

RESEARCH NOTE

Analysis of Tumor Necrosis Factor- α Serum Level in Brazilian Patients with Dengue-2

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After a long period of absence in Rio de Janeiro, Dengue-1 was introduced in 1986 (HG Schatzmayr et al. 1986 *Mem Inst Oswaldo Cruz* 81: 245-246) and Dengue-2 in 1990 (RMR Nogueira et al. 1993 *Epidemiol Infect* 111: 163-170) causing two widespread epidemics. In the years of 1986 and 1987 about 84,000 cases were notified and in 1990/1991 about 98,000 cases. During the Dengue-2 epidemic 17 deaths due to dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS) were reported (Health Secretary of Rio de Janeiro State 1992, Annual Report).

DHF/DSS is the most severe form of dengue which includes laboratory findings not present in classic dengue fever (DF), such as hemoconcentration due to plasma leakage, results in hypovolemia and in some cases shock and death. Most of the DHF/DSS patients are already IgG-seropositive for dengue during the first days of infection, supporting the theory that this severe form of disease is related to sequential infections by different serotypes (SB Halstead 1980 p. 107-173. In RW Schlesinger, *The Togaviruses: Biology, Structure and Replication*, Academic Press, New York). Antiviral non-neutralizing antibodies from a previous heterotypic infection would bind to the virus enhancing its penetration through Fc receptors on mononuclear phagocyte surfaces. Infected cells would release immunological mediators that could be involved in the severe symptoms of the disease (SB Halstead 1989 *Rev Infect*

Dis 11 (Suppl.4): S830-S839). Therefore, to examine the possible role of tumor necrosis factor- α (TNF- α) secreted by infected monocytes we investigated the presence of this monokine in sera of dengue patients with well characterized clinical symptoms.

The levels of TNF- α were determined in sera of patients with symptoms of dengue admitted to the Hospital Evandro Chagas, IOC, FIOCRUZ and Hospital Orêncio de Freitas, Niterói, State of Rio de Janeiro. The following groups were tested: (i) patients with symptoms of DF from which Dengue-2 virus was isolated and had high hemagglutination inhibition titers (higher than 1/2560), indicating a secondary infection by dengue virus following rules from the World Health Organization (1985 Technical Report Series: Viral Haemorrhagic Fever, 721 Geneva); (ii) patients with symptoms of DHF/DSS with increasing grades of severity (II, III and IV), following classification criteria from the World Health Organization (*loc. cit.*) and high hemagglutination inhibition (except for one patient); (iii) patients with no virus isolation and no hemagglutination inhibition titer in two paired samples were used as controls. Most patients were adults, except two children of eleven: one DHF grade II and one DHF grade IV. Samples were taken from day 2 to day 7 post infection except for DHF-grade IV patient which was taken on day 10.

The sera were transported on ice and kept at -70°C and suffered about three thawing procedures.

ELISA assays were run using aliquots of sera in duplicate in order to detect the presence of TNF- α . Kits were a kind gift from Dr H Van Heuverswyn (Innogenetics Gent, Belgium), and used as described by the manufacturer.

All duplicates from the TNF- α kit standard curve and from most tested samples had very small standard deviation (SD) values of optical density: the few sera with SD higher than 15% of the mean were discarded.

TNF- α was detected in two out of eight DF sera, none of the eight DHF grade II, two out of five DHF grade III and in the one DHF grade IV, who died one day after serum was collected. Values in picograms (pg) of TNF- α are shown in Table. Statistical analysis was not possible regarding the small sampling. Nevertheless one can observe the tendency of higher average in groups with DHF grade III and IV.

TNF- α is a well known factor which plays an important role in endotoxic shock during bacterial infections (B Beutler et al. 1987 *Science* 229: 869-871, K Tracey et al. 1987 *Nature* 330: 662-

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TABLE

TNF- α determination in sera from dengue patients

| Control | DF | DHF-II | DHF-III | DHF-IV |
|--------------------|------------|-------------|-------------|---------------------|
| 24.75 ^a | 7.63 | 0.00 | 0.00 | 957.31 ^b |
| 7.73 | 8.70 | 28.22 | 16.01 | |
| 0.00 | 12.56 | 0.76 | 32.34 | |
| 19.23 | 32.73 | 23.29 | 56.96 | |
| | 33.16 | 3.77 | 160.02 | |
| | 35.09 | 17.93 | | |
| | 42.70 | 6.99 | | |
| | 62.25 | 0.00 | | |
| 12.93 \pm | 9.35 \pm | 10.12 \pm | 53.07 \pm | 957.31 |
| 11.16 ^c | 18.90 | 11.37 | 63.39 | |

a: individual values of TNF- α are reported in picograms per milliliter of serum.

b: individual values marked in italics were considered positives because were higher than control mean + 2SD (35.25), according to Chebyshev's theorem (RE Walpole 1968 p. 72 In *Introduction to Statistics*. Collier-Macmillan Limited, London).

c: average and standard deviation of TNF- α amounts detected for each patient group are represented in the last row.

664) and is involved in symptoms resembling those of DHF/DSS, such as development of hemoconcentration and hypovolemic shock (A Cerami, B Beutler 1988 *Immunol Today* 9: 28-31). Recently, TNF- α and Interleukin-6 were detected in dengue

patients from Asia (Hober et al. 1993 *Am J Trop Med Hyg* 48: 324-331) and Central America (G Kuno, RE Bailey 1994 *Mem Inst Oswaldo Cruz* 89: 179-182) and correlation of symptom severity with the production of both cytokines was suggested by authors.

From the work presented here using Brazilian patients it is clear that TNF- α can be detected during dengue disease including its more severe forms. Even though only a few patients were tested, results support the believe that TNF- α production is related to immunopathologic mechanisms underlying DHF/DSS: the high level of TNF- α detected in our fatal case was not described in the previous papers and reinforces the possible role of this factor in the hemorrhagic disease and shock. No correlation of age or time of infection with TNF- α production was possible because of small sampling.

Interleukin-6 determination was also performed in sixteen patient sera but only one was positive (data not shown), leading us to inconclusive results. Thus, further determinations of TNF- α , Interleukin-6 and other cytokines in sera of Brazilian dengue patients should provide a clearer picture of the role of these immunomodulators in DHF/DSS.

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