Downregulation of Interferon-β in Antibody-Dependent Enhancement of Dengue Viral Infections of Human Macrophages Is Dependent on Interleukin-6

To the Editor—Dengue virus (DENV) is a major arthropod-borne virus affecting millions across Asia, Africa, and any tropical areas where vector mosquitoes find optimal breeding conditions. A feature of DENV infection is that individuals who have been previously infected with DENV are at increased risk of developing severe dengue hemorrhagic fever (DHF) following subsequent infection. Despite intensive research, the mechanisms underlying disease exacerbation following secondary infections are still unclear.

The recent work by Ubol et al identified possible mechanisms to explain how previous dengue infection might lead to disease enhancement [1]. The authors suggested that antibody-dependent enhancement (ADE) of infection is a key mechanism in the pathophysiological changes that occur during DHF. ADE in DENV infection was first described in the mid-1970s [2], and has since been described during infection with a broad range of viruses [3]. ADE is believed to involve enhanced virus entry and replication in the

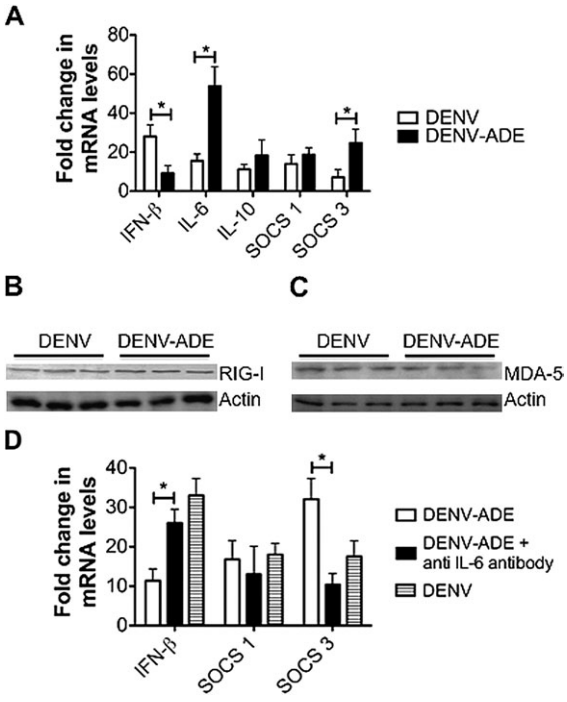

Figure 1. Dengue virus (DENV) 2 NGC strain was used to infect monolayer of human macrophage cell cultures at a multiplicity of infection (MOI) of 1. For antibody-dependent enhancement (ADE) of infection, DENV was incubated with a 10^{-3} dilution of DENV-immune serum for 1 hour at room temperature (DENV-ADE), while virus for control DENV infection (non-ADE) was incubated with a 10^{-3} dilution of normal human serum prior to macrophage infection. The virus inoculum was discarded and cell monolayer rinsed with medium. Fresh medium, RPMI, was added to the monolayer, and the infected cultures were incubated for 12 hours. *A,* Quantitative real-time polymerase chain reaction (PCR) analysis of transcript for interferon (IFN)-β, interleukin (IL)-6, IL-10, suppressor of cytokine signaling 1 (SOCS1), and suppressor of cytokine signaling 3 (SOCS3) messenger RNA (mRNA) in cells 12 hours after infection with DENV-ADE or DENV. Data represent the mean fold change in mRNA levels, compared with uninfected controls. GAPDH was used as a reference gene. Significant differences in expression are marked with an asterisk, * $P < .05$. *B* and *C,* Western blot analysis of retinoic acid–inducible gene I (RIG-I) (*B*) and melanoma differentiation–associated antigen 5 (MDA-5) (*C*) expression in macrophages infected with DENV-ADE or DENV (1 MOI) for 12 hours. The cell lysates were examined by Western blotting with antibodies to RIG-I or MDA-5 (both from Cell Signaling). Control included the detection of host cell protein expression by anti-α actin antibody (Santa Cruz Biotechnology). *D,* Macrophage cultures were pretreated with .15 μg/mL antihuman IL-6 antibody (R&D Systems) for 1 hour at 37°C. The cells were then infected with DENV-ADE for 1 hour at 37°C. Cells infected with DENV-ADE in the absence of antihuman IL-6 antibody treatment were used as a control. The virus inoculum was discarded and cells were rinsed with medium. Fresh medium containing .15 μg/mL antihuman IL-6 antibody was added to the monolayer for an additional 12 hours at 37°C. Quantitative real-time PCR was performed for the analysis of transcript for IFN-β, SOCS1, and SOCS3 mRNA in cells 12 hours after infection with DENV-ADE (with or without antihuman IL-6 antibody treatment) or DENV. Data represent the mean fold change in mRNA levels, compared with uninfected controls. GAPDH was used as a reference gene. Significant differences in expression are marked with an asterisk, * $P < .05$.

presence of a subneutralizing concentration of virus-specific antibodies. However, ADE has largely been described in vitro, and it has been difficult to demonstrate its clinical relevance to dengue infection.

Ubol et al used a DENV-antibody complex to induce ADE in a monocytic cell line (THP-1), and then analyzed the expression of interferon (IFN)-β and antiviral intracellular signaling pathways. Retinoic acid–inducible gene I (RIG-I), melanoma differentiation-associated antigen 5 (MDA-5), and IFN-β were upregulated following DENV infection, but the induction of these proteins was prevented in cells infected with the DENV-ADE complex. In parallel, the authors noted that expression of interleukin (IL)-10 and suppressor of cytokine signaling 3 (SOCS3) was markedly enhanced in the DENV-ADE cells. The authors noted that these changes may also occur in vivo; peripheral blood mononuclear cells (PBMCs) from DHF patients expressed lower levels of RIG-I, MDA-5, and IFN-β than those from patients with mild dengue fever, while IL-10 and SOCS3 were detected at higher levels in DHF patients than in patients with mild dengue fever.

In view of the findings by Ubol et al, we aimed to determine whether similar mechanisms are active in primary monocyte–derived human macrophages as these cells are major targets of dengue virus infection [4]. We obtained blood from healthy individuals and isolated PBMCs following separation using Ficoll. Cells were then washed in phosphate-buffered saline, resuspended in culture medium, and allowed to adhere for 2 hours. For the generation of macrophages, PBMCs were cultured for 5 days in the presence of macrophage colony stimulating factor as described previously [5]. The identity of the cells as macrophages was confirmed by flow cytometry. Cells were then infected with DENV in the absence (non-ADE) or presence of sub-neutralizing anti-DENV antibodies with ADE activity (ADE).

DENV-ADE–infected human macrophages showed increased infectivity and enhanced virus titers compared with non-ADE infection at 12 and 24 hours after infection (data not shown). Cells were collected at 12 hours after infection and the messenger RNA (mRNA) transcripts for IFN-β, IL-6, IL-10, supressor of cytokine signaling 1 (SOCS1), and SOCS3 were determined by real-time polymerase chain reaction. Cells were also collected for Western blotting to analyze RIG-I and MDA-5 protein levels. IFN-β mRNA expression was significantly lower ($P < .05$) in DENV-ADE–infected macrophages compared with those infected with

DENV (Figure 1*A*). In contrast, IL-6 and SOCS3 mRNA transcripts were significantly ($P < .05$) higher in DENV-ADE–infected cells compared with non–ADE-infected cells (Figure 1*A*). Interestingly, IL-10 mRNA expression (Figure 1*A*) and RIG-I (Figure 1*B*) and MDA-5 (Figure 1*C*) protein levels were no different between the culture groups. Since IL-6 signaling involves the activation of SOCS3 [6], and SOCS3 inhibits JAK-STAT signaling [7], we hypothesized that the low levels of IFN-β in DENV-ADE–infected macrophages is mediated by IL-6. To test this hypothesis, we performed blocking studies using antihuman IL-6 antibody. Neutralization of IL-6 led to a significant increase in the expression of IFN-β mRNA, and a significant decrease in SOCS3 mRNA, in DENV-ADE–infected cells (Figure 1*D*). This result indicates that IL-6 is a critical regulator of ADE in DENV-infected macrophages.

The downregulation of IFN-β and upregulation of SOCS3 in DENV-ADE–infected macrophages (Figure 1*A*) is consistent with the findings of Ubol et al in THP-1 cells [1]. In contrast to Ubol et al, we did not observe any changes in the expression of IL-10, SOCS1, RIG-I, or MDA-5 in DENV-ADE infection, a difference that may be due to our use of primary human monocyte–derived macrophages. While the findings of Ubol et al are of interest, the identification of possible mechanisms and mediators responsible for severe DHF needs to be viewed with caution as differential responses are expected in different cell types. In summary, we demonstrate for the first time a role for IL-6 in suppressing antiviral responses via SOCS3 in DENV-ADE–infected macrophages, suggesting a plausible mechanism by which DENV-ADE leads to an impaired antiviral response.

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