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A strong interferon response correlates with a milder dengue clinical condition

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ABSTRACT

Background: Type 1 interferon (IFNα/β) has a significant role in establishing protection against virus infections. It has been well documented by *in vitro* studies that dengue virus (DENV) activates a robust IFNα/β response. However, DENV also induces a down-regulation of the JAK/STAT pathway, inhibiting the induction of interferon regulated genes. As a consequence, the role played by the IFN type 1 response in the protection of dengue patients is not fully understood.
Objective: To compare IFN-α levels in dengue patients with dengue fever (DF) or dengue hemorrhagic fever (DHF) undergoing primary or secondary infections.
Study design: Two hundred and four serum samples were analyzed for IFN-α level by cytometric bead array. Patients' clinical condition was assigned following the WHO 1997 criteria and specific IgG and IgM antibodies were measured using commercial assays to determine primary and secondary infections. The infecting serotype was determined by qRT-PCR.
Results and conclusion: The IFN-α levels were found significantly higher in DF than DHF patients irrespective of the infecting serotype (DENV1 or 2), and were found to decline rapidly at day 3 after fever onset. For DENV2 infections, higher IFN-α level was found during primary than secondary infections. These results suggest that an early strong interferon response correlates with a better clinical condition.

1. Background

Nowadays, dengue is the most important mosquito borne viral disease in humans. It is endemic in more than 100 countries, causing more than 50,000 million cases and approximately 25,000 fatalities every year [1]. The clinical manifestations of dengue can range from dengue fever (DF) to severe dengue. DF patients show high fever, headache, retro-orbital pain, myalgia and arthralgia, among other clinical symptoms. Persons typically convalesce after 5 or 6 days. However, on occasions patients can progress to severe dengue, characterized by an increase in capillary permeability that can induce shock and death. Although the pathogenesis and the mechanisms involved in the severe forms of dengue infection have been extensively studied, the process is not yet fully understood [2–4]. Secondary infections are recognized as one important risk factors for the development of severe dengue and antibody-dependent enhancement (ADE) of viral infection has been postulated to be the mechanism responsible for the association of secondary infections and severe dengue [5–7]. It has been described that during ADE infection of human monocytic cell lines, a proinflammatory cytokine response is produced, with capacity to disturbs the apical junction complex *in vitro* and cause increase in vascular permeability *in vivo* [8].

Type 1 interferon (IFNα/β), secreted by mammalian cells very early after viral infection, has a significant role in establishing

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protection against viruses [9,10]. In the last years, it has been clearly established that dengue virus (DENV) activates most of the cellular mechanisms, which sense viral infection and triggers a robust IFNα/β response. However, DENV nonstructural proteins are capable of a down-regulation of the JAK/STAT pathway; thus inhibiting the interferon regulated gene expression [11–17].

Since IFNα/β is induced early during DENV infection but such a response may be later modulated by DENV non-structural proteins, we wanted to study the levels of IFN-α in patients sera during the course of DENV infection and to correlate those levels with disease severity. We observed that IFN-α levels are rapidly modulated after fever onset and that higher IFN-α levels correlate with a better clinical condition.

2. Objectives

To compare IFN-α levels in dengue patients with dengue fever (DF) or dengue hemorrhagic fever (DHF) undergoing primary or secondary infections.

3. Study design

A total of 204 samples of human serum collected between, September 2009 to December 2009, were provided by the Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE) in Mexico City, Mexico. Although informed consent was not required because the diagnosis of dengue disease is an obligatory report in Mexico, the confidentiality of patients was conserved. Epidemiological data were obtained from the clinical history of each case, where DF or DHF conditions were defined. The samples were taken from patients during the first 5 days post fever onset. Day 0 was defined as the day where the fever was detected. Samples from healthy blood donors were used as negative controls. All sera were stored at −70 °C until used. To determine the infecting virus serotype, serum samples were tested for viral RNA by real-time reverse transcription-polymerase chain reaction (RT-PCR) as previously described [18].

The IgM and IgG anti-DENV antibodies were determined using Dengue IgM and IgG Capture ELISA (Panbio). The sample value was determined for each sample by dividing the OD of the test sample by the average OD of the cutoff control provided by manufacturers. A sample was defined as positive for IgM when $(OD/CV) \times 10 \geq 11$ for IgM-Cap ELISA, and was defined as positive for IgG when $OD/CV \times 10 \geq 22$ for IgG-Cap ELISA. In addition, a sample was defined as primary infection when it was negative for IgG-Capture ELISA, or secondary infection when it was positive for IgG-Capture ELISA [19,20].

The levels of IFN-α were measured using a BD cytometric bead array (CBA) with human soluble protein flex sets and human soluble protein master buffer kits including particles with discrete fluorescence intensities to detect soluble analytes (BD CBA flex set; BD Biosciences). Briefly, patients' sera were immunoprecipitated by using a captured-bead array coated with specific antibodies against IFN-α. Then, phycoerythrin (PE)-conjugated detection reagents were added, and quantification was carried out by flow cytometry assay. Data acquisition and analyses were done by using a BD FACSCalibur flow cytometer using BD CellQuestPro software (BD Biosciences). Supplied standard was used to construct a standard curve ($R^2 > 0.9957$) (BD CBA flex set; BD Biosciences). The resulting mean fluorescence intensity (MIF) allowed sample analysis and soluble protein quantification by using the FCAP Array software program, version 3.0 (BD Biosciences, CA, USA).

The *Mann–Whitney U* test was used to compare median values between groups. Graphs were generated with the Graph Pad Prism 5 software.

4. Results

Based on real time PCR results [18], of the total of 204 samples of human serum analyzed, 124 were identified as DENV1 and 80 DENV2 infections. Based on the clinical story, 116 cases were classified as DF and 88 as DHF. Additionally, based on the positivity to IgG anti-DENV antibodies, 55 (68.75%) out of the 80 cases of DENV2 were classified as primary infections, while 116 (93.54%) out of the 124 cases of DENV1 were classified as primary infections.

Circulating levels of IFN-α were found significantly higher in patients with DF than with DHF. For DENV1 patients with DF the median IFN-α concentration was 64.88 pg/mL (25–75% IQR = 28.73–112.8 pg/mL, range = 18.79–517.8 pg/mL) while the median for those patients with DHF was 36.4 pg/mL (25–75% IQR = 25.31–77.51 pg/mL, range = 18.46–478 pg/mL). Similarly, for DENV2 infected patients showing DF, the median IFN-α concentration was 49.45 pg/mL (25–75% IQR = 32.85–99.16 pg/mL, range = 21.24–646.9 pg/mL) *versus* a median concentration of 38.1 pg/mL, (25–75% IQR = 28.55–55.6 pg/mL, range = 19.78–148.5 pg/mL) for those showing DHF. The differences in mean concentration of IFN-α between DF and DHF fever patients were found statistically significant for both serotypes ($p = 0.0052$ and $p = 0.0233$ for DENV1 and DENV2, respectively) (Fig. 1A). As expected, very low concentrations of IFN-α were observed in the sera of blood donors used as controls.

Since levels of IFN-α in sera may be dynamic and vary according to illness day, the levels of IFN-α in patients infected with DENV1 and DENV2 were determined according to the day of sera collection after fever onset (Fig. 1B). For DENV1 infected patients, sera concentration of IFN-α were observed significantly higher ($p < 0.0001$) during the first two days of infection (median = 94.42 pg/mL, 25–75% IQR = 35.6–133.6 pg/mL, range = 19.27–478.0 pg/mL) than in days 3–5 after fever onset (median = 36.12 pg/mL, 25–75% IQR = 25.39–61.3 pg/mL, range = 18.46–517.8 pg/mL). Likewise for DENV2 infected patients, levels of IFN-α were found significantly higher ($p = 0.0239$) in sera collected 0–2 days after fever onset than in those collected 3–5 days (median = 53.39 pg/mL, 25–75% IQR = 32.43–142.3 pg/mL, range = 20.4–646.9 pg/mL *versus* median = 38.25 pg/mL, 25–75% IQR = 32.02–55.6 pg/mL, range = 19.78–335.3 pg/mL).

Finally, we compared IFN-α levels is sera during primary or secondary infections from patients infected with DENV2. Patients with DENV1 infection were excluded from the analysis since very few secondary infections with DENV1 were observed ($n = 8$). Primary infection showed significantly ($p < 0.0001$) higher concentrations of IFN-α than secondary infections (median = 53.39 pg/mL, 25–75% IQR = 34.93–134.0 pg/mL, range = 20.4–646.9 pg/mL *versus* median = 32.79 pg/mL, 25–75% IQR = 28.75–39.0 pg/mL, range = 19.78–106.3 pg/mL). Results are shown in Fig. 2.

5. Discussion

One of the main challenges in dengue patients' healthcare is to establish criteria for early detection of severe forms of the disease. Parameters that could help in the diagnostic are: viremia, levels of circulating NS1 or concentrations of proinflammatory cytokines such as TNF-α. Although one of the most robust responses induced during DENV infection is the IFN response, several viral proteins have been shown to negatively modulate this response [12,15]. Despite this inhibitory mechanism, DENV infection is able to induce a IFN type 1 response in patients, suggesting that this response may be regulated at different levels by different pathogen-sensing mechanisms. Thus, in the present study, the levels of IFN-α were