Cytokine Responses to Dengue Infection among Puerto Rican Patients

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Recently, a strong correlation between high concentration of tumor necrosis factor (TNFα) in blood and severity of dengue hemorrhagic fever/dengue shock syndrome has been reported from Asia and the Pacific. We wished to determine if a similar relationship could be found in dengue patients in the Americas where adult patients with severe syndromes have been observed more frequently than in Asia where severe cases have been observed mostly among children.

The concentrations of interleukin-1 (IL-1β) in hospistalized adult groups were significantly lower than that in outpatient adults. In contrast, the levels of interleukin 6 (IL-6) were significantly higher in hospitalized adults and children than in the corresponding outpatients. Levels of TNFα were higher in hospistalized children than in outpatient children or hospistalized adults. There was no significant difference in the levels of these three cytokines among hospitalized patients with or without hemorrhagic manifestations. Thus, an elevated IL-6 level was positively associated with severity of dengue infection in both children and adults, but IL-1β level was negatively associated with severity in adults.

Key words: dengue - cytokine - interleukin-1 - interleukin- 6 - tumor necrosis factor

The severe forms of dengue fever, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which have been endemic for many years primarily in some parts of southeast Asia, have recently been more frequently observed in the Americas (Kouri et al. 1989, Anonymous 1990). Although several hypotheses and risk factors have been proposed to explain the pathogenicity associated with these syndromes, thus far no consensus or definitive *in vivo* evidence has been obtained.

Recently, elevated levels of tumor necrosis factor (TNFα) in blood were found to be positively correlated with the development of the severe dengue syndromes in Asia and Pacific (Vitarana et al. 1991, Yadav et al. 1991, Iyingkaran et al. 1992, Hober et al. 1993). Therefore, it is important to investigate the phenomenon among dengue patients in the Western Hemisphere.

In this study, we measured the levels of three cytokines, interleukin-1 (IL-1ß), interleukin-6 (IL-6), and TNFα, in Puerto Rican patients with different degrees of disease severity and statistically analyzed the relationship between cytokine level and disease severity.

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* The use of trade names or commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

MATERIALS AND METHODS

Patients - Two hundred thirty-three patients with laboratory-confirmed dengue in Puerto Rico in 1991 were selected and classified into six groups by age (children ≤15 yrs and adults), presence or absence of hemorrhagic manifestations, and whether or not they were hospitalized (Table I). The patients with hemorrhagic manifestations had at least one of the following signs: hematemesis, epistaxis, petechia, purpura, ecchymosis, gum bleeding, vaginal bleeding, frank hematuria, melena, and prolonged bleeding from skin puncture.

Determination of cytokine levels - We determined the concentrations of three cytokines (IL-1ß, IL-6, and TNFα) in serum samples, collected within seven days after the onset of illness with commercial enzyme-linked immunosorbent assay kits (for IL-1ß and TNFα, Cistron Biotechnology, Pine Brook, NJ; for IL-6, R&D Systems, Minneapolis, MN).* Concentrations were calibrated with the use of recombinant cytokines. Sensitivity limits ranged between 3.5 and 10 pg/ml among products; however, for convenience, a cut-off limit was set at 10 pg/ml for all cytokines.

TABLE I

Presence of detectable cytokines in Puerto Rican patients with dengue infections by severity and age

Group[a]	No. patients	IL-1B[b]		IL-16[b]		TNFα[b]	
		No. pos	%	No. pos.	%	No. pos.	%
A	8	4	50	4	50	5	62
B	48	25	52	23	48	36	75
C	42	14	33	22	52	25	60
D	47	22	47	21	45	27	57
E	44	19	43	10	23	16	36
F	44	27	61	4	9	25	57

[a]: abbreviations: A: children with hemorrhage (hospitalized); B: children without hemorrhage (hospitalized); D: adults without hemorrhage (hospitalized); E: children (outpatients); F: adults (out patients).
[b]: IL - interleukin; TNF-tumor necrosis factor.

TABLE II

Cytokine levels (pg/ml) in the blood samples of dengue patients in Puerto Rico

Group[a]	No. Patients	Cytokine[b]	Mean	SD[c]
A	8	IL-1β	16.88[d]	22.13
		IL-6	232.63	506.21
		TNFα	58.75	107.69
B	48	IL-1β	35.13	81.18
		IL-6	53.17	142.29
		TNFα	69.92	152.48
C	42	IL-1β	28.98	59.70
		IL-6	49.48	80.38
		TNFα	26.12	53.86
D	47	IL-1β	67.45	177.61
		IL-6	80.17	184.34
		TNFα	24.19	28.09
E	44	IL-1β	41.41	59.92
		IL-6	12.59	18.63
		TNFα	46.18	70.24
F	44	IL-1β	74.02	96.09
		IL-6	9.16	20.62
		TNFα	66.14	79.03

[a]: for description of groups, see Table I
[b]: cytokines: IL-Interleukin; TNF-Tumor necrosis factor
[c]: standard deviation
[d]: values expressed in pg/ml

TABLE III

Significance of the differences in cytokine levels in different groups of Puerto Rican patients with dengue[a]

Groups e compared[b]	p-Value IL-β[c]	IL-6[c]	TNFα[c]
A and B	--[d]	--	--
C and D	--	--	--
E and F	--	--	--
A and C	--	--	--
B and D	--	--	--
A and E	--	--	--
B and E	--	0.005[e]	0.038[e]
C and F	0.003[f]	<0.001[e]	--
D and F	--	<0.001[e]	--
AB and CD	--	--	0.027[e]
AC and BD	--	--	--
AB and E	--	0.004[e]	0.041[e]
CD and F	0.005[f]	<0.001[e]	0.035[f]
ABCD and EF	0.024[f]	<0.001[e]	--
ABE and CDF	--	--	--

[a]: based on all values including negative specimens.
[b]: see Table I for description of groups.
[c]: cytokines: IL=Interleukin; TNF=Tumor necrosis factor
[d]: p>0.05. P - values were determined using the Wilcoxon (Mann-Whitney) test for comparison between two groups.
[e]: first group in comparison has a higher cytokine level.
[f]: second group in comparison has a higher cytokine level.

RESULTS

Cytokine levels in serum specimens of the patients were analyzed qualitatively by the proportion of cytokine-positive patients and quantitatively by the concentration of cytokine in each patient group or a combination of groups.

In the qualitative analysis of the hospitalized children or adults, no significant difference in cytokine positivity was observed between the patients with and without hemorrhagic manifestations (Table I). However, the proportion (61%) of outpatient adults positive for IL-1ß was significantly higher than the proportion (40%) of the hospitalized adults (x^2 5.17; $p<0.05$). On the other hand, the proportion of hospitalized children (73%) was significantly higher for TNFα (x^2 13.65; $p<0.001$) than that of the outpatient children (36%). For both children and adults, a significantly higher proportion of patients with detectable IL-6 was observed in hospitalized groups than in outpatients (for children 48 vs 23% with x^2 6.87; $p<0.01$, and for adults 48% vs 9% with x^2 19.82; $p<0.001$). In all other comparisons, the differences in proportion of cytokine positivity were not statistically significant.

The concentrations of the three cytokines in the six groups of dengue patients are presented in Table II. The level of IL-1ß in the hospitalized adults with hemorrhagic manifestations was significantly lower than that in the outpatient adults (Wilcoxon test; $p < 0.01$), reflecting the lower proportion described earlier. A similar result was obtained when the IL-1ß level in either all hospitalized adults (a combination of the patients with and without hemorrhagic manifestations) or all hospitalized patients (combined groups of all hospitalized adults and children) was compared with that of outpatient adults or all outpatients, respectively (Table III).

On the other hand, the concentrations of IL-6 were significantly higher ($p<0.001$) in the groups of hospitalized adults and hospitalized children without a hemorrhagic manifestation than in the corresponding outpatients. The same result was obtained when the IL-6 levels of all hospitalized children or all hospitalized patients were compared with those of outpatient children or all outpatients, respectively (Table III).

Significantly higher ($p<0.05$) concentrations of TNFα were noted in the hospitalized children without hemorrhagic manifestations (as compared with outpatient children), in all hospitalized children (as compared with outpatient children), and in all hospitalized children (as compared with all hospitalized adults) (Table III). TNFα level in all hospitalized adults was significantly ($p<0.05$) lower than that of outpatient adults.

DISCUSSION

Although several hypotheses and risk factors, such as pre-existing enhancing antibody, ethnic factor, presence of underlying unrelated illness or infection, and variation of virulence among virus strains, have been proposed to explain the exact pathogenetic mechanism(s) of DHF/DSS, the exact pathophysiological changes leading to manifestation of severe syndromes remain unresolved.

We focused our attention on the roles of three cytokines. They are known to be involved in the development of pyretic response, inflammatory reaction, change in vascular permeability and hemorrhagic manifestations, hypotension, and/or shock in many infectious diseases (Beutler & Cerami 1989, Dinarello & Savage 1989, Van Snick 1990). These same signs and symptoms are also characteristics of DHF/DSS.

Thus, it is important to review the recent reports from Asia and Pacific correlating TNFα levels and severity of DHF/DSS, since we found a significantly higher level of TNFα in the hospitalized children than in outpatient children. In one study, the TNFα levels in DHF patients (dengue grade unspecified) were reported to be significantly higher than the levels of those patients with classic dengue fever (Vitarana et al. 1991). However, our statistical analysis by Wilcoxon 2-sample test of the original data presented in that report revealed that the difference was insignificant, whether or not the value of one DHF patient with the lowest level of TNFα was removed from calculation, as suggested by the authors. In other reports (Yadav et al. 1991, Iyngkaran et al. 1992), concentrations of TNFα were extremely high, often exceeding 1 µg/ml. Those values are highly unusual, because the levels of TNFα in infectious diseases, such as malaria and AIDS, rarely exceed 1 nanogram/ml (Reddy et al. 1988, Kern et al. 1992). In our study, the levels were generally lower than 500 pg/ml.

In our study, TNFα levels were higher in hospitalized children than in outpatient children or in all hospitalized adults. However, a positive association of TNFα levels was not observed among adults. On the other hand, significantly higher levels of IL-6 were associated with both age groups that were hospitalized. In contrast to TNFα and IL-6, levels of IL-1ß were negatively correlated with hospitalization, in particular, among adults. Further, hemorrhagic manifestations and serum cytokine levels were not correlated for any cytokine. Thus, our results generally agree more with the report from Tahiti in which higher levels of TNFα and IL-6 in children were associated with increased severity (grades III and IV of DHF/DSS) but no such an association was observed for IL-1ß (Hober et al. 1993).

One peculiar observation of DHF patients in the Americas, which is markedly different from the situation in southeast Asia, is the higher proportion of adults among DHF patients. In Asia, DHF patients are predominantly children, although DHF/DSS cases in adults have been documented in Asia, too (Rulukruedej 1988). For example, in one study in Thailand (Kitayaporn et al. 1989), DHF morbidity rates between 1983 and 1987 ranged from 198 to 1,203 per 100,000 persons for children (5-9 yrs) and from 4.3 to 31.5 per 100,000 for adults (≥25 yrs). On the other hand, during the 1981 epidemic in Cuba, in one hospital study, 65% of DHF/DSS patients were adults (≥15 yrs) (Guzman et al. 1984), and in the 1989-90 DHF/DSS outbreak in Venezuela, one-third of the fatal cases were adults (Anonymous 1990). Thus, it was of interest to determine if cytokine levels in dengue-infected adults in Puerto Rico were different from those in children. As shown in the results, no significantly higher value was observed for any cytokine among adults as compared with children.

In our study, we evaluated the presence of hemorrhagic manifestations and hospitalization as possible indicators of the severity of dengue infection. We chose these factors, rather than the DHF/DSS grading criteria established by the

World Health Organization (WHO) (World Health Organization 1986), because the number of available serum specimens from Puerto Rico representing each of four WHO grades was insufficient. Despite the subjective character of hospitalization as an indicator of disease severity, it proved to be highly useful, because it was significantly correlated with the levels of IL-6 in both age groups and of TNFα in the hospitalized children without hemorrhage. These results corroborate and reinforce a prior report that disease severity is better correlated with IL-6 level rather than with TNFα level (Dinarello 1992).

Because of the multiple functions of these cytokines and the complexity of interaction among many cytokines and other related molecules, including the lymphokines (Kurane et al. 1989), neither the studies on macrophage-derived cytokines alone nor the analyses on lymphokines alone may explain the severity of dengue infection. Nevertheless, accumulation of data on all relevant cytokines and related mediators may eventually facilitate the elucidation of the mechanism(s) of the manifestation of severe syndromes in dengue infection.

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