Interleukin (IL-2) Levels in Past Dengue Infection

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Abstract
Several reports relate the role of different cytokines in the pathogenesis of dengue. Here, we report the production, after trigger with dengue virus, of IL-2 by peripheral blood mononuclear cells of individuals with history of dengue infection 20 years ago.

Key words: Dengue, cytokines, dengue virus, Havana, Cuba

Introduction
Dengue haemorrhagic fever (DHF) is the extreme and severe manifestation of the dengue infection entailing a rapid clinical deterioration, circulatory collapses and shock. The severe disease mostly depends on the intrinsic immune response of the host, which is responsible for additional damages that are qualitatively different to those provoked by the virus itself[1].

Interleukin-2 (IL-2) is the major autocrine growth factor for T lymphocytes. This cytokine stimulates the activation, differentiation and proliferation of T, B, NK cells and monocytes. Levels of IL-2 and soluble CD4+ and CD8+ molecules in plasma from DHF patients are significantly higher than those from dengue fever patients (DF)[2,3]. IL-2 is capable of stimulating either human T cell (HT-2) or murine (CTLL-2) cell lines to grow. Quantification of CTLL-2 proliferation is a standardized assay to indirectly measure IL-2 concentration[4].

The memory T cell response to dengue virus is not very well understood. Based on these facts, the present research was designed to study a long-lasting memory T cell response through the detection and quantification of IL-2 in supernatants of dengue-stimulated human lymphocyte
cultures from dengue 1 or 2 Cuban immune individuals that suffered their primary infection 21 years (DEN-1) and 17 years (DEN-2) ago.

The unique Cuban epidemiological situation on dengue allows us to perform this study\(^5\). In 1977, Cuba suffered a DEN-1 epidemic that affected the whole country with more than 400,000 reports. This epidemic was followed by a DHF outbreak in 1981 caused by the serotype 2\(^6\). More than 344,000 reports, 10,000 DHF and 158 fatal cases were reported. After more than 14 years without dengue circulation, in 1997, a small DEN-2 outbreak was observed in Santiago de Cuba municipality, where 3012 cases were confirmed with 205 DHF and 12 fatal cases, all in adults\(^7\). Taking into account these antecedents, all dengue-immune Cuban individuals have been infected in either the 1977 epidemic (DEN-1), and/or during the DEN-2 epidemics (1981 and 1997).

Considering the probable role of IL-2, both in protection and/or in the pathogenesis of DHF\(^8\), we decided to quantify its production in dengue-stimulated PBMC from DEN-1 or DEN-2 immune individuals.

**Methodology**

In 1998, 20 healthy adult volunteers from Havana city were bled. These individuals could have been infected during the 1977 (DEN-1) and/or 1981 (DEN-2) epidemics. Serum samples were tested for quantifying the presence of total anti-dengue virus antibodies using an inhibition immunoenzymatic assay\(^9\). Neutralizing antibodies were determined in those positive sera by plaque reduction neutralization assay on BHK21 clone 15 cells according to Morens et al.\(^{10}\) with minor modifications. DEN-1 (Hawaii strain) and DEN-2 (A15 Cuban strain) viruses were employed\(^{11}\). Those DEN-1 or DEN-2 immune individuals were enrolled in the study.

Peripheral blood mononuclear cells (PBMC) (3x10\(^6\)/well), isolated by the Boyum method\(^{12}\), were incubated at 37\(^\circ\)C in 5% CO\(_2\) atmosphere with DEN-1 (Hawaii strain) and DEN-2 (NGC strain) antigens at a concentration of 184.5\(\mu\)g/ml / 2.1x10\(^3\) pfu and 260\(\mu\)g/ml/4.5x10\(^3\) pfu, respectively. The supernatants were collected for IL-2 quantification after 48h incubation. Cell cultures with media were used as negative control.

The IL-2 assay consisted in culturing the IL-2-dependent cytotoxic T lymphocyte cell line CTLL2 (CTLL-2 ATCC TIB 214, 302 catalog Cell Lines & Hybridomas; 7\(^{th}\) edition, 1992, edited by ATCC Maryland) in the presence of either non-stimulated or antigen-stimulated culture supernatants. CTLL-2 proliferation was detected by a non-radioactive cell proliferation assay (Cell Titer 96\(^\text{TM AQ}\)ueous Non-Radioactive Cell Proliferation Assay, Promega).

**Results and discussions**

Total dengue antibodies were detected in 5/20 (25%) individuals. Table 1 shows the neutralizing antibody titer to DEN-1 or DEN-2 viruses in those dengue-immune individuals.

Levels of IL-2 (7.27-78.23 U/ml) were detected in the supernatant of stimulated PBMC from dengue-immune individuals (Table and Figure). A serotype-specific stimulation in all cases and a slight cross-reactivity in some of them were detected.
The presence of IL-2 in the supernatants of dengue-stimulated PBMC from immune individuals is in line with the results previously published by Kurane et al.\textsuperscript{(2)} who detected IL-2 in the sera of children with dengue. Our results are also in agreement with the previous results found by Pérez et al.\textsuperscript{(13)} who detected memory lymphocytes to dengue after long periods of the primary infection. These results could explain at least in part the recent epidemiological observation done in 1997 in Santiago de Cuba municipality\textsuperscript{(14)} where after more than 20 years of the DEN-1 primary infection, a DHF outbreak caused by DEN-2 virus was reported. It seems that there is no time-limit for the sensitization after a primary dengue infection.

The study of other cytokines in dengue-stimulated PBMC from immune individuals could clarify the immunological cascade disruption and their role in the pathogenesis of this disease.

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References