, Heise MT, de Silva AM, and Baric RS (2016). Dengue Virus Envelope Dimer Epitope Monoclonal Antibodies Isolated from Dengue Patients Are Protective against Zika Virus. MBio 7, e01123–16. [PubMed: 27435464]

Tang WW, Grewal R, and Shresta S (2015). Influence of antibodies and T cells on dengue disease outcome: insights from interferon receptor-deficient mouse models. Curr. Opin. Virol 13, 61–66. [PubMed: 26001278]

Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, Wheeler DK, Sette A, and Peters B (2019). The Immune Epitope Database (IEDB): 2018 update. Nucleic Acids Res. 47, D339–D343. [PubMed: 30357391]

Weiskopf D, Yauch LE, Angelo MA, John DV, Greenbaum JA, Sidney J, Kolla RV, De Silva AD, de Silva AM, Grey H, et al. (2011). Insights into HLA-restricted T cell responses in a novel mouse model of dengue virus infection point toward new implications for vaccine design. J. Immunol 187, 4268–4279. [PubMed: 21918184]

Weiskopf D, Angelo MA, de Azeredo EL, Sidney J, Greenbaum JA, Fernando AN, Broadwater A, Kolla RV, De Silva AD, de Silva AM, et al. (2013). Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells. Proc. Natl. Acad. Sci. USA 110, E2046–E2053. [PubMed: 23580623]

Weiskopf D, Angelo MA, Sidney J, Peters B, Shresta S, and Sette A (2014). Immunodominance changes as a function of the infecting dengue virus serotype and primary versus secondary infection. J. Virol 88, 11383–11394. [PubMed: 25056881]

Weiskopf D, Bangs DJ, Sidney J, Kolla RV, De Silva AD, de Silva AM, Crotty S, Peters B, and Sette A (2015a). Dengue virus infection elicits highly polarized CX3CR1+ cytotoxic CD4+ T cells associated with protective immunity. Proc. Natl. Acad. Sci. USA 112, E4256–E4263. [PubMed: 26195744]

Weiskopf D, Cerpas C, Angelo MA, Bangs DJ, Sidney J, Paul S, Peters B, Sanches FP, Silvera CG, Costa PR, et al. (2015b). Human CD8+ T-Cell Responses Against the 4 Dengue Virus Serotypes Are Associated With Distinct Patterns of Protein Targets. J. Infect. Dis 212, 1743–1751. [PubMed: 25980035]

Weiskopf D, Angelo MA, Grifoni A, O'Rourke PH, Sidney J, Paul S, De Silva AD, Phillips E, Mallal S, Premawansa S, et al. (2016). HLA-DRB1 Alleles Are Associated With Different Magnitudes of Dengue Virus-Specific CD4+ T-Cell Responses. J. Infect. Dis 214, 1117–1124. [PubMed: 27443615]

Wen J, and Shresta S (2017). T Cell Immunity to Zika and Dengue Viral Infections. J. Interferon Cytokine Res 37, 475–479. [PubMed: 29135369]

Wen J, and Shresta S (2019). Antigenic cross-reactivity between Zika and dengue viruses: is it time to develop a universal vaccine? Curr. Opin. Immunol 59, 1–8. [PubMed: 30884384]

Wen J, Elong Ngono A, Regla-Nava JA, Kim K, Gorman MJ, Diamond MS, and Shresta S (2017a). Dengue virus-reactive $CD8^+$ T cells mediate cross-protection against subsequent Zika virus challenge. Nat. Commun 8, 1459. [PubMed: 29129917]

Wen J, Tang WW, Sheets N, Ellison J, Sette A, Kim K, and Shresta S (2017b). Identification of Zika virus epitopes reveals immunodominant and protective roles for dengue virus cross-reactive $CD8^+$ T cells. Nat. Microbiol 2, 17036. [PubMed: 28288094]

Yauch LE, Zellweger RM, Kotturi MF, Qutubuddin A, Sidney J, Peters B, Prestwood TR, Sette A, and Shresta S (2009). A protective role for dengue virus-specific CD8+ T cells. J. Immunol 182, 4865–4873. [PubMed: 19342665]

Yauch LE, Prestwood TR, May MM, Morar MM, Zellweger RM, Peters B, Sette A, and Shresta S (2010). CD4+ T cells are not required for the induction of dengue virus-specific CD8+ T cell or antibody responses but contribute to protection after vaccination. J. Immunol 185, 5405–5416. [PubMed: 20870934]

Yu CY, Chang TH, Liang JJ, Chiang RL, Lee YL, Liao CL, and Lin YL (2012). Dengue virus targets the adaptor protein MITA to subvert host innate immunity. PLoS Pathog. 8, e1002780. [PubMed: 22761576]

Zellweger RM, Tang WW, Eddy WE, King K, Sanchez MC, and Shresta S (2015). CD8+ T Cells Can Mediate Short-Term Protection against Heterotypic Dengue Virus Reinfection in Mice. J. Virol 89, 6494–6505. [PubMed: 25855749]

Highlights

- Vaccination with DENV/ZIKV-cross-reactive peptides elicits a Th1 $CD4^+$ T cell response
- Peptide vaccine-elicited cross-reactive $CD4^+$ T cells protect against ZIKV infection
- Cross-reactive $CD4^+$ T-cell-mediated protection is dependent on IFNγ or TNF
- Cross-reactive $CD4^+$ T-cell-mediated protection occurs in the absence of antibodies

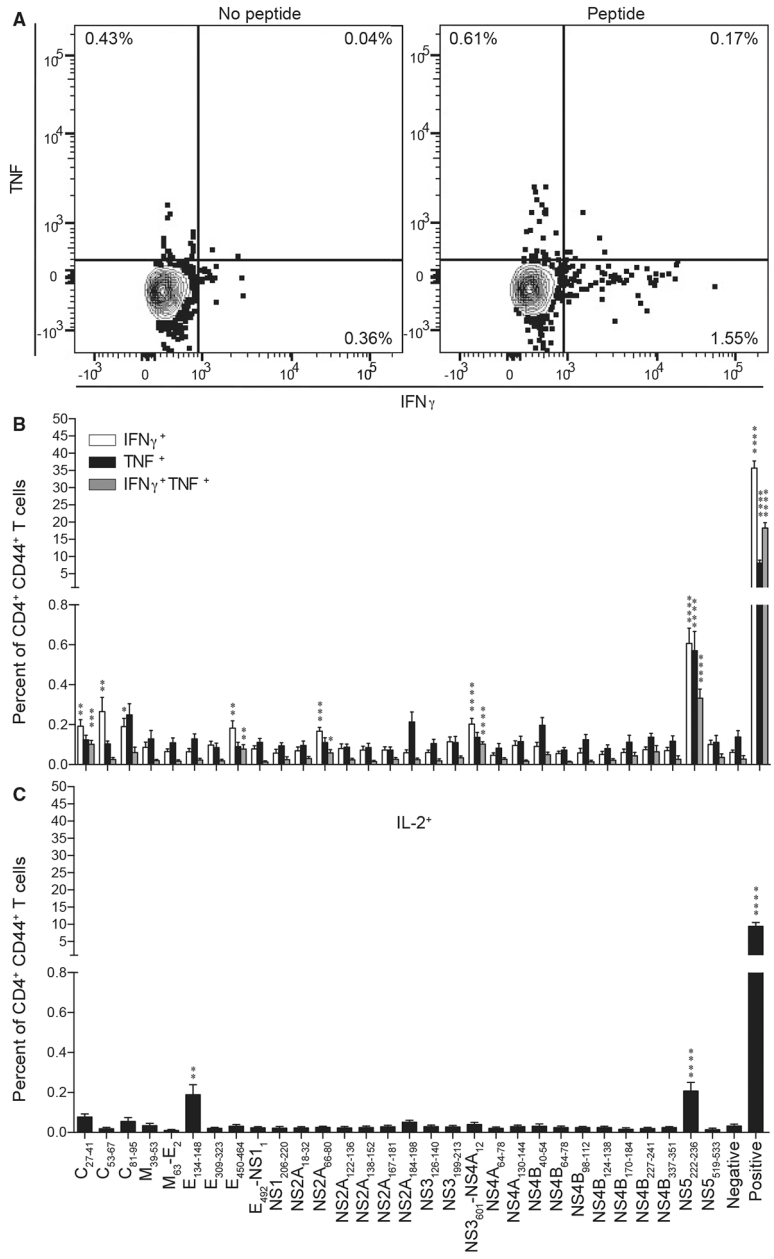


Figure 1. Cytokine Secretion Pattern of ZIKV-Peptide-Specific HLA-DRB1*0101-Restricted CD4$^+$ T Cells

Ifnar1$^{-/-}$HLA-DRB1*0101 mice were infected retro-orbitally with 1 × 10^2 focus-forming unit (FFU) of ZIKV strain SD001 for 7 days. Splenocytes were isolated and stimulated in vitro for 6 h with the indicated ZIKV-derived peptides that were predicted to bind HLA-DRB1*0101, and the frequencies of cytokine-producing cells were detected using the ICS assay.

(A) Representative flow cytometry contour plots.

(B and C) Quantification of CD4$^+$ T cells producing IFNγ, TNF, or IFNγ plus TNF (B) or IL-2 (C). Data represent the mean ± SEM of four independent experiments (n = 3–5 mice/experiment; total 16 mice). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-

tailed Mann-Whitney test with a Benjamini-Hochberg (B-H) adjusted p value (FDR) < 10%. Negative and positive refer to cells incubated in medium alone or with phorbol-12-myristate-13-acetate (PMA) and ionomycin, respectively.

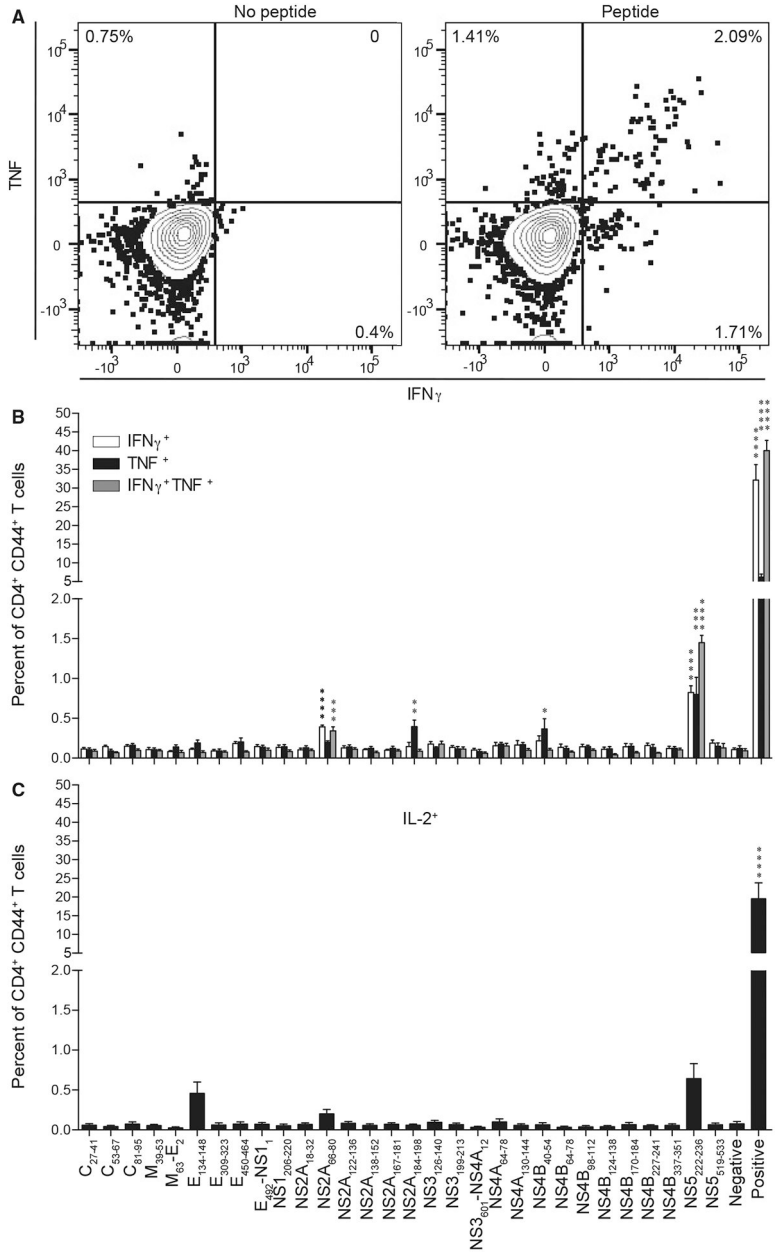


Figure 2. Cross-Reactivity of DENV2-Primed CD4$^+$ T Cells to HLA-DRB1*0101-Binding ZIKV Peptides

Ifnar1$^{-/-}$HLA-DRB1*0101 mice were infected intraperitoneally with 2 × 10^3 FFU of DENV2 strain S221 for 7 days. Splenocytes were stimulated in vitro for 6 h with the indicated ZIKV-derived peptides that were predicted to bind HLA-DRB1*0101, and the frequencies of cytokine-producing cells were detected using the ICS assay.

(A) Representative flow cytometry contour plots.

(B and C) Quantification of CD4$^+$ T cells producing IFNγ, TNF, or IFNγ plus TNF (B) or IL-2 (C). Data represent the mean ± SEM of three independent experiments (n = 3–4 mice/experiment; total 10 mice). **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-tailed Mann-

Whitney test with a B-H adjusted p value (FDR) < 10%. Negative and positive refer to cells incubated in medium alone or with PMA and ionomycin, respectively.

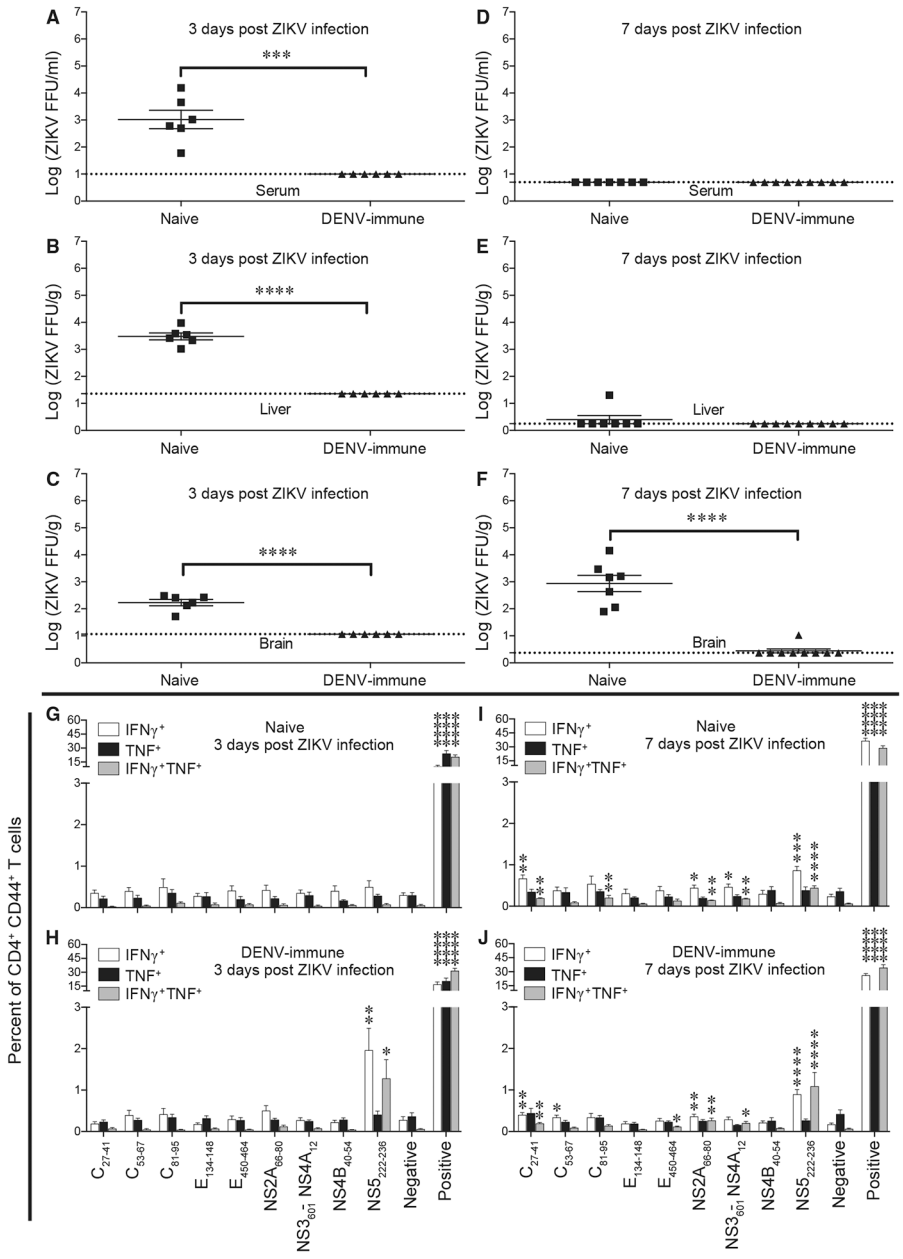


Figure 3. Reduction in Viral Load in DENV-Immune Mice after ZIKV Infection

Ifnar1$^{-/-}$HLA-DRB1*0101 mice were infected intraperitoneally with 2×10^3 FFU of DENV2 S221 for 4 weeks. Naive mice and DENV2-immune mice were infected retro-orbitally with 1×10^4 FFU of ZIKV strain SD001 for 3 (A–C and G–H) or 7 (D–F and I–J) days.

(A–F) Mouse blood (A and D), liver (B and E), and brain (C and F) were harvested, and levels of infectious ZIKV were determined using the FFA. Each point represents an individual mouse.

(G–J) Splenocytes were isolated from mice at 3 dpi (G and H) or 7 dpi (I and J) and stimulated *in vitro* for 6 h with the indicated ZIKV-specific and DENV2/ZIKV-cross-reactive peptides, and the frequencies of $CD4^+$ T cells producing IFNγ, IFNγ plus TNF, or

TNF were detected using the ICS assay. Negative and positive refer to cells incubated alone or with PMA and ionomycin, respectively. Data represent the mean ± SEM of two independent experiments (n = 3–5 mice/experiment).
(A–F) ***p < 0.001, ****p < 0.0001 by two-tailed Mann-Whitney test.
(G–J) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-tailed Mann-Whitney test with a B-H adjusted p value (FDR) < 10%.

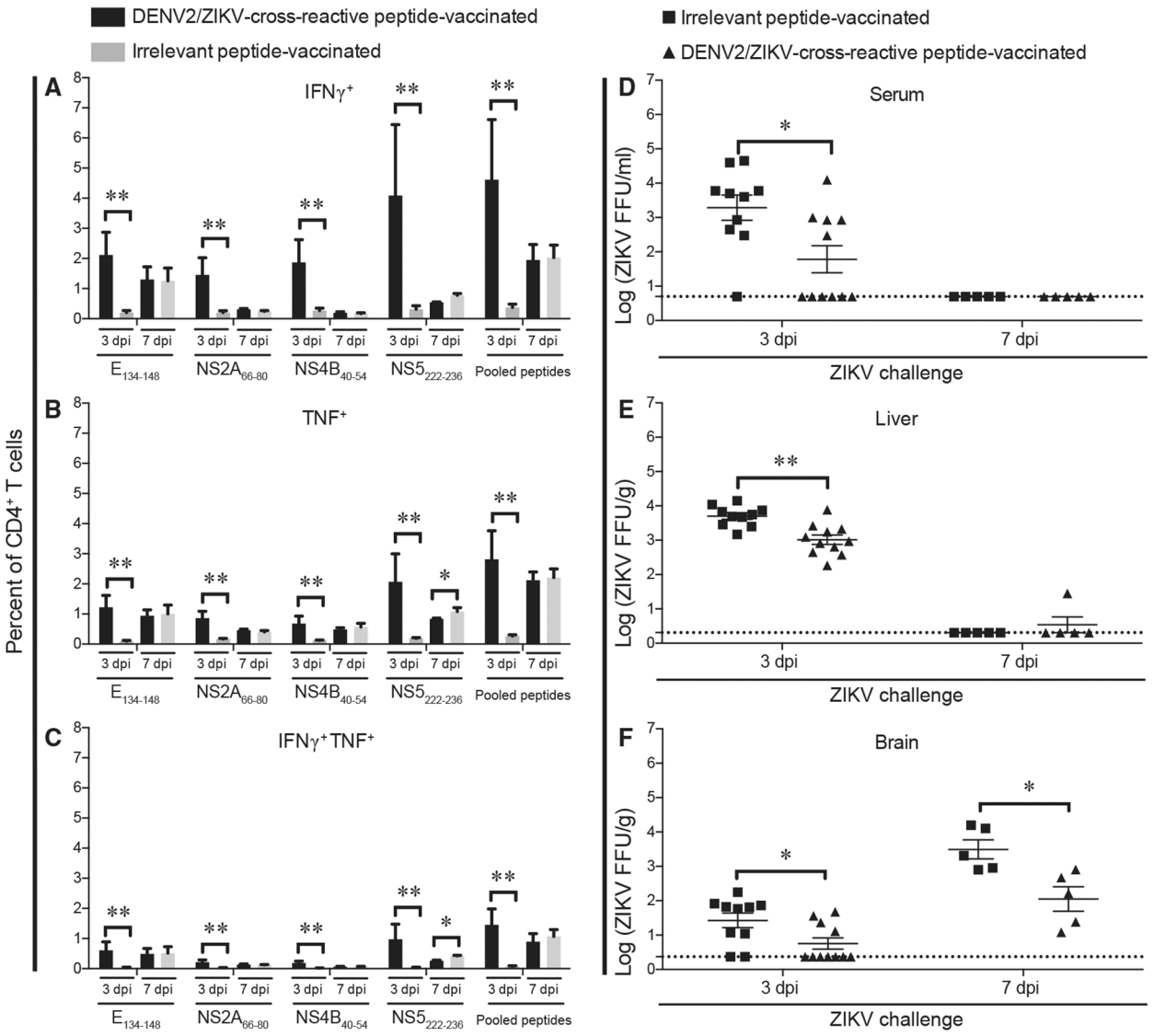


Figure 4. Expansion of CD4$^+$ T Cells and Suppression of Viral Burden in Mice Infected with ZIKV 4 Weeks after Vaccination with DENV2/ZIKV-Cross-Reactive Peptides

Ifnar1$^{-/-}$HLA-DRB1*0101 mice were injected subcutaneously with four irrelevant peptides or four DENV2/ZIKV-cross-reactive peptides ($E_{134–148}$, $NS2A_{66–80}$, $NS4B_{40–54}$, and $NS5_{222–236}$) and boosted in the same manner 2 weeks later. Two weeks after boosting, all mice were infected by retro-orbital injection with 1×10^4 FFU of ZIKV strain SD001. Three or 7 days later, mice were sacrificed.

(A–C) Splenocytes were isolated and stimulated for 6 h *in vitro* with the indicated DENV2/ZIKV-cross-reactive peptides, and the frequencies of CD4$^+$ T cells producing IFNγ (A), TNF (B), or IFNγ plus TNF (C) were detected using the ICS assay.

(D–F) Blood (D), liver (E), and brain (F) were harvested, and levels of infectious ZIKV were determined using the FFA. Data represent the mean ± SEM of two independent experiments (n = 3–6 mice/experiment). *p < 0.05, **p < 0.01, ***p < 0.001 by two-tailed Mann-Whitney test.

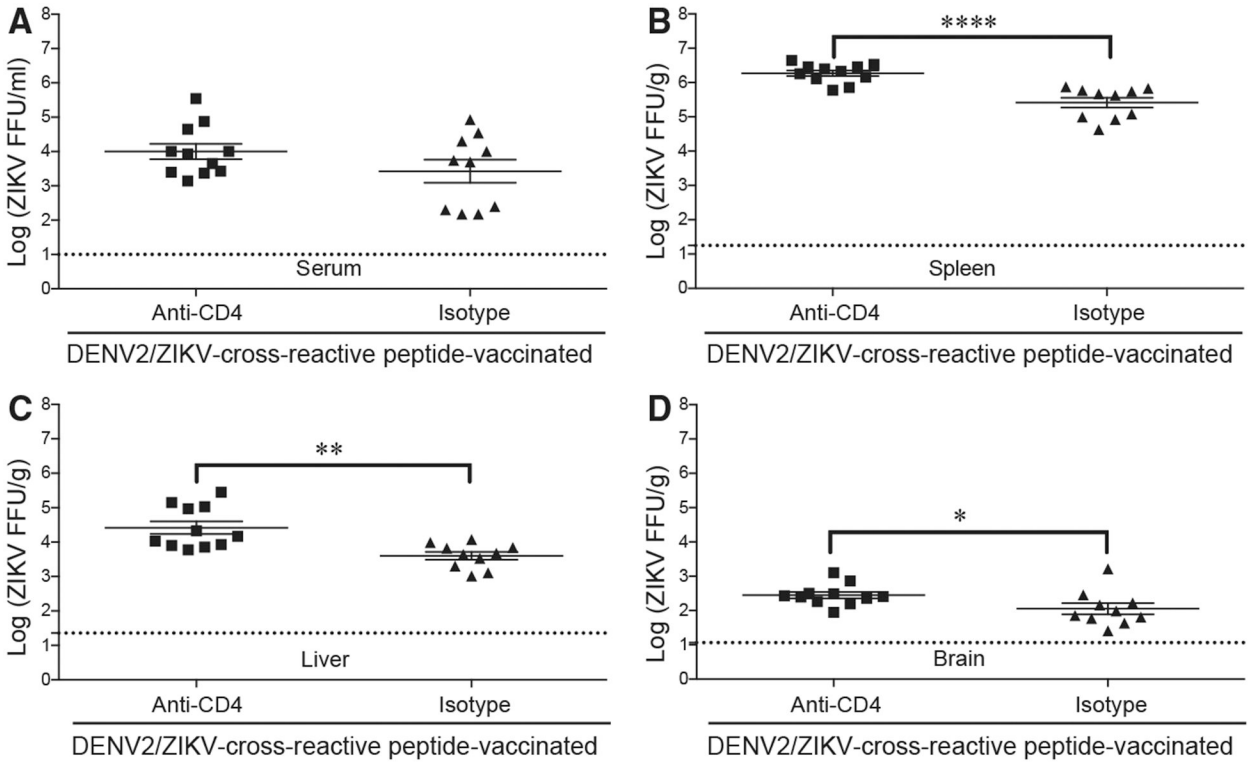


Figure 5. Reduction in Protective Immunity against ZIKV Infection in Mice Vaccinated with DENV2/ZIKV-Cross-Reactive Peptides and Depleted of CD4$^+$ T Cells

Ifnar1$^{-/-}$ HLA-DRB1*0101 mice were injected subcutaneously with four DENV2/ZIKV-crossreactive peptides ($E_{134–148}$, $NS2A_{66–80}$, $NS4B_{40–54}$, and $NS5_{222–236}$) and boosted in the same manner 2 weeks later. Two weeks after boosting, all mice were infected by retro-orbital injection with 1×10^4 FFU of ZIKV strain SD001. Mice were injected intraperitoneally with isotype control Ab or anti-CD4 Ab days 3 and 1 before and day 1 after ZIKV infection. At 3 dpi, levels of infectious ZIKV in serum (A), spleen (B), liver (C), and brain (D) were determined using the FFA. Each point represents an individual mouse. Data represent the mean ± SEM of two independent experiments (n = 5–6 mice/experiment). *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ by two-tailed Mann-Whitney test.

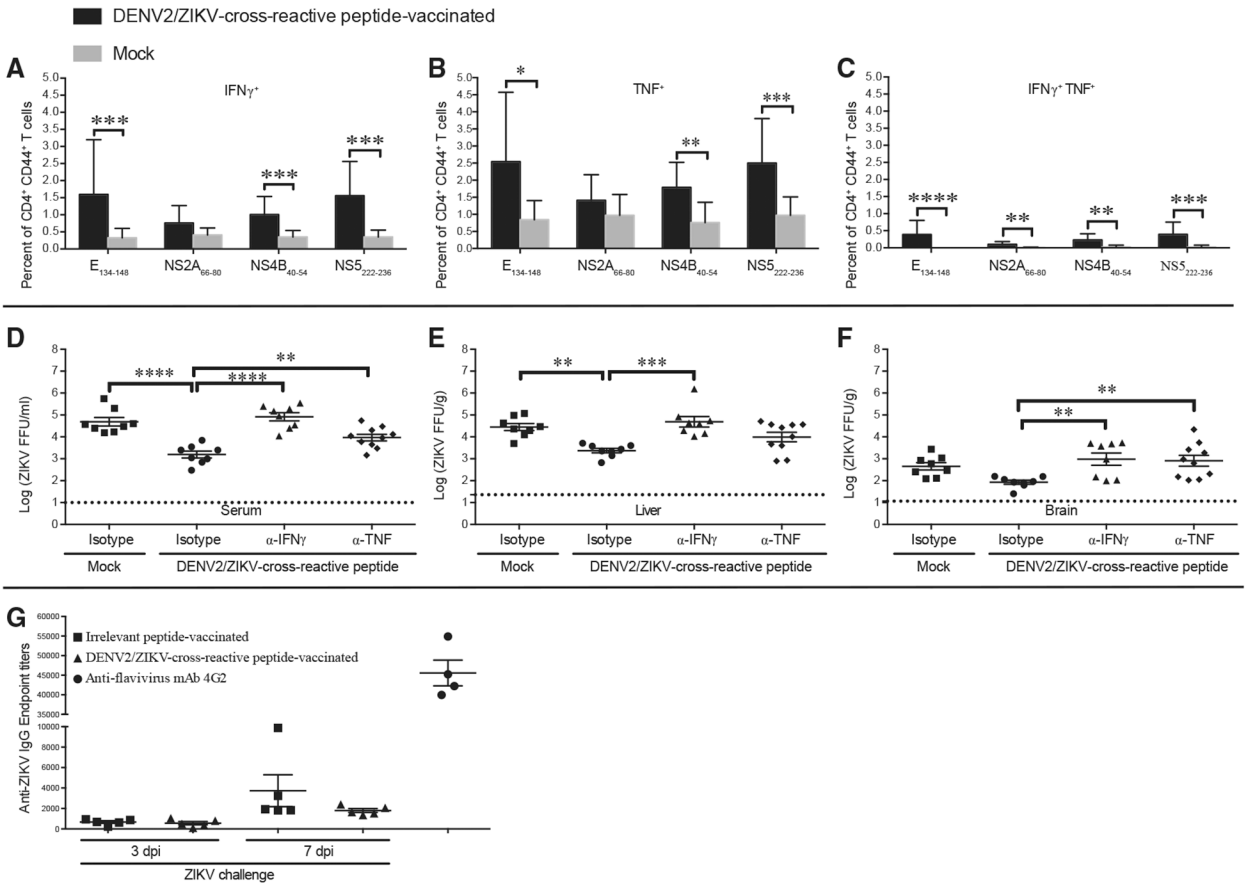


Figure 6. Anti-ZIKV Immune Response in Mice Depleted of IFNγ or TNF and Infected with ZIKV 4 Weeks after Vaccination with DENV2/ZIKV-Cross-Reactive Peptides

Ifnar1$^{-/-}$HLA-DRB1*0101 mice were injected subcutaneously with adjuvant alone (mock) or four irrelevant peptides or four DENV2/ZIKV-cross-reactive peptides ($E_{134–148}$, $NS2A_{66–80}$, $NS4B_{40–54}$, and $NS5_{222–236}$) and boosted in the same manner 2 weeks later. Two weeks after boosting, mice were infected by retro-orbital injection with 1×10^4 FFU of ZIKV strain SD001. Three days later, mice were sacrificed and blood and tissues were harvested.

(A–C) Splenocytes were stimulated for 6 h *in vitro* with the indicated DENV2/ZIKV-cross-reactive peptides, and the frequencies of $CD4^+$ T cells producing IFNγ (A), TNF (B), and IFNγ plus TNF (C) were detected using the ICS assay.

(D–F) Mice were injected intraperitoneally with a neutralizing Ab against TNF or IFNγ or an isotype control Ab on days 3 and 1 before and 1 day after ZIKV infection. Levels of infectious ZIKV in the serum, liver, and brain at 3 dpi were determined using the FFA. Each point represents an individual mouse.

(G) Sera were tested for the presence of ZIKV E-reactive IgG by ELISA. Data represent the mean ± SEM of two independent experiments, with a total of 10 mice per group (A–C) or with 10 mice (8 females, 2 males) for a-TNF, 8 mice (2 females, 6 males) for α-IFNγ, 8 mice (1 female, 7 males) for isotype, or 8 mice (8 males) for mock (D–F) groups. *p < 0.05,

**p < 0.01, ***p < 0.001, ****p < 0.0001 by two-tailed Mann-Whitney test (A–C) or Kruskal-Wallis one-way ANOVA with Dunn’s correction (D–F).

Table 1.

Zika-Virus-Derived Potential HLA-DRB1*0101-Restricted Epitopes

Peptides[a]	Sequences	IEDB Prediction Percentile_Rank	% $CD4^+$ INF_γ^+	$CD44^+$ TNF^+	T Cells (ZIKV) $INF_\gamma^+TNF^+$	$IL\text{-}2^+$	% $CD4^+$ INF_γ^+	$CD44^+$ TNF^+	T Cells (DENV2) $INF_\gamma^+TNF^+$	$IL\text{-}2^+$
$C_{27–41}$*	FGGLKRLPAGLLLGH	0.25	0.19	0.12	0.10	0.08	0.11	0.11	0.09	0.06
$C_{53–67}$	FLRFTAIKPSLGLIN	0.19	0.27	0.10	0.03	0.02	0.15	0.09	0.07	0.04
$C_{81–95}$	IKKFKKDLAAMLRII	3.02	0.19	0.25	0.06	0.06	0.15	0.16	0.10	0.07
$M_{39–53}$	NPGFALAAAAIAWLL	0.13	0.09	0.13	0.02	0.03	0.11	0.11	0.09	0.05
M_{63}-E_2	YLVMILLIAPAYSIR	0.96	0.07	0.11	0.02	0.01	0.08	0.14	0.07	0.03
$E_{134-148}$*†	NLEYRIMLSVHGSQH	2.27	0.06	0.13	0.02	0.19	0.11	0.19	0.07	0.46
$E_{309–323}$	TAAFTFTKIPAETLH	1.58	0.10	0.08	0.02	0.02	0.09	0.09	0.08	0.06
$E_{460-464}$	GAAFKSLFGGMSWFS	2.51	0.18	0.09	0.08	0.03	0.18	0.20	0.08	0.07
E_{492}-$NS1_1$	GGVLIFLSTAVSADV	1.99	0.08	0.11	0.01	0.02	0.15	0.14	0.10	0.07
$NS1_{206–220}$	NDTWRLKRAHLIEMK	2.73	0.06	0.09	0.02	0.02	0.14	0.14	0.08	0.05
$NS2A_{18-32}$	TTKIIISTSMAVLVA	3.72	0.07	0.10	0.03	0.02	0.10	0.13	0.09	0.07
$NS2A_{66-80}$*†	LALIAAFKVRPALLV	1.81	0.17	0.11	0.06	0.03	0.39	0.20	0.34	0.20
$NS2A_{122-136}$	ALAWLAIRAMWPRT	1.24	0.08	0.09	0.02	0.02	0.13	0.14	0.11	0.08
$NS2A_{138–152}$	NITLAILAALTPLAR	2.51	0.07	0.09	0.02	0.02	0.11	0.12	0.07	0.06
$NS2A_{167-181}$	GGFMLLSLKGKGSVK	2.27	0.07	0.07	0.03	0.03	0.10	0.12	0.09	0.07
$NS2A_{184-198}$	LPFVMALGLTAVRLV	0.28	0.06	0.21	0.02	0.05	0.14	0.40	0.09	0.06
$NS3_{126–140}$	CGRVIGLYGNGWIK	4.77	0.06	0.11	0.02	0.03	0.18	0.13	0.18	0.09
$NS3_{199–213}$	RLRTVILAPTRWAA	1.81	0.11	0.11	0.04	0.03	0.14	0.12	0.11	0.06
$NS3_{6O1}$-$NS4A_{12}$*	GAAFGVMEALGTLPG	3.95	0.20	0.14	0.10	0.04	0.10	0.08	0.06	0.03
$NS4A_{64-78}$	GIFFVLMRNKGIGKM	0.42	0.05	0.08	0.03	0.02	0.16	0.18	0.15	0.10
$NS4A_{13O-144}$	QMAIIIMVAVGLLGL	0.96	0.10	0.12	0.02	0.03	0.17	0.17	0.10	0.06
$NS4B_{40-54}$†	WAIYAALTTFITPAV	3.95	0.09	0.20	0.05	0.03	0.22	0.37	0.10	0.06
$NS4B_{64-78}$	NYSLMAMATQAGVLF	1.71	0.06	0.07	0.01	0.02	0.14	0.12	0.08	0.03
$NS4B_{98-112}$	IGCYSQLTPLTLIVA	0.25	0.06	0.13	0.01	0.02	0.14	0.15	0.10	0.04
$NS4B_{124-138}$	IPGLQAAAARAAQKR	1.06	0.05	0.08	0.02	0.02	0.11	0.12	0.04	0.04
$NS4B_{170-184}$	MGQVLLIAVAVSSAI	2.05	0.06	0.11	0.04	0.02	0.14	0.15	0.07	0.07
$NS4B_{227-241}$	FRGSYLAGASLIYTV	4.77	0.08	0.14	0.06	0.02	0.16	0.13	0.07	0.05
$NS4B_{337-351}$	GWSYYAATIRKVQEV	1.53	0.07	0.12	0.03	0.02	0.12	0.12	0.11	0.06
$NS5_{222–236}$*†	RAIWYMWLGARFLEF	0.88	0.61	0.57	0.33	0.21	0.82	0.80	1.45	0.64
$NS5_{519–533}$	HRRDLRLMANAICSS	1.9	0.10	0.11	0.04	0.01	0.19	0.15	0.13	0.06
Negative	no peptide		0.06	0.14	0.03	0.03	0.11	0.12	0.09	0.07
Positive	PMA/ionomycin		35.69	8.14	18.28	9.40	32.13	6.21	40.02	19.54

[a]The position of the peptides was determined according to the amino acid sequences of ZIKV strain FSS13025. The peptides denoted with an asterisk (*) were identified as ZIKV epitopes using the ICS assay. The peptides denoted with a dagger (†) were identified as DENV2/ZIKV-cross-reactive epitopes. The peptides underlined were used to immunize mice.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rat monoclonal anti-mouse CD16/CD32 (clone 2.4G2)	3D Biosciences	Cat#553141; RRID:AB_394656
Rat monoclonal anti-mouse CD49d (clone R1–2), Brilliant Violet 605	3D Biosciences	Cat#740341; RRID:AB_2740074
Rat monoclonal anti-mouse CD3 (clone 145–2C11), PerCP-Cy5.5	Tonbo Biosciences	Cat#65–0031; RRID:AB_2621872
Rat monoclonal anti-mouse IFNg (clone XMG1.2), FITC	Tonbo Biosciences	Cat#35–7311; RRID:AB_2621724
Rat monoclonal anti-mouse CD44 (clone IM7), Brilliant Violet 785	BioLegend	Cat#103041; RRID:AB_11218802
Rat monoclonal anti-mouse CD11a (clone M17/4), PE	BioLegend	Cat#101107; RRID:AB_312780
Rat monoclonal anti-mouse CD25 (clone PC61), PE	BioLegend	Cat#102008; RRID:AB_312857
Rat monoclonal anti-mouse IL-2 (clone JES6–5H4), PE	BioLegend	Cat#503807; RRID:AB_315301
Rat monoclonal anti-mouse IL-2 (clone JES6–5H4), Brilliant Violet 421	BioLegend	Cat#503825; RRID:AB_10895901
Rat monoclonal anti-mouse IL-2 (clone JES6–5H4), Brilliant Violet 711	BioLegend	Cat#503837; RRID:AB_2564225
Rat monoclonal anti-mouse IL-4 (clone 11B11), APC	BioLegend	Cat#504105; RRID:AB_315319
Rat monoclonal anti-mouse CD279 (clone 29F.1A12), Brilliant Violet 605	BioLegend	Cat#135219; RRID:AB_11125371
Rat monoclonal anti-mouse IL-17A (clone TC11–18H10.1), Brilliant Violet 510	BioLegend	Cat#506933; RRID:AB_2562668
Rat monoclonal anti-mouse CD185 (CXCR5) (clone SPRCL5), PE	Thermo Fisher Scientific	Cat#12–7185-82; RRID:AB_11217882
Rat monoclonal anti-mouse CD185 (CXCR5) (clone SPRC15), biotin	Thermo Fisher Scientific	Cat#13–7185-82; RRID:AB_2572800
Rat monoclonal anti-mouse CD4 (clone GK1.5), APC-eFluor780	Thermo Fisher Scientific	Cat#47–0041-82; RRID:AB_11218896
Rat monoclonal anti-mouse CD62L (clone MEL-14), Alexa Fluor 700	Thermo Fisher Scientific	Cat#56–0621-82; RRID:AB_494003
Rat monoclonal anti-mouse TNF (clone MP6-XT22), Alexa Fluor 700	Thermo Fisher Scientific	Cat#56–7349-42; RRID:AB_10671335
Rat monoclonal anti-mouse TNF (clone MP6-XT22), APC	Thermo Fisher Scientific	Cat#17–7321-82; RRID:AB_469508
Rat monoclonal anti-mouse IL-5 (clone TRFK5), PE	Thermo Fisher Scientific	Cat#12–7052-82; RRID:AB_763587
Rat monoclonal anti-mouse FoxP3 (clone FJK-16 s), Alexa Fluor 700	Thermo Fisher Scientific	Cat#56–5773-82; RRID:AB_1210557
Rat monoclonal anti-mouse IFNg (clone XMG1.2), neutralizing antibody	Thermo Fisher Scientific	Cat#16–7311-81; RRID:AB_469242
Rat monoclonal anti-mouse CD4 (clone GK1.5), CD4+ T cell-depleting antibody	Bio X Cell	Cat#BP0003–1; RRID:AB_1107636
Rat isotype control monoclonal antibody (clone LTF-2)	Bio X Cell	Cat#BP0090; RRID:AB_1107780
Rat monoclonal anti-mouse TNF (clone XT3.11), neutralizing antibody	Bio X Cell	Cat#BP0058; RRID:AB_1107764
Isotype control monoclonal antibody (clone HPRN)	Bio X Cell	N/A
Pan-flavivirus envelope protein-specific monoclonal antibody 4G2 (clone D1–4G2–4-15)	Absolute Antibody	Cat#Ab00230–2.0; RRID:AB_2715504
Goat polyclonal anti-mouse IgG, horseradish peroxidase	Sigma-Aldrich	Cat#A0168; RRID:AB_257867
Bacterial and Virus Strains		
ZIKV strain SD001	Carlin et al., 2018	N/A
DENV2 strain S221	Yauch et al., 2009	N/A
Chemicals, Peptides, and Recombinant Proteins		
Live/Dead blue viability stain	Thermo Fisher Scientific	Cat#50-112-1524
Cell Stimulation Cocktail (containing phorbol-12-myristate-13-acetate(PMA)/ionomycin)	Thermo Fisher Scientific	Cat#00-4970-93

REAGENT or RESOURCE	SOURCE	IDENTIFIER
TMB chromogen solution	Thermo Fisher Scientific	Cat#00-202-3
Brilliant Violet 421-conjugated streptavidin	BioLegend	Cat#405225
Cytofix/Cytoperm solution	BD Biosciences	Cat#554722
Protein transport inhibitor (containing Brefeldin A)	BD Biosciences	Cat#555029
Carboxymethyl cellulose	Sigma-Aldrich	Cat#9004-32-4
TrueBlue peroxidase substrate	KPL	Cat#5510-0030
ZIKV E protein	The Native Antigen	N/A
Experimental Models: Cell Lines		
Baby hamster kidney (BHK)-21 cells	ATCC	CCL-10
Mouse: *Ifnar1*$^{-/-}$ HLA-DRB1*0101 mice	Weiskopf et al., 2011	N/A
Software and Algorithms		
Immune Epitope Database and Analysis Resource (IEDB-AR)	Vita et al., 2019	http://www.iedb.org/home_v3.php