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Enhancement of Zika Infection by Dengue Virus–Specific Antibody Is Associated With Low Levels of Antiviral Factors

To the Editor—Zika virus (ZIKV) has recently emerged as an important human pathogen because of its association with congenital defects including microcephaly and adult-onset Guillain-Barré syndrome [1]. In Brazil in 2015, an association was reported between ZIKV infection and an increased number of infants born with microcephaly in Zika-affected areas [1]. The virus has continued to spread in the Americas and by April 2017, 48 countries and territories in the region have reported transmission of ZIKV and 26 countries have reported congenital syndrome.

Antibody-dependent enhancement of infection (ADE) is a phenomenon in which virus infectivity is markedly increased by virus-specific antibodies, particularly under nonneutralizing conditions [2]. ADE involves increased virus uptake (known as extrinsic ADE) and/or modulation of antiviral signaling (intrinsic ADE), and is usually mediated through Fc receptors [2]. Antibody cross-reactivity can contribute to ADE. Early studies suggested that anti–dengue virus (DENV) antibodies can promote ADE of ZIKV infection [3], and these observations have been extended recently by Castanha et al in *The Journal of Infectious Diseases* [4]. These authors confirmed that DENV-specific antibodies enhanced infection of K562 cells (FcgRII positive) with a Brazilian isolate of ZIKV. They also showed that serum from DENV-immune pregnant women can mediate ZIKV ADE.

We and others have shown that ADE of DENV infection is associated with downregulation of antiviral factors such as type I interferon (IFN) and nitric oxide (NO) [5, 6]. K562 cells do not produce type I IFN; instead, we used primary human macrophages as a suitable cellular model to test whether ZIKV ADE leads to modulation of antiviral molecules. DENV targets macrophages, and data are emerging that ZIKV also infects macrophages [7]. Placental macrophages are susceptible to ZIKV and can facilitate spread of infection within the placenta and possibly to the fetus itself. Peripheral blood mononuclear cells obtained from healthy individuals (Griffith University ethics number BDD/01/12/HREC) were suspended in culture medium and allowed to adhere for 2 hours. The adherent cells were cultured with macrophage colony-stimulating factor for 5 days to generate macrophages, as described previously [8]. Flow cytometry confirmed macrophage differentiation. Macrophages were infected with ZIKV in the presence of subneutralizing anti-DENV antibodies or normal human serum.

At 12 hours postinfection, ZIKV-infected macrophages cultured with DENV-specific antibodies (ZIKV-ADE group) showed increased virus titers compared to cultures without DENV-specific antibodies (non-ADE group) (Figure 1A). In ZIKV-ADE macrophages, levels of IFN-β and reactive nitrogen intermediates were lower than in non-ADE macrophages ($P < .05$; Figure 1B and 1C). Interleukin (IL) 6 levels were significantly ($P < .05$) higher in ZIKV-ADE macrophages (Figure 1D), as was STAT3 activity, measured by electrophoretic mobility shift assay (Figure 1E). There was no difference in IL-10 production between ADE and non-ADE cultures (data not shown). SOCS3 expression, measured by Western blot, was also higher in the ADE group (Figure 1F). We hypothesized that downregulation of IFN-β in ZIKV-ADE–infected macrophages was mediated by IL-6. IL-6 can activate transcription factor STAT3, which results in the induction of SOCS3 that has inhibitory activity on JAK-STAT signaling [9]. The downregulation of IFN-β in ZIKV-ADE–infected macrophages is consistent with the findings of Ubol et al with DENV infection of THP-1 cells [5] and our own findings with DENV-ADE in primary human macrophages [6]. It would be interesting to know whether intrinsic ADE is also active in the experimental system described by Castanha et al [4]. Although the K562 cells used by these authors do not produce type I IFN, it would be informative to assess expression of other factors such as IL-6, NO, or chemokines to determine whether, similar to our data with primary human macrophages, ADE also regulates production of inflammatory mediators in these cells.

One caveat to our data is that the described mechanisms may not operate in all cell types; it will be important to probe numerous cell types with and without Fc receptors to determine the extent of the ADE mechanism. In addition, the specific inflammatory mediators affected by ADE of DENV differ among different cell types [8], and this should also be examined in ADE of ZIKV infection. We previously observed that ADE of DENV infection was associated with downregulation of type I IFN, which was mediated via IL-6 [6]. We speculate that a similar mechanism may be involved in ADE of ZIKV infection. Another potential mechanism by which ADE of ZIKV infection could lead to reduced IFN production involves increased uptake of ZIKV virions, some of which have an intrinsic capacity to interfere with host IFN production pathways [10]. These potential mechanisms are not mutually exclusive and more than one pathway may contribute to downregulation of antiviral factors.

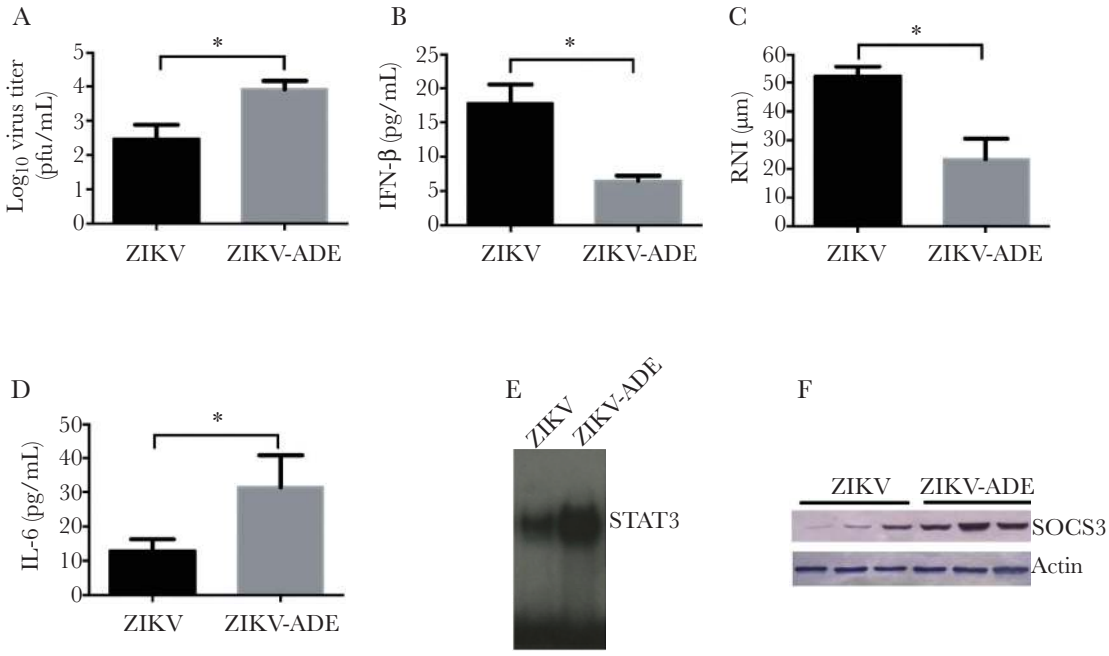

Figure 1. Dengue-immune sera induce antibody-dependent enhancement of infection (ADE) of Zika virus (ZIKV) infection in macrophage and low levels of antiviral factors. Human macrophage monolayers were infected with ZIKV MR 766 at a multiplicity of infection of 1. Prior to infection, the ZIKV inoculum had been incubated at room temperature for 1 hour with dengue virus (DENV)–immune serum (10^{-3} dilution, ZIKV-ADE) or normal human serum (10^{-3} dilution, non-ADE). After infection, the inoculum was removed and the monolayer was rinsed and replenished with fresh media. *A*, Virus titers (plaque-forming units [pfu]) were determined by plaque assay using Vero cells at 12 hours postinfection. *B–D*, Interferon beta (IFN-β), reactive nitrogen intermediates (RNI), and interleukin 6 (IL-6) levels in culture supernatant 12 hours after infection. IFN-β (PBL Assay Science) and IL-6 (R&D Systems) concentrations were determined by enzyme-linked immunosorbent assay according to the manufacturer's instructions. Significant differences in expression are marked with an asterisk (*$P < .05$). Data are presented as mean ± standard error of the mean. The 2-tailed unpaired Student *t* test was used to determine statistical significance. Statistical analyses were performed with GraphPad Prism software. *E*, electrophoretic mobility shift assay analysis of STAT3 in ZIKV-ADE and non-ADE cultures at 12 hours after infection. Nuclear extracts were analyzed for STAT3 homodimer DNA-binding activity. *F*, Western blot analysis of SOCS3 in ZIKV-ADE and non-ADE cultures at 12 hours after infection. Cell lysates were probed by Western blotting with antibodies specific to SOCS3 (Cell Signaling). The control was α-actin expression, detected with an anti-α actin antibody (Santa Cruz Biotechnology).

Notes

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Enhancement of Zika Infection by Dengue-Specific Antibodies Does Not Alter the Production of Interleukin 6 in FcγRII-Expressing K562 Cells

To the Editor—We thank Hueston and colleagues for their comments about our manuscript and for sharing their results [1]. Indeed, antibody-dependent enhancement (ADE) of virus infection is a complex phenomenon. The mechanism is triggered by the attachment of immune complexes to Fcγ receptors, leading to an increased number of virus-infected cells (extrinsic ADE) and/or to the modulation of the antiviral signaling pathway (intrinsic ADE) [2].

In dengue, the relevance of ADE in driving the severe outcomes of the disease have been demonstrated in experimental studies conducted in vitro and in vivo [2, 3]. Additionally, it has been established that secondary infection with a heterologous dengue virus (DENV) serotype is a risk factor for the development of severe disease [4]. Another unique example of ADE in mediating severe outcomes is the fact that infants born to DENV-immune mothers might develop severe dengue during a primary infection when maternally transferred dengue antibodies have waned to below protective levels [5].

In children and adults, the spectrum of the clinical manifestations of Zika virus (ZIKV) infection is normally much less symptomatic when compared to dengue, and there is no equivalent to severe dengue (dengue hemorrhagic fever and dengue shock syndrome). However, ZIKV has the ability to infect embryos and fetuses inside the uterus, causing devastating pathology. The mechanisms underlying this severe outcome of ZIKV infection remain unknown, and several studies have focused on investigating whether ADE might have contributed to the expanded ZIKV pathogenesis [1, 6–8]. Collectively, these studies have confirmed (in vitro and in vivo) that ADE of ZIKV infection by dengue-specific antibodies not only facilitates viral uptake, as demonstrated by our study and by others, but also modifies antiviral mechanisms, resulting in increased ZIKV replication, as interestingly explored by Hueston and colleagues [1].

Notably, these studies have used different cell types to explore intrinsic and extrinsic ADE properties. The FcγRII-expressing K562 cell line does not produce type I interferon (IFN) and, thus, is not suitable for studying intrinsic ADE, as correctly pointed out by Hueston and colleagues [1]. Instead, this cell line has been widely used to measure extrinsic

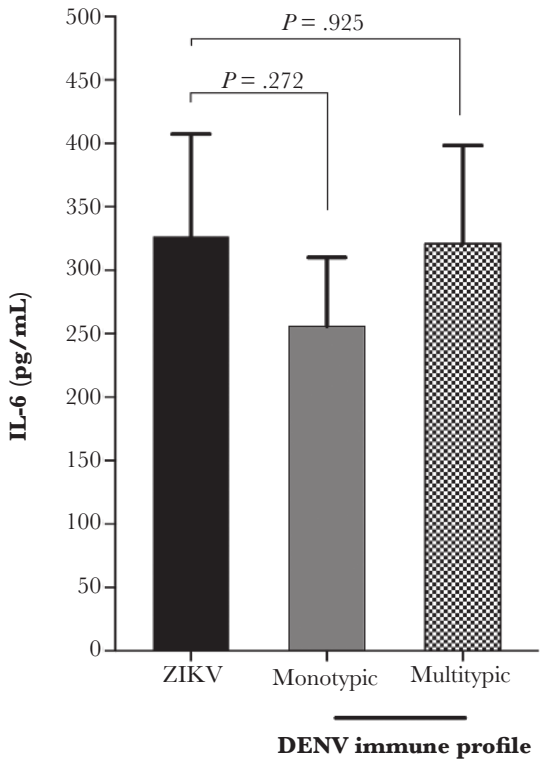

Figure 1. Antibody-dependent enhancement of Zika virus (ZIKV) infection by dengue virus (DENV)–specific antibodies and levels of interleukin 6 (IL-6). FcγRII-expressing K562 cells were infected with ZIKV PE/243 in the absence of antibodies or in the presence of a panel of serum samples from pregnant women with different dengue immune status, as determined by plaque reduction neutralization test: monotypic (DENV-3) (n = 10) and multitypic (DENV-3 and DENV-4) (n = 10). Cell culture supernatants were collected 48 hours postinfection, and levels of IL-6 were determined by Citometric bead array (BD CBA Human Th1/Th2/Th17 Cytokine Kit) following the manufacturer's instructions. Mann-Whitney test was used to determine statistical significance. Statistical analysis was performed using Graph Pad Prism software, version 7.0a.