Immunology Letters 161 (2014) 89–95

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Dynamics of interleukin-21 production during the clinical course of primary and secondary dengue virus infections

H. Vivanco-Cid[a,c,*,1], M.J. Maldonado-Rentería[a,1], L.A. Sánchez-Vargas[a], I.Y. Izaguirre-Hernández[a], K.G. Hernández-Flores[a], R. Remes-Ruiz[b]

[a] Instituto de Investigaciones Medico-Biológicas, Universidad Veracruzana, Veracruz, México
[b] Hospital Regional de Alta Especialidad de Veracruz, Servicios de Salud de Veracruz, México
[c] Universidad del Valle de México, campus Villa Rica, Facultad de Medicina "Dr. Porfirio Sosa Zárate", México

ARTICLE INFO

Article history:
Received 29 December 2013
Received in revised form 22 April 2014
Accepted 7 May 2014
Available online 22 May 2014

Keywords:
Dengue
Interleukin 21
Antibody response
Cellular immunity

ABSTRACT

Previous studies have revealed the clinical relevance of pro-inflammatory cytokine production during dengue virus (DENV) infections. In this study, we evaluated the production of interleukin-21 (IL-21), a key soluble mediator mainly produced by CD4+ T cells.

The aim of this study was to investigate the role of IL-21 production during the clinical course of primary and secondary DENV infections and the potential association of IL-21 serum levels with the disease pathogenesis. Blood samples from DENV-infected patients were collected on different days after the onset of symptoms. Patients were classified according to their phase of disease (acute vs. convalescent phases), the type of infection (primary vs. secondary), and the clinical severity of their disease (dengue fever (DF) vs. dengue hemorrhagic fever (DHF)). IL-21 levels were measured using a quantitative capture ELISA assay. The levels of IL-21 were significantly elevated in the disease group compared with the control group. IL-21 was detected in primary and secondary DENV infections, with a significantly higher concentration in the convalescent phase of primary infections. IL-21 levels were significantly higher in patients with secondary acute DHF infections when compared with those with secondary acute DF infection. There was a relationship between the elevated serum levels of IL-21 and the production of DENV-specific IgM and IgG antibodies. Taking together, our results show for the first time the involvement of IL-21 during the clinical course of DENV infections.

We speculate that IL-21 may play a protective role in the context of the convalescent phase of primary infections and the acute phase of secondary infections.

1. Introduction

Dengue fever is the most common transmitted arboviral disease worldwide. The infection is caused by one of the four serotypes of dengue virus, the etiologic agents, all of which are members of the Flavivirus group into the family *Flaviviridae* [1]. The pathophysiology of DF/DHF/DSS in humans is complex and multi-factorial, involving the interplay of host and viral factors that influence disease severity and the clinical outcome [2]. With respect to host factors, the immune response to DENV plays a dual role in terms of both protection and the pathogenesis of the severe forms of this viral disease. A cascade of pro-inflammatory cytokines is released during acute dengue infections, including cytokines from innate immunity cell sources, such as TNF-α, IL-1, IL-6, and IFN-α, and chemokines, such as MIP1-α, IP-10 and IL-8. Pro-inflammatory cytokines are implicated in host protection roles such as activating innate and adaptive immune cells, chemotaxis and inhibition of viral replication. Some detrimental roles for the high inflammatory cytokine levels that are often observed in severe disease include the induction of cell death (apoptosis and necrosis), endothelial activation, vascular leak and shock [3–7]. Cytokines produced by adaptive immune cells, such as CD4+ and CD8+ T cells, also play a major role with respect to viral spread and further support the development of effector functions, including humoral, cell-specific and memory immune responses. During the CD4+ T cell response to DENV, Th1 and Th2 cells participate in the viral disease with the production of IFN-γ, TNF-β, and IL-2 or IL-4, IL-5, IL-6 and IL-10, respectively [8]. A shift from the Th1 to Th2 response has been suggested in the

* Corresponding author at: Laboratorio Multidisciplinario en Ciencias Biomédicas, Instituto de Investigaciones Medico-Biológicas, Universidad Veracruzana, Avenida Iturbide S/N, Col. Centro, C.P. 91700 Veracruz, México. Tel.: +52 2299318011x120; fax: +52 2299318011.
E-mail addresses: hvivanco@uv.mx, vivacid@hotmail.com (H. Vivanco-Cid).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.imlet.2014.05.006

clinical pathogenesis of DHF on the basis of *in vitro* studies and the *ex vivo* analysis of the cytokine patterns in the serum samples of patients with severe disease [5,8–10].

In this study, we evaluated the interleukin-21 (IL-21) production in sera from DENV infected patients, a key pro-inflammatory and soluble mediator mainly produced by CD4+ T cells. To date, IL-21 has not previously been studied during the clinical course of DENV infections. IL-21 is a cytokine member of the common γ-chain family, which includes IL-2, IL-4, IL-7, IL-9 and IL-15 [11]. IL-21 is produced by various subsets of activated CD4+ T cells, which include Th17 cells, T-follicular helper (Tfh) and CD4+ natural killer (NK) T cells, and exerts multiple and pleiotropic effects on various innate and adaptive immune cells, such as NK cells, monocytes, macrophages, B cells, and CD4+ and CD8+ T lymphocytes, as well as on non-immune cells such as keratinocytes [12], fibroblasts [13,14] and vascular endothelial cells [15]. The aim of this work was to investigate whether the serum levels of IL-21 are elevated in dengue-infected patients, analyzing the production during primary and secondary infections in comparison with healthy individuals and the potential association of IL-21 serum levels with the disease pathogenesis.

2. Materials and methods

2.1. Subjects

A clinical cohort of patients with suspected cases of dengue were recruited in the city of Veracruz México during outbreaks that occurred from June 2010 through November 2012. A total of 360 febrile patients were laboratory confirmed for dengue infection and enrolled in our study. Blood samples from patients were collected in three urban health centers ("Anastasio Iturralde", "21 de Abril", and "El Coyol"), Hospital Regional de Alta Especialidad de Veracruz and Hospital Naval de Veracruz. Dengue patients had not received any drug or treatments before the sample collection. Blood samples were tested and diagnosed by reverse transcription-polymerase chain reaction (RT-PCR) and ELISA techniques to confirm the cases. To rule out other viral or bacterial infections, further serological tests for leptospirosis, influenza A, hepatitis A and *Salmonella typhi* were carried out at the clinical admission time. Patients were classified as having dengue fever or dengue hemorrhagic fever according to clinical and laboratory criteria of the World Health Organization (WHO 1997). Clinical case definition of dengue fever included an acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, arthralgia, myalgia, rash, leukopenia and supportive molecular or serology diagnosis. Clinical case definition of dengue hemorrhagic fever included all of the above criteria for DF plus evidence of hemorrhagic tendencies (epistaxis, gingival bleeding, petechiae, a positive tourniquet test). Vascular leakages were documented by chest X-ray and estimating hypovolemia from multiple hematocrit determinations. Other clinical and laboratory findings included thrombocytopenia (<100,000 counts/mm^3), hypotension and hypoproteinemia. No fatal cases were included in the present study.

Seventy-eight serum samples from healthy individuals without clinical signs or manifestations suggestive of dengue (no febrile symptoms) or other apparent illnesses in the previous 3 months were included as controls. All serum samples were frozen at −70 °C until analysis.

2.2. Ethics statement

Written consent to participate in the study was obtained from each participant after a full explanation of the study was provided and in accordance with the guidelines of the Ethics Committee of the Instituto de Investigaciones Medico-Biológicas, Universidad Veracruzana (Veracruz, México). The study protocol was approved by the institutional ethics review board.

2.3. Dengue diagnosis

Serum samples were tested and diagnosed by reverse transcription-polymerase chain reaction (RT-PCR) and three reference anti-dengue enzyme-linked immunosorbent assays (ELISA): Platelia Dengue NS1 Ag Test (BIORAD Laboratories, France), Dengue IgM (Panbio, Australia) and, to discriminate among primary and secondary infections, a commercial kit (capture IgG ELISA, Panbio) that is used for the quantitative detection of elevated titers of IgG antibodies to dengue virus in patients with secondary infection according to previously established criteria [16].

2.4. Classification of samples

Based on the time of clinical illness, we classified dengue patients into acute (1–7 days after disease onset) and convalescent (samples collected on days 8–10 after disease onset) phases. Primary dengue infection was defined for patients with positive results for RT-PCR, NS1 and/or IgM tests and negative IgG ELISA results. Secondary dengue infection was defined for IgG ELISA positive results (equivalent to a hemagglutination inhibition titer of >2560) regardless of RT-PCR, NS1 and IgM results.

2.5. Measurement of serum IL-21 levels

The IL-21 concentrations in the sera from dengue-positive patients and healthy controls were measured by sandwich ELISA kit from PeproTech Inc. (Rocky Hill, NJ) according to the manufacturer's instructions. The optical density at 405 nm with a reference filter at 650 nm was measured by using a microplate reader (Awareness Technology Inc., Palm City, FL, USA).

2.6. Measurement of serum DENV-specific IgM and IgG levels

The serum samples from dengue-infected patients were tested for DENV IgM and IgG antibodies by ELISA at a 1:100 dilution. Polysorb 96-well microplates (Nalge Nunc International, Rochester, NY) coated with recombinant antigens provided in the Dengue IgM kit and the commercial kit capture IgG ELISA (Panbio, Australia) were used to measure the DENV specific antibody levels. The serum samples were incubated for 1 h at 37 °C. After incubation, serum samples were removed, and horseradish peroxidase-conjugated monoclonal anti-human IgM or IgG were added for 1 h at 37 °C, followed by the addition of 3,3′,5′-tetramethyl benzidine chromogen solution. One hundred microliters of Stop Solution was added to each well plate. The antibodies levels of each serum samples were represented as an index value, according to the manufacturer's instructions. The OD results were corrected by subtracting the OD values of blank samples which are included in every test kit. The ratio with a standard positive sample, the cut-off calibrator, is expressed as an index value (IV). Index value is calculated dividing the optical density of the sample by the cutoff value.

2.7. Statistical analysis

The data were represented as the means ± SD or as medians. The Chi square test was used to compare categorical variables. The non-parametric Kruskal–Wallis test was used to comparison more than two groups. Comparisons among each patient group were performed using Dunn's multiple comparison tests. Statistical significance between two individual groups was determined with the Mann–Whitney *U* test using GraphPad Prism software V