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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

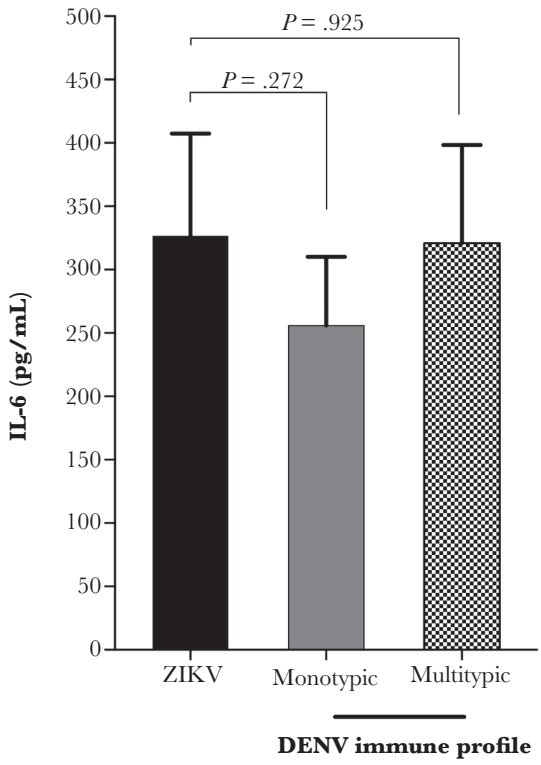

Figure 1. Antibody-dependent enhancement of Zika virus (ZIKV) infection by dengue virus (DENV)–specific antibodies and levels of interleukin 6 (IL-6). FcγRII-expressing K562 cells were infected with ZIKV PE/243 in the absence of antibodies or in the presence of a panel of serum samples from pregnant 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.

ADE properties [2]. We acknowledge that measuring the production of inflammatory mediators—as suggested by Hueston et al—would be very informative. In fact, we observed no differences in interleukin 6 (IL-6) production between K562 cells infected with ZIKV in the absence of antibodies or in the presence of a panel of sera with different dengue immune profile (monotypic and multitypic) (Figure 1). In contrast, Hueston et al demonstrated increased IL-6 levels on human macrophages infected with ZIKV preincubated with dengue-immune sera [1]. These dissimilar findings probably reflect variations on the production of inflammatory mediators among different cell types under ADE conditions [2]. Of note, both experiments were based on a single short time point after infection (24 and 48 hours postinfection for Hueston et al and our experimental system, respectively); thus, a complete time course experiment after infection would probably represent a better picture of K562 cell inflammatory responses. However, investigating intrinsic ADE and expression of different cytokines was beyond the scope of our manuscript.

It is notable that considerable progress has been made in a short amount of time toward understanding the role of ADE of ZIKV infection by dengue antibodies. However, it remains necessary to determine the relevance of the ADE mechanism in the epidemiological context [9, 10], particularly by analyzing how previous dengue immunity affects virus transmission and the development of congenital ZIKV syndrome. Studies addressing this issue will have important implications for ZIKV and DENV vaccine development.

Notes

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