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Lower activation-induced T-cell apoptosis is related to the pathological immune response in secondary infection with hetero-serotype dengue virus

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ABSTRACT

The available evidence suggests that dengue virus-specific T lymphocytes and cytokine storm play a pivotal role in the immunopathogenesis of plasma leakage. Investigations are underway to identify the immune profiles associated with increased or decreased risk for severe disease. In this study, CD14+ cells from the peripheral blood mononuclear cells (PBMCs) of patients who recovered from DENV-1 infection were infected with DENV-1 or DENV-2 and co-cultured with memory T cells. We found that secondary infection with DENV-2 suppresses the cell reproductive capacity but forms more cell clones and more functional cells to produce more proinflammatory factors (IFN-γ, TNF-α, IL-6, IL-8, IL-12 and IL-17) and less regulatory cytokines (IL-10, TGF-β) which results in higher viral replication compared to secondary infection with DENV-1. Memory dengue virus-specific T cells which are induced in a primary dengue virus infection are reactivated by the heterologous serotype of dengue virus and antigen-presenting cells (APCs) during a secondary infection. Dramatically, less apoptosis and more continuous activation of T cells in secondary infection with hetero-serotype DENV were observed. This discovery which has not been reported previously may be the reasonable and vital interpretation for the cytokine storm and severe symptoms observed in secondary infection with DENV. In summary, secondary infection with hetero-serotype DENV elicits the relatively pathological immune response while secondary infection with homologous-serotype DENV induces the relatively protective immune response by activation-induced cell death (AICD) of T cells.

1. Background

Dengue virus (DENV), a mosquito-transmitted member of the Flaviviridae family, consists of four antigenical distinct serotypes (DENV1–4). About two fifths of the world's population lives in areas where there exists a high risk of dengue infection, and dengue virus infects about 50 million people annually, leading to more than 25,000 deaths (Gubler and Meltzer, 1999). The severity of DENV infection and its mortality rate is a major health concern for people in tropical and subtropical areas.

The majority of DENV infections are asymptomatic and many cases present as dengue fever (DF), which is an acute febrile illness. A small percentage of cases will develop to dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS), which results in plasma leakage and hemorrhage depending on the degree of fever and clearance of viraemia (Vaughn et al., 1997; Vaughn et al., 2000). The development of DHF/DSS is dependent on host and viral

Abbreviations: DENV, dengue virus; AICD, activation-induced cell death; APC, antigen-presenting cell; DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; DC, dendritic cell; PBMC, peripheral blood mononuclear cell; PI, post-infection; FACS, fluorescence-activated cell sorting; RT-PCR, real-time reverse-transcription polymerase chain reaction; ADE, antibody dependent enhancement; BFA, Brefeldin A; TCR, T cell receptor; MOI, multiplicity of infection; SEM, standard error of the mean; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; TNF-α, tumor necrosis factor-alpha; IL-6, IL-10, IL-12, IL-17, interleukins-6, 10, 12, 17; IFN-γ, interferon gamma; TGF-β, transforming growth factor beta; CFSE, carboxyfluorescein succinimidyl ester; ELISA, enzyme linked immunosorbent assay; RT, room temperature.

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factors. Some prospective cohort studies have shown that secondary infection with a heterologous DENV serotype could be a major risk factor (Sangkawibha et al., 1984). Additionally, it has been demonstrated that the order of infections modulates the risk of developing DHF/DSS (Anantapreecha et al., 2005; Suwandono et al., 2006; Nisalak et al., 2003). DHF/DSS has also been associated with heterologous secondary infection which could induce cytokine storms (Rothman, 2011). Secondary DENV infections can lead to the production of proinflammatory cytokines induced by the memory T cells and dendritic cells (DCs). These proinflammatory cytokines and other chemical inflammatory mediators affect the endothelial cells and can lead to angiorrhexis, which causes plasma leakage. The activation of the T cells is dependent on the effects of APCs, especially DCs, whose maturity is influenced by the DENV infections. Profound T-cell activation and the production of proinflammatory cytokines may be the main reason for the systemic disturbance leading to DHF (Mongkolsapaya et al., 2003). Further study of the T cell activated by DENV infected-DCs might give us more clues to understand the mechanism of DHF.

Guang Dong Province in southern China has a high incidence of dengue diseases. There were more than 40,000 cases of infection in the summer of 2014 (Zhao et al., 2014). Over the past 20 years, DENV-1, DENV-2 and DENV-4 were prevalent. Currently, however, DENV-1 is the most prevalent (Messina et al., 2014; Sanchez-Vegas et al., 2013). To find out the relationship between the response of the DCs and T cells in secondary homologous or heterologous DENV infections, we collected blood samples and isolated peripheral blood mononuclear cells (PBMCs) of patients who recovered from DENV infection and stimulated them with DENV-1 and DENV-2 ex vivo.

2. Methods

2.1. Ethics statement

All blood samples were collected at Guangzhou Armed Police General Hospital, Guangzhou Province, China. Written informed consent was obtained for this study in agreement with protocols approved by Guangzhou Armed Police General Hospital Human Ethics Committee.

2.2. Study subjects and blood samples

In the study, we collected blood samples from 20 volunteers (11 females and 9 males) aged 20–60, who recovered after one month or so from DENV-1 primary infection at Guangzhou Armed Police General Hospital. All donors were detected by real-time reverse-transcription polymerase chain reaction (RT-PCR)-based dengue gene identification or DENV IgM & IgG captured by Enzyme Linked Immunosorbent Assay (ELISA) to make sure there was no existing dengue gene or virus protein in the blood samples. The sera IgM & IgG titers were determined by IgM & IgG captured Sandwich ELISA kit.

2.3. DENV cultures

Serotype DENV-1 and DENV-2 were grown and propagated in C6/36 mosquito cells, and virus supernatant was collected and stored at −80 °C. To calculate the multiplicity of infection (MOI) for infection assays, virus titers were determined by flow cytometry using a method similar to that described previously Zhao et al. (2012). Briefly, vero cells were plated in 24-well plates and infected with serial dilutions of DENV stock. At 24 h post-infection (PI), cells were fixed, permeabilized, and intracellular staining was done with E protein mAb (ab41349, Abcam) and secondary FITC-labeled goat anti-mouse-IgG (Cat. #349031, Becton Dickinson, Biosciences USA) and analyzed by flow cytometry. The titer of the virus was determined using the following formula: fluorescence-activated cell sorting (FACS) infectious units/mL = [(% of infected cells) 3 (total number of cells per well) 3 (dilution factor)]/(volume of inoculum added to cells).

2.4. DENV stimulation

$CD14^+$ monocytes and T cells were isolated using the EasySep™ human monocyte enrichment kit (Cat. #19059, STEMCELL™ technologies), human T-cell enrichment (Cat. #19051, STEMCELL™ technologies) and a human $CD4^+$ memory T-cell enrichment kit (Cat. #19157, STEMCELL™ technologies) respectively. Isolated cells were cultured with RPMI 1640 medium with 5% human sera. Monocytes from each subject were infected with DENV-1 or DENV-2, and incubated for 3–5 days at 37 °C, 5% CO_2. Controls represent monocytes cultured with supplemented medium without virus. All cultures were performed in triplicate. Intracellular viral antigens were detected by an indirect immunofluorescence assay using a DENV-specific polyclonal antibody (Chemicon/Millipore, Billerica, MA). Uninfected cultures from each group were used as negative controls.

2.5. Measurements of cytokines by Bio-Plex assays

Ten cytokines were assessed simultaneously using the Bio-Plex system. The selection of specific cytokines in the study was based on previously available reports and the detection of these cytokines in assays was performed in duplicate by following the standard operating protocol provided by the Bio-Plex Multiplex cytokine assay (Kumar et al., 2012). Briefly, lyophilized cytokine standard was resuspended in standard diluent. Serial dilution series to generate standard curves for each cytokine were performed. The bead mixture, specific for cytokines, was incubated for 30 min at room temperature (RT) with 50 μL standard or cell supernatant samples. Several washing steps were performed with 100 μL wash buffer/well. After addition of secondary biotinylated antibody mix for 30 min at RT and three more washing steps, streptavidin-conjugated R-phycoerythrin (SAPE) was added for 10 min at RT. After three final washing steps, beads were resuspended with 125 μL assay buffer, acquired and analyzed by the BioPlex Manager 6.0 software.

2.6. Flow cytometry

The cell-staining protocol used here has been described previously Mangada et al. (2004). In brief, cells were stained with directly conjugated monoclonal antibodies specific to CD4, CD3, CD45, CD11c, CD14 and HLA-DR or with appropriate isotype control (BD Pharmingen). For the detection of the intracellular cytokines TNF-α, IL-17, IFN-γ and DENV E protein, cells were permeabilized and fixed in BD Perm/Wash Buffer (BD Biosciences). Flow cytometric analysis was performed using a FACS CantoII apparatus with Flowjo software (BD).

2.7. Carboxyfluorescein succinimidyl ester (CFSE) labeling and proliferation protocol

The CFSE labeling protocol used here has been described previously Mannering et al. (2003). Briefly, single-cell suspensions from the PBMCs were counted and stained with CFSE dye at 1.25 μM at room temperature in the dark with continuous rocking. After 5 min, staining was quenched with heat-inactivated FBS and the cells were washed, counted, resuspended in complete medium, and used for in-vitro cultures.

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