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TREM-1 modulation during early stages of dengue virus infection

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ABSTRACT

Uncontrolled and intricate production of inflammatory factors is the characteristic feature of dengue infection. The triggering receptor expressed in myeloid cells-1 (TREM-1), expressed on the surface of monocytes and neutrophils, is capable of enhancing and regulating the inflammatory response via the production of different mediators in bacterial and viral infections. Here, both the expression of TREM-1 on human monocytes and neutrophils from peripheral blood of dengue infected individuals, as well as the levels of the soluble form of TREM-1 (sTREM-1) in the sera of these patients were compared against healthy controls. A significant reduction of TREM-1 expression was observed in neutrophils during the first days of infection, followed by a gradual recovery throughout the course of infection. Also, sera from DENV-infected patients exhibited significantly higher sTREM-1 levels than healthy individuals. The difference was more pronounced during the first 5 days after the onset of symptoms. These findings highlight the dynamic process of TREM-1 expression during DENV infection. We hypothesized that increment of free sTREM-1 could be a compensatory mechanism aiming to counteract the inflammatory process elicited during DENV infection.

1. Introduction

DENV infection is a major cause of infectious disease in tropical and subtropical areas, with an estimated 50 million of cases occurring each year and more than 2.5 billion people being at risk of infection [1]. Infection with any of the DENV serotypes (DENV-1, -2, -3, and -4) is generally asymptomatic, but many cases develop dengue fever (DF) or result in severe forms of the disease, known as dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) [2,3]. Cases of dengue are characterized by an uncontrolled inflammatory response [2,4], with massive production of soluble factors such as TNF-<alpha>, IFN-<alpha>, IL-6, IL-8, IL-10 and HMGB1, which are related to disease severity [5–10]. Monocytes are considered as the main target of DENV [11] and recently, Wong et al. demonstrated that different subpopulations of these cells ($CD16^+$ and $CD16^-$) are permissive to infection and capable of support the production of new infective virus particles. Likewise, both subsets had the capacity to produce IFN-<alpha>, CXCL-10 and TRAIL [12]. In addition, it is suggested that monocytes may serve as a vehicle for spreading, as well as a major source of pro-inflammatory cytokines and chemokines capable of compromising the integrity of the vascular tissue leading to the massive leak of plasma, a distinctive feature of severe dengue disease [13,14]. Although neutrophils are not permissive to DENV-infection [15], elevated levels of IL-8, neutrophilic elastase constituent of the azurophilic granules of neutrophils, and lactoferrin are found in children with DENV infection, suggesting the involvement of neutrophils during DENV infection [16]. In addition, Butthed et al. demonstrated that neutrophils may interact with infected cells, which may impact on the activation and secretion of different proteins possibly enhancing the inflammatory status that characterizes the disease [17].

The molecular mechanisms responsible for regulating this response are less known. Triggering receptor expressed in myeloid cells-1 (TREM-1) is selectively expressed on blood neutrophils and monocytes [18], which have emerged as a very important pro-inflammatory molecule in several bacterial and viral infections

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Table 1
Classification of dengue infected patients according to phase and disease severity.

Phase	DF (n = 29)		DHF (n = 27)	
	Primary n (%)	Secondary n (%)	Primary n (%)	Secondary n (%)
Acute	14 (82.3%)	8 (66.7%)	5 (71.4%)	9 (45%)
Convalescent	3 (17.7%)	4 (33.3%)	2 (28.6%)	11 (55%)
Total (n)	17	12	7	20

DF: dengue fever; DHF: dengue hemorrhagic fever.

[19,20]. Engagement of TREM-1 on myeloid cells such as monocytes induces the increased production of pro-inflammatory chemokines and cytokines while in neutrophils promotes the release of myeloperoxidase and MCP-1 [18,21]. Here, the soluble form and the surface expression of TREM-1 on myeloid cells were studied during the course of dengue infection. As far as we know, no studies analyzing the involvement of TREM-1 during DENV infection have been conducted.

2. Materials and methods

2.1. Patients

Peripheral blood samples from untreated DENV-infected patients were collected in the City of Veracruz, in the Gulf of Mexico coast, Mexico, during the 2012 outbreak (Table 1). A total of 56 patients with dengue symptoms and without clinical evidence of other infectious diseases were enrolled in the study, 21 were men (37.5%, mean age 33.44 years, range 14–71 years), 35 women (62.5%, mean age 28.96 years, range 12–61 years). All of them were diagnosed and classified according to the World Health Organization (WHO) system, *i.e.*, dengue fever patients by the presence or combination of high fever, headache, retro-orbital pain, muscle and or joint pains, nausea and vomiting, and rash; dengue hemorrhagic fever patients by hemorrhages or signs consistent with plasma leakage, and marked thrombocytopenia ($<50 \times 10^9$ platelets/L) [3]. None of the patients with hemorrhagic manifestations included in this study fulfilled criteria established by the WHO to be considered as DSS [3]. Dengue infection was laboratory-confirmed by SD BIOLINE Dengue Duo kit (Standard Diagnostic, Korea) and standard serological assays for dengue specific IgM, IgG and NS1 ELISA kits (Panbio, Australia). To rule out presence of other viral or bacterial infections, further serological tests for leptospirosis, influenza A, hepatitis A and *Salmonella* spp. were carried out at the clinical admission time. In male patients, DF was present in 36.4% of cases and DFH in 63.6% of them, while in female patients, DF was detected in 68.6% and DFH in 31.4%. Twenty healthy controls (12 men, 8 women, mean age 26.3 years, range 19–51 years) with no clinical signs or serological markers of dengue were recruited from the State Blood Center of Veracruz. Accordingly to the guidelines of Ethics Committee of the Biological Medical Research Institute, Universidad Veracruzana (Veracruz, Mexico), written consent to participate in the study was obtained from each participant after a full explanation of the protocol.

2.2. Analysis of TREM-1-positive cells

Thirty-nine samples from select DENV-infected patients were analyzed by flow cytometry, the remaining samples were discarded due to insufficient volume or to be unsuitable for analysis. One hundred μL of total blood were incubated with pycoerythrin (PE)-conjugated mouse monoclonal anti-human TREM-1 (R&D Systems, Minneapolis, MN) and peridinin-chlorophyll-protein complex (PerCP)-conjugated mouse anti-human CD14 antibody (BD Bio-Sciences, San Jose, CA) or with PE and PerCP conjugated isotype controls (BD Bio-Sciences, CA). After 30 min incubation, samples were analyzed using an Accuri™ C6 Flow cytometer (Becton-Dickinson, Franklin Lakes, NJ). At least 5000 events corresponding to CD14+ were captured. Flow data analysis for frequencies and median fluorescence intensity (MFI) values were calculated using Cflow sampler analysis software V1.0.227.4 from single events (FSC-A vs. FSC-H dot plot) and according to their side scatter and CD14 antibody staining patterns as has been reported [18,22–24], total monocytes (FSC^{med}, SSC^{med} CD14+) and neutrophils ($FSC^{med/hi}$, SSC^{hi}CD14−) were gated (Fig. 1).

2.3. Determination of soluble TREM-1 in sera from patients with acute dengue infection

Serum soluble TREM (sTREM)-1 level was measured using a commercial assay following the manufacturer instructions (Mybiosource, San Diego, CA).

2.4. Statistical analyses

Mann–Whitney test or ANOVA one way was employed depending on data characteristics. Correlation was analyzed with Spearman's correlation coefficient, p values below 0.05 were considered with statistical significance. GraphPad Prism version 5.0 (Graph-Pad Software, San Diego CA) was used for statistical analyses.

3. Results

3.1. TREM-1 expression is differentially regulated on human neutrophils and monocytes during dengue infection

The percentage of monocytes was increased in DENV-infected patients compared to those present in the healthy subjects group (12.4 ± 0.04 vs. 6.1 ± 1.3; $p < 0.0001$). Moreover, there were not found significant changes in neutrophils percentage observed in patients, compared to healthy subjects (69.4 ± 2.4 vs. 60.9 ± 4.8; $p = 0.1407$). Similarly, no differences were found in monocytes and neutrophils TREM-1 positive percentages among the healthy subjects and DENV-infected patients (monocytes $^{TREM\text{-}1+}$: 98.86 ± 0.1884 vs. 98.89 ± 0.2344; $p = 0.6188$; neutrophils $^{TREM\text{-}1+}$: 93.39 ± 0.8836 vs. 88.86 ± 1.834; $p = 0.5063$). Additionally, similar levels of TREM-1 expressed on the surface of monocytes were observed among dengue-infected patients, either with DF or DHF, and healthy controls (Fig. 2A and 2C). In contrast, TREM-1 expression on peripheral blood neutrophils was notably down regulated in DENV-infected patients in comparison with controls (Fig. 2B). No differences in TREM-1 expression on monocytes and neutrophils were observed when different clinical presentations of dengue (DF or DHF) were taken into account (Fig. 2C and 2D). To perform a detailed analysis of the expression of TREM-1, the MFI results from the patients were grouped according to days after the onset of fever. The levels of TREM-1 on monocytes of dengue patients were significantly higher during the very early infection period (days 2 and 7) in comparison with the healthy controls (Fig. 2E). In contrast, the expression of TREM-1 on neutrophils decreased significantly during the early stage of DENV infection, maintaining a