SHORT REPORT: $T_{H2}$ IMMUNE RESPONSE IN PATIENTS WITH DENGUE DURING DEFERVERSCENCE: PRELIMINARY EVIDENCE

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Abstract. A shift from a $T_{H1}$ to a $T_{H2}$ immune response has been observed in vitro during dengue virus infection. Estimation of plasma IgE level ($n = 28$), CD4:CD8 lymphocyte ratio, and the intracellular interferon-$\gamma$ (IFN-$\gamma$):interleukin-4 (IL-4) ratio ($n = 9$) was conducted in patients with various severities of dengue around the time of defervescence. The CD4:CD8 lymphocyte ratio was significantly lower (median = 0.45, interquartile range [IQR] = 0.4–0.47 versus 1.3, 1.0–1.9; $P = 0.001$), and IgE levels were significantly higher (mean = 300.4, SD = 252.5 versus 143.7, 117.9 IU/mL; $P = 0.004$) in patients than in healthy controls. The intracellular IFN-$\gamma$:IL-4 ratio was significantly lower in patients compared with controls (0.28, 0.13 versus 0.99, 0.4; $P = 0.001$). These findings suggest that a $T_{H2}$ immune response occurs in patients with dengue around the time of defervescence.

INTRODUCTION

If symptomatic, dengue virus infection usually causes a self-limited illness, namely dengue fever (DF). At times, severe forms of illness such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) occur that are characterized by increased vascular permeability. Although it is well recognized that inflammatory mediators elaborated in response to dengue virus infection are responsible for the development of complications, the nature of immune response in humans with dengue is not well studied. Earlier studies have found increased levels of $T_{H1}$ cytokines such as tumor necrosis factor-$\alpha$ and interferon-$\gamma$ (IFN-$\gamma$) in patients with dengue and their correlation with disease severity. However, in vitro studies suggest that dengue virus infection induces a predominant $T_{H1}$ immune response early in the course of infection that is replaced by a $T_{H2}$ response after a period of approximately three days. To find out whether such a predominance of a $T_{H2}$ immune response also occurs in vivo, we studied IgE levels, T lymphocyte subsets, and intracellular levels of the cytokines interleukin-4 (IL-4) and IFN-$\gamma$ in peripheral blood mononuclear cells (PBMCs) of patients with various severities of dengue around the time of defervescence.

An epidemic of DF occurred in New Delhi, India and surrounding areas in northern India from October to December in 2003, resulting in more than 2,000 laboratory-confirmed cases. Dengue-2 (DEN-2) and DEN-3 are the major circulating dengue viral serotypes in this population. An earlier epidemic in 1996 was caused by the DEN-2 serotype. The epidemic under study involved transmission of both the DEN-3 and DEN-2 serotypes. Twenty-eight adult patients (21 [75%] males) with various severities of dengue (DF = 18, DHF/DSS = 10) as per the World Health Organization case definition who were admitted to the All India Institute of Medical Sciences Hospital in New Delhi during this epidemic were included in the study. None of the patients were atopic or had risk factors for human immunodeficiency virus (HIV) infection. After informed consent was obtained, blood samples were drawn into heparinized tubes at the time of hospitalization. Estimation of IgE levels was done in all patients, while estimation of T lymphocyte subsets and levels of intracellular cytokines was done in a subgroup of patients ($n = 9$: DF = 6, DHF/DSS = 3). The study was conducted in compliance with the ethical guidelines for biomedical research on human subjects issued by the Indian Council of Medical Research (New Delhi, India).

Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation within six hours of blood collection and resuspended in RPMI 1640 medium supplemented with 10% inactivated fetal calf serum. Immunostaining was done as per the manufacturer’s specifications (BD Biosciences, San Jose, CA), using phycoerythrin-labeled monoclonal antibodies to CD4, IFN-$\gamma$, or an IgG1 isotypic control, and fluorescein isothiocyanate–labeled monoclonal antibodies to CD8, IL-4, or an IgG2a isotypic control. Briefly, after two washes with staining buffer containing 0.5% bovine serum albumin, PBMCs were incubated with respective antibodies for surface immunostaining of CD4 and CD8. For intracellular immunostaining (IL-4 and IFN-$\gamma$), PBMCs were fixed in 4% buffered formalin and permeabilized with 0.3% saponin, followed by incubation with respective antibodies. Subsequently, samples were run in a two-color flowcytometer (FACSVantage; BD Biosciences) and analyzed using Cell Quest software. IgE levels in plasma were estimated by a sandwich enzyme-linked immunosorbent assay (Bethyl Laboratories, Inc., Montgomery, TX).

Normal values established in our laboratory in HIV-negative, age-matched, non-atopic healthy controls for the CD4:CD8 lymphocyte ratio ($n = 60$), IgE levels ($n = 60$), and the intracellular IFN-$\gamma$:IL-4 ratio in PBMCs ($n = 9$) were used for comparison with those of the patients. Data are presented as the mean (SD) when normally distributed and as the median (interquartile range) if the distribution was skewed. Continuous variables were compared between patients and controls using an independent $t$-test or the Mann-Whitney U test as appropriate. Comparison of continuous variables involving more than two groups was done with the Kruskal-Wallis test. Statistical analyses were done using SPSS for Windows, version 10.0.1 (SPSS Inc., Chicago, IL). All $P$ values were two-sided and values < 0.05 were considered statistically significant.

The mean (SD) age of the study group was 29 (13) years versus 30 (14) years in controls, and the mean (SD) duration of fever at presentation was 6 (2) days. The subgroup that underwent T lymphocyte subset and intracellular cytokine estimation was comparable to the rest of patients in the study group (age = 31 [6] versus 29 [16] years), and no significant differences were noted with respect to any of the clinical fea-
It stimulates T1. CD4:CD8 lymphocyte ratio in peripheral blood of patients with dengue. Among the patients, those with milder illness (DF) had higher mean levels of IgE than those with severe illness (DHF/DSS). However, this difference was not statistically significant ($P = 0.175$). None of the patients had peripheral blood eosinophilia (absolute eosinophil count = 124 [58]/µL, range = 23–252; controls = 144 [99]/µL; $P = 0.27$). The proportion of IFN-γ positive PBMCs was significantly lower in patients compared with controls (0.15 [0.12] versus 0.47 [0.37]; $P = 0.03$), whereas the proportion of IL-4-positive PBMCs was similar in both two groups (0.51 [0.32] versus 0.49 [0.34]; $P = 0.89$). This resulted in a significantly lower intracellular IFN-γ:IL-4 ratio in patients than the controls (0.28 [0.13] versus 0.99 [0.4]; $P = 0.001$). Among patients with various severities of illness (DF and DHF/DSS), the differences in the CD4:CD8 lymphocyte ratio and intracellular IFN-γ:IL-4 ratio were not statistically significant.

Severe dengue infection is seen primarily among children. However, the present study included a considerable number of adults with severe forms of dengue (10 of 28, 36%). The probable reason was that in northern India the level of dengue endemicity is relatively low and for the same reason, many of these patients experienced a second episode of dengue virus infection only as adults. Our results confirm that inversion of the CD4:CD8 lymphocyte ratio occurs in patients with dengue. Inversion of this ratio and impaired proliferation of T lymphocytes in response to mitogens are known to occur in patients with dengue. Earlier studies have reported the levels of various cytokines in the serum of patients with dengue. In the present study, intracellular levels of cytokines, rather than serum levels, were studied since serum levels of cytokines may not truly reflect their expression in patients with DHF/DSS due to increased vascular permeability.

Elevated IgE levels along with low IFN-γ:IL-4 ratios suggest a predominance of a T1,2 immune response in these patients. Interleukin-4 is an important cytokine involved in the T1,2 immune response. It stimulates T1,2 helper T cell differentiation and also mediates the class switch to IgE synthesis by B lymphocytes. Conversely, IFN-γ inhibits IL-4-dependent induction of IgE synthesis. Increased IgE levels in patients with dengue, as observed in the present study, is probably due to a reversal of inhibition of IL-4-dependent IgE synthesis, resulting from decreased levels of IFN-γ during defervescence. In the present study, all patients presented at the time around defervescence, as shown by the duration of fever at presentation (typically fever lasts for 5–7 days followed by defervescence). This is consistent with the view that the cytokine response was waning away when blood specimens were collected.

It is possible that the elevation of IgE levels in patients with dengue was due to undiagnosed parasitic infections that are endemic in this population. However, the fact that IgE levels were still higher when compared with controls in the local population reasonably excludes this possibility. A recent study has shown that IgE levels correlate with disease severity in patients with dengue. Basophils and mast cells are thought to play an important role in the pathogenesis of dengue. IgE mediates antibody-enhanced cellular entry of dengue virus into basophils and mast cells, and subsequently leads to elaboration of vasoactive mediators by the latter. The present study did not find a significant relationship between IgE levels and disease severity. Conversely, a T1,2 immune response may have a protective role against the development of complications in patients with dengue. A balanced immune response instead of one that is polarized towards a T1,1 response might be what underlies uncomplicated disease resolution. Observation of relatively lower levels of IgE in severe forms of dengue (DHF/DSS) compared with those in milder illness (DF) in the present study is supportive of this hypothesis.

Thus, the findings of the present study suggest that a T1,2 immune response occurs in patients with dengue around the time of defervescence, as has been observed in vitro. Further studies involving larger number of patients are required to confirm these findings and to evaluate the causal relationship between a T1,2 immune response and the development of severe forms of dengue (DHF/DSS) compared with those in milder illness (DF) in the present study is supportive of this hypothesis.
complications in patients with dengue. Moreover, these studies should involve sampling at multiple time points, starting early in the course of illness so as to find whether a shift from a $T_{H1}$ to a $T_{H2}$ immune response occurs in patients with dengue.

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