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INFLAMMATORY MEDIATORS IN DENGUE VIRUS INFECTION IN CHILDREN: INTERLEUKIN-6 AND ITS RELATION TO C-REACTIVE PROTEIN AND SECRETORY PHOSPHOLIPASE A2

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Abstract. To assess the potential role of interleukin-6 (IL-6) in the pathogenesis of dengue virus infection, levels of this cytokine were measured in children with dengue virus infection on admission to the hospital. As presumed surrogate markers of IL-6, C-reactive protein (CRP) and secretory phospholipase A2 (sPLA2) were measured. Three groups were studied: 33 apparently healthy children as negative controls, 11 children with bacterial infections as positive controls, and 186 children with serologically documented dengue virus infection. One-hundred and fifteen patients had dengue fever (DF) and 71 had dengue hemorrhagic fever (DHF). Compared with healthy controls, dengue shock syndrome (DSS) patients had significantly higher levels of IL-6 on admission ($P < 0.05$), comparable with those in positive controls. Dengue patients with shock had significantly higher levels of IL-6 than normotensive patients ($P < 0.001$) and higher levels of IL-6 were associated with a higher incidence of ascites. C-reactive protein concentrations in dengue patients and in healthy children were not different, but lower than in children with bacterial infections ($P = 0.008$). Secretory phospholipase A2 levels were higher in dengue patients than in apparently healthy children ($P \leq 0.05$) and similar to those in children with bacterial infection. Dengue shock syndrome patients had significantly higher sPLA2 concentrations than normotensive patients ($P = 0.02$). These data indicate that IL-6 and sPLA2 may have a pathogenetic role only in the most severe forms of dengue virus infection.

INTRODUCTION

Dengue is an acute febrile disease resulting from an infection by dengue virus, a member of the family of Flaviridae. Four antigenically distinct subtypes of these RNA-viruses are designated as dengue types 1–4. Transmission involves ingestion of viremic blood by mosquitoes and subsequent passage to a susceptible human host, the principal vector being *Aedes aegypti*.[1] Dengue virus infections can manifest as dengue fever (DF), an uncomplicated febrile illness, or dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), the severe form.[2–5] The essential pathophysiologic features in DHF are increased permeability and abnormal hemostasis, including thrombocytopenia. The severity of DHF is graded according to World Health Organization (WHO) criteria:[6] grade I: fever, accompanied by non-specific constitutional symptoms, and a positive tourniquet test as the only hemorrhagic manifestation; grade II: grade I plus spontaneous bleeding; grade III: circulatory failure manifested by cold and clammy skin, restlessness, rapid and weak pulse, narrowing of pulse pressure, and hypotension; and grade IV: deep shock with undetectable pulse and blood pressure.[3]

In DHF, fluid accumulation in body cavities, thrombocytopenia, and coagulopathy frequently occur. However, the mechanisms by which these pathophysiological changes occur is incompletely understood.[7,8] Some evidence suggests an abnormal immune response and a disturbance in immune regulation as the basis of the pathogenesis.[9] The lack of pathological findings in major organs in most cases of fatal DHF/DSS and rapid recovery of those who survive the critical period, suggest that physiologic dysfunction is secondary to the action of soluble mediators as these are capable of producing severe illness with minimal structural injury.[8,10,11] Macrophages are an important target of dengue virus infection,[2,12] which are known to produce various cytokines upon stimulation.[13,14] In dengue virus infection, cytokines may be released either directly from virus-infected cells such as monocytes/macrophages or upon interactions of virus-infected cells with other immune cells such as activated T lymphocytes.[13,14]

The production of Interleukin 6 (IL-6), a cytokine of central importance can be induced by IL-1 and TNF, and other cytokines. IL-6 stimulates the synthesis of acute-phase proteins such as C-reactive protein (CRP) and secretory phospholipase A2 (sPLA2) type II by the liver.[15–18] Increased plasma levels of IL-6 are present in most patients with various bacterial infections and are related to clinical outcome.[15–18] There are only limited data on the importance of IL-6 in dengue-infected patients.[19–23] These data are somewhat conflicting. Some studies show a correlation between severity of illness and IL-6 levels, i.e., highest levels in DHF/DSS.[19–21] In one large prospective study,[22] IL-6 levels tended to be lower in patients in shock than those not in shock. Also there are discrepancies with respect to the proportion of patients showing elevated IL-6 levels.[19,23] Therefore the potential role of this cytokine in the pathogenesis of severe forms of dengue fever remains unclear. In these previous studies, considering the fast clearance of IL-6 and delays in hospital admission, it is possible that significant IL-6 responses may have been missed. This study was designed to detect IL-6 and indirect parameters for IL-6 production: CRP and sPLA2 production are induced by IL-6 and last longer in serum than IL-6.[24–26] In one study CRP levels were measured in addition to IL-6 levels but their correlation was not assessed.[22] To our knowledge, type II sPLA2 has been measured only in one dengue study, in which elevated levels were present in 90% of these patients. No correlation with other mediators was mentioned.[27]

In the present study we examined circulating levels of IL-6 as well as those of CRP and sPLA2 in children with den-

gue virus infection. The main goal of the study was to analyze whether levels of these circulating inflammatory mediators on admission are related to the severity of illness and the occurrence of complications.

PATIENTS, MATERIALS, AND METHODS

Patients. The children included in the study were admitted to the Department of Pediatrics of the Dr. Sardjito General Hospital in Yogyakarta, Indonesia, between September 1995 and May 1996. Entry criteria were fever lasting for 2–7 days and elevated IgM antibodies with or without detectable IgG antibodies against a dengue virus according to criteria described.[6] A clinical diagnosis of DF or DHF was assigned to the patients who fulfilled the WHO criteria before the results of serologic studies were known. The severity of illness was graded according to the WHO criteria for dengue hemorrhagic fever.[6]

There were two control groups. Eleven patients with a bacterial infection (bacterial meningitis, sepsis, or typhoid fever) were positive controls. The diagnosis of these infections was based on clinical symptoms in combination with bacteriologic examination of blood or cerebrospinal fluid samples, or a positive Widal test, respectively. Apparently healthy children (n = 33) in the outpatient department served as negative controls.

Clinical signs were recorded at the time of phlebotomy. All patients were treated with standard supportive therapy. The protocol was approved by the Medical Ethics Committee of the Faculty of Medicine, Gadjah Mada University, Dr. Sardjito Hospital and by the Medical Ethics Committee of the Academic Hospital Free University, Amsterdam, the Netherlands. Informed consent was obtained from the parent of each patient included in the study.

Blood sampling. Blood was obtained from each patient within 24 hr after admission and in some patients during subsequent days during hospital stay. Blood was collected in tubes that contained EDTA (10 mM/L, final concentration), soybean trypsin inhibitor (100 pg/ml) and benzamidine (10 mM) to prevent *in vitro* activation of plasma cascade systems. The tubes were centrifuged for 10 minutes at 1,300 *g*. Plasma was stored at −70°C and transported to Amsterdam on dry ice.

Laboratory investigations. IgM and IgG antibodies against dengue virus were measured by enzyme linked immunosorbent assay (ELISA).[28] Interleukin-6 (IL-6) was measured with an ELISA (Pelikine IL-6 assay, Dept Immune Reagents; CLB; Amsterdam, the Netherlands) according to the manufacturer's instructions. Levels exceeding 10 pg/ml were considered elevated. C-reactive protein was measured with an ELISA in which a monoclonal antibody against human CRP was the capture antibody and biotinylated purified polyclonal rabbit antibodies against human CRP were the detecting antibodies.[29] Results obtained from plasma samples were compared with those of a commercial CRP standard (Behringwerke AG, Marburg, Germany); a level exceeding 5 mg/L was considered to be elevated. sPLA2 was measured with an ELISA as described previously.[18] A level exceeding 5 ng/ml was considered elevated.[18] Plasma protein was measured with a microcapillary method: heparinized blood was centrifuged for 10 minutes at 1,300 *g*, and the supernatant was analyzed for protein content with a refractometer (Atago SPRN, Atago CO Ltd, Japan).

TABLE 1
Demographics of dengue patients

		Sex (%)		Age (year)	
Group	n	Male	Female	Median	Range
DF	115	52	48	9	<1–14
DHF1	22	68	32	10	4–15
DHF2	20	40	60	8.5	3–14
DHF3	18	33	67	8	3–14
DHF4	11	54	46	7	4–11

DF = dengue fever; DHF = dengue hemorrhagic fever.

Data analysis. Differences between groups with respect to age were assessed by the analysis of variance test. Differences in levels of IL-6, CRP, and sPLA2 between cases and controls as well as between shock and normotensive patients were analyzed by the Wilcoxon-Mann-Whitney U Rank Sum test (WMU). Differences in the proportion of patients with elevated levels of IL-6, CRP, and sPLA2, and the distribution of gender between DF and DHF were analyzed by the Chi-square test. Comparisons of clinical and laboratory variables between groups with normal and those with elevated plasma IL-6, CRP, and sPLA2 were performed using the Chi-square test for proportional data and the Student's *t*-test for continuous data. A two-tailed *P*-value of less than 0.05 was considered to represent a significant difference. A significant number of patients had elevated levels of CRP and sPLA2. A Spearman's rank correlation coefficient was calculated for these variables. Since IL-6 levels were elevated in only a small proportion of patients, the WMU test was performed to test whether patients with elevated IL-6 levels had higher CRP or sPLA2 levels than patients with normal IL-6 levels. All statistical calculations were done using SPSS 6.0 program for Windows 95.

RESULTS

Patients. Two hundred and thirty-five patients with fevers of 2–7 days duration were initially included in this study. Of these, 186 children were confirmed dengue cases. Fifty-three of these were IgM-positive and IgG-negative; 131 patients were IgM-positive and IgG-positive, and two patients were IgM-positive whereas IgG results are missing. Accordingly, 53 patients had a suspected primary infection, 131 patients had a suspected secondary infection, and two patients could not be classified. Seventy-one patients fulfilled the WHO criteria for dengue hemorrhagic fever (DHF): 22 cases had DHF1 (11.8%), 20 cases had DHF2 (10.7%), 18 cases had DHF3 (9.6%), and 11 cases had DHF4 (5.9%). Most of the patients (n = 115) had dengue fever (DF) (61.8%). One patient in the DSS group died, all others survived. The median age (year) and the gender distribution in the various groups are presented in Table 1. There was no statistically significant difference in age and gender distribution between those group ($P = 0.24$, and $P = 0.21$, respectively). The age and gender distribution in both control groups was in the same range as in the children with dengue virus infection.

IL-6 levels. Sufficient plasma was available for 185/186 patients for IL-6 and CRP measurement. Levels of IL-6

TABLE 2
Levels of IL-6, CRP, and sPLA2 on admission in the various groups of dengue patients

Group	IL-6 (pg/ml)			CRP (mg/l)			sPLA2 (ng/ml)		
	n	Median (range)	% elevated	n	Median (range)	% elevated	n	Median (range)	% elevated
Dengue virus infection	185	<10 (<10–108)	13	185	3 (<1–93)	32.4	186	28 (<5–821)*	92.5†
DF	115	<10 (<10–49)	9.6	114	2.6 (<1–55)	28.1	115	25 (<5–821)*	91.3†
DHF1	21	<10 (<10–38)	4.8	22	4.3 (<1–30)	45.5	22	26.5 (<5–290)*	90.9†
DHF2	20	<10 (<10–13)	10	20	4.4 (<1–26)	40	20	35 (8.2–324)*	100†
DHF3	18	<10 (<10–30)*	27.8†	18	2.4 (<1–93)	27.8	18	47 (<5–567)*	88.9†
DHF4	11	<10 (<10–108)*	45.5†	11	4 (<1–48)*	45.5†	11	59 (10–643)*	100†
Bacterial infection	11	10 (<10–48)	54.5	11	27 (<1–117)	81.8	11	42 (<5–410)	90.9
Healthy children	33	<10 (<10–16)	3.3	33	1.1 (<1–40)	30.3	33	<5 (<5–53)	42.4

* $P < 0.05$ for levels of IL-6, CRP and sPLA2 in groups compared to healthy children, and $P > 0.05$ compared to bacterial infection.
† $P < 0.05$ for proportion of elevated levels of IL-6, CRP and sPLA2 in groups compared to healthy children, and $P > 0.05$ compared to bacterial infection.

higher than 10 pg/ml were found in 13% of 185 samples obtained on admission from patients with dengue virus infection (Table 2, Figure 1). In the negative control group 3.3% had elevated levels of IL-6. In contrast, 54.5% of the patients with a bacterial infection had increased levels of IL-6. The more severe the DHF classification, the higher the percentage of patients with increased IL-6 levels (Table 2, Figure 1). The highest levels of IL-6 were found in patients with DHF4. IL-6 was measured the day after admission (Day 2) in 82 patients, and in 34 on Day 3, and were elevated in 13.4% and 14.7%, respectively. Peak levels of IL-6 were usually found on the day of admission and subsequently typically decreased. Levels of IL-6 in patients with shock (DHF3 and DHF4) were significantly higher than in healthy children, and were comparable with those in patients with bacterial infection (Table 2, Figure 1). The plasma levels of IL-6 were higher in the shock group (DHF3, DHF4) than in the normotensive group (DF, DHF1, DHF2); ($P < 0.0001$). Also within the DHF group IL-6 concentrations were higher in patients with shock (DHF3 + DHF4) than in those without shock (DHF1 + DHF2) ($P < 0.05$). However, although IL-6 levels were somewhat higher in the DHF patients than in the DF patients, this did not reach statistical significance.

C-reactive protein levels. On admission, 32.4% of 185 patients with dengue virus infection had elevated levels of CRP. These levels were comparable with those of healthy children (Table 2, Figure 1). Levels of CRP in patients with bacterial infection were higher than those in patients with dengue virus infection except in DHF4 (Table 2, Figure 1). The highest median level of CRP occurred in dengue patients with DHF2. Levels of CRP in patients with shock were similar to those of normotensive patients, but CRP concentrations were higher in the DHF patients than in patients with DF ($P < 0.05$).

Secretory phospholipase A2 levels. Levels of sPLA2 were elevated in 92.5% of all samples from patients with confirmed dengue virus infection on admission (Table 2, Figure 1). In groups DHF2 and DHF4 all patients had elevated levels of sPLA2 (Table 2, Figure 1). However, 42.4% of the healthy children also had elevated levels of sPLA2 (Table 2). Levels as well as the proportion of patients with elevated levels of sPLA2 were significantly higher in all dengue groups than in healthy children and were comparable to those in patients with bacterial infection (Table 2). The highest median levels of sPLA2 occurred in patient group DHF4. Levels of sPLA2 in patients with shock were significantly higher than in normotensive patients ($P = 0.02$). Also, patients with DHF had higher sPLA2 concentrations than those with DF ($P < 0.05$).

Relationships between IL-6 and CRP, sPLA2. We found higher levels of sPLA2 in patients with elevated IL-6 levels than in those with an IL-6 level of less than 10 pg/ml ($P = 0.04$, WMU test). In contrast, there was no difference in CRP levels between patients with elevated and those with normal IL-6 concentrations. There was a positive correlation between levels of CRP and of sPLA2 ($r = 0.4$, $P < 0.01$, Spearman's rank test).

Relationship between IL-6, C-reactive protein, and secretory phospholipase A2 levels with clinical and laboratory parameters. In patients with an elevated IL-6 concentration on admission a higher incidence of ascites and gastrointestinal bleeding was noted during the course of disease than in those with a normal level ($P < 0.05$). Also, the maximal body temperature and leukocyte count measured during the observation period were higher ($P < 0.05$) in patients with an increased IL-6 than in those with a normal concentration. Patients with an elevated CRP level on admission had an increased incidence of gastrointestinal bleeding during the course of their illness compared to those with normal CRP ($P = 0.02$). However, no difference was found concerning other studied clinical and laboratory variables between patients with elevated and those with normal CRP concentrations. Also, the incidence of clinical abnormalities and mean values for maximal heart rate and body temperature as well as for laboratory variables were not different between patients with an elevated sPLA2 and those with a normal sPLA2 level on admission. However, almost all patients (92.5%) had sPLA2 levels considered abnormal in a normal Dutch adult population and nearly half (42.4%) of the presumed healthy children had elevated concentrations. Using another cut-off value (25 ng/ml) we found a statistically significant ($P < 0.05$) higher incidence of ascites, pleural effusion and gastrointestinal bleeding in patients with an elevated sPLA2 ($n = 105$) using this definition. Also, maximal mean heart rate and body temperature, were higher ($P < 0.05$) and minimal platelet count and plasma protein concentration were lower ($P < 0.05$) in patients with an sPLA2 level ≥ 25 ng/ml (data not shown).

Relationship of plasma levels of IL-6, C-reactive protein, and secretory phospholipase A2 and sequential infection. In patients with a suspected primary infection ($n = 53$) 19.6% had elevated levels of IL-6 on admission. This

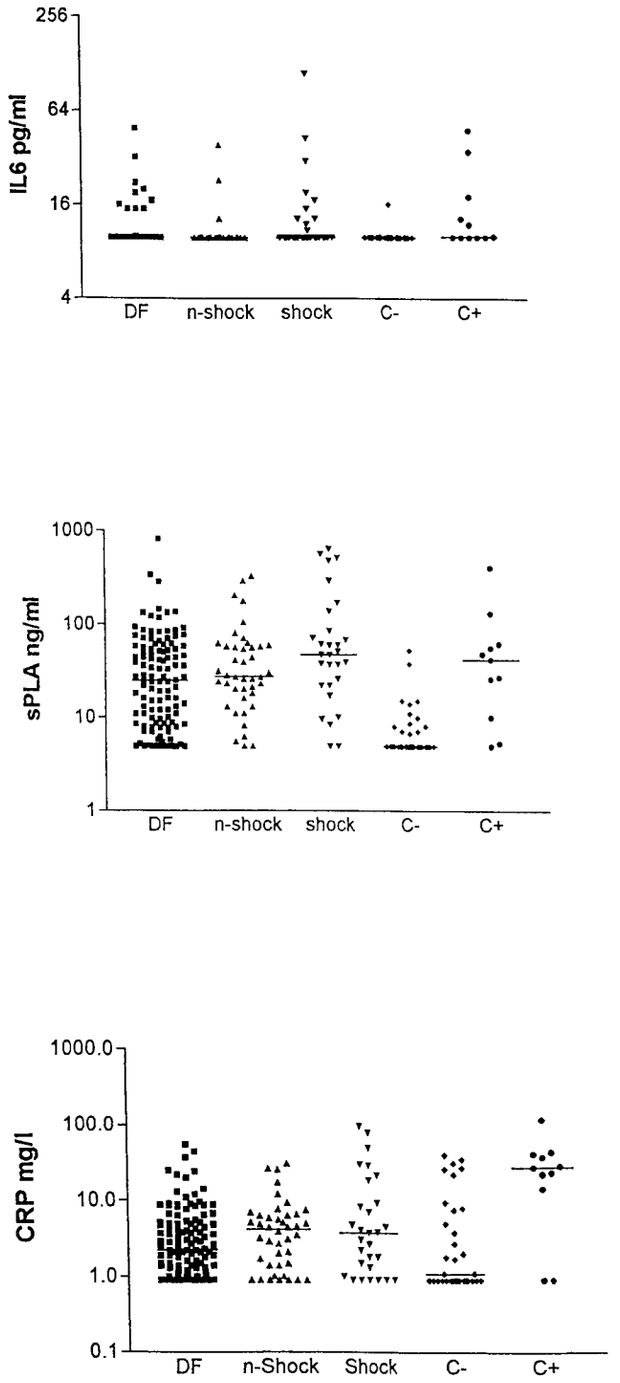

FIGURE 1. Levels on admission of IL-6, CRP, and sPLA2 in children with dengue virus infection and in control groups. DF = dengue fever; DHF = dengue hemorrhagic fever; n-shock = normotensive DHF (DHF1+DHF2); shock: DHF with shock (DHF3+DHF4); C−: negative control; C+: positive control; line indicates median.

was the case in 10.8% of patients with suspected secondary infection (n = 131) and no statistically significant difference was found in plasma IL-6 levels between children with primary or secondary dengue virus infection. Thirty-five percent of patients with primary infection and 32% of those with secondary infection had increased CRP concentrations on admission. Proportions of increased sPLA2 were similar in patients with primary infection (88.5%) and those with secondary infection (94%). Levels of sPLA2 and CRP were similar in both groups.

DISCUSSION

The main goal of this study was to determine whether circulating levels of IL-6 and the IL-6 induced mediators CRP and sPLA2 were associated with the severity of illness and the incidence of complications in dengue virus-infected children. Such knowledge may provide insight into the pathogenetic role of IL-6, since the CRP and sPLA2 are present in blood longer than IL-6.[24–26] In dengue patients, 13% had an elevated IL-6 level on admission, 32.4% elevated CRP levels, and 92.5% elevated sPLA2 levels. IL-6 levels were highest on admission and subsequently declined. IL-6 levels on admission were significantly higher in patients with DSS than in normotensive patients, indicating that IL-6 reflected severity of illness. These findings are consistent with previous results from a study of DHF, where the highest levels of IL-6 were observed in patients with shock.[19] In contrast, another study in 226 children with DHF showed that those with shock tended to have lower levels of IL-6 than normotensive children. After correction for albumin levels as a marker of capillary leakage, no significant correlation between levels of IL-6 and disease severity was found.[22] The maximal level of IL-6 in our study was less than 110 pg/ml. Similar IL-6 levels have been reported in another study in DHF.[19] In comparison with sepsis, the IL-6 levels we found in dengue patients are considerably lower.[15] In addition, in sepsis, by far the majority of patients exhibit elevated IL-6 concentrations.[15] Since only 13% of our dengue patients had only moderately elevated IL-6 levels, it seems that the IL-6 response is relatively mild in the dengue patients reported here. In patients with sepsis the highest concentrations of IL-6 occur at the time of diagnosis and decrease thereafter, independently of outcome.[15,16] Thus, the relatively low levels of IL-6 in our patients may be related to the fact that most patients were not hospitalized before the third day of the disease.

The most important primary targets of dengue virus infection are monocytes.[2,12] Infected monocytes may stimulate the production of mediators such as cytokines including IL-6. IL-6 exerts multiple effects on different types of target cells, but its major biological activities include induction of acute phase proteins, induction of terminal differentiation of B cells and activation of T cells.[30] Among the IL-6-induced acute phase proteins are CRP and sPLA2.[30–32] We found, however, no difference in CRP concentrations between patients with increased IL-6 and those with normal IL-6 levels, whereas levels of sPLA2 were higher in the former than in the latter group. It is possible that the generation of IL-6 was not abundant enough to generate high levels of CRP, which were elevated in only about one-third of the dengue patients and not different between DSS and non-shock patients. sPLA concentrations were increased in the majority of dengue patients. Also in other studies elevated levels of sPLA2 have been found in 90% of dengue-infected patients.[27]

DSS patients had higher sPLA2 levels than dengue patients without circulatory failure. This finding suggests that

sPLA2 levels reflect, to some extent, severity of illness. Patients with septic shock have higher circulating sPLA2 concentrations than normotensive septic patients.[33] Although the sPLA2 levels in our dengue patients were moderately increased compared to those found in septic shock patients,[34] our findings may indicate, similar to sepsis, that this mediator could be involved in the pathogenesis of circulatory failure in dengue. Interpretation of the sPLA2 data is, however, confounded by the finding that in almost half of the apparently healthy Indonesian children, elevated sPLA2 levels were present. In addition, other mediators such as interferon-gamma (IFN-γ) are involved in the induction of sPLA2 synthesis.[18] High levels of IFN-γ have been reported in patients with DHF.[35] Since 10 out of 33 apparently healthy children had an increased CRP level, it seems likely that this group of children could have chronic immune stimulation, such as parasite infestations which are very common in the area where the study was performed. This speculation could also account for higher sPLA2 levels.

Individuals who have a second dengue infection with a different serotype are at significant risk for developing DHF or DSS.[1,36–39] However, some patients with primary dengue infection develop DHF or DSS.[40,41] A minority (9%) of our patients with DHF or DSS had primary dengue. There were no statistically significant differences in plasma levels of IL-6, CRP, or sPLA2 between patients with a first or subsequent dengue infection. Rather, shock occurred in both groups (although more frequently in patients with a second dengue infection) and the highest levels of those mediators were found in patients with shock. These data suggest that sequential infection by itself has no effect on the levels of IL6, of CRP, and of sPLA2 in dengue virus infection.

An important issue is the mechanism by which these mediators could potentially cause tissue injury. IL-6 has both anti- and pro-inflammatory properties, including the induction of CRP and sPLA2 synthesis which both are involved in the pathogenesis of sepsis.[30] There is experimental evidence that IL-6 is a factor in the activation of coagulation in endotoxemia.[42] It remains speculative whether IL-6 could be involved in the coagulation abnormalities that occur in DHF. In this study, patients with elevated IL-6 concentrations had a significantly higher incidence of ascites. Since plasma leakage is an important pathogenetic mechanism in the development of shock, this observation is consistent with the hypothesis that IL-6 contributes to circulatory failure in dengue. Because hospital admissions are often delayed, the highest IL-6 levels may have been missed. IL-6, most likely exerts pro-inflammatory effects by induction of mediators such as sPLA2. Phospholipase A2 plays a role in phospholipid digestion, host defense, and signal transduction.[43,44] High circulating levels of sPLA2 have been found in patients with various inflammatory states such as acute respiratory distress syndrome, septic shock, experimental endotoxemia, and multiple organ failure.[45–48] High levels of sPLA2 can induce lung injury,[49] and promote platelet-activating factor or eicosanoid production that mediate tissue injury,[48–50] and could contribute to circulatory failure in septic shock.[34] Recently, it has been shown that sPLA2 is involved in the production of pro-inflammatory cytokines and nitric oxide.[51] Patients with the highest sPLA2 levels had a higher incidence of ascites and pleural effusion and lower plasma protein concentrations than patients with lower sPLA2 levels. Although our findings only represent associations, they are consistent with the hypothesis that sPLA2 could be involved in the pathogenesis of plasma leakage.

The findings in this study indicate that the highest levels of IL-6 and sPLA2 are found in DSS patients. In patients with elevated IL-6 levels the incidence of ascites was increased. In patients with the highest sPLA2 levels, the incidence of both pleural effusion and ascites was increased, both of which are markers of plasma leakage. Circulating levels of both IL-6 and sPLA2 are correlated with severity of illness and the occurrence of complications, and suggest that IL-6 and sPLA2 are involved in the pathogenesis of severe dengue.

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REFERENCES

1. Henchal EA, Putnak JR, 1993. The dengue viruses. *Clin Microbiol Rev 3:* 376–396.
2. Halstead SB, 1980. Immunological parameters of togavirus disease syndrome. Schlesinger RW, ed. *The Togavirus: Biology, Structure, Replication.* New York: Academic Press, 107–173.
3. Halstead SB, 1992. The XXth century dengue pandemic: need for surveillance and research. *World Health Stat Q 45:* 292–298.
4. Krishnamurti C, Alving B, 1989. Effect of dengue virus on procoagulant and fibrinolytic activities of monocytes. *Rev Infect Dis 11* (*suppl 4*)*:* 843–846.
5. Shekhar KC, Huat OL, 1992/1993. Epidemiology of dengue/dengue hemorrhagic fever in Malaysia—a retrospective epidemiological study 1973–1987. Part I: dengue hemorrhagic fever (DHF). *Asia Pac Public Health 6:* 15–25.
6. World Health Organization, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control*. Geneva: World Health Organization.
7. Halstead SB, 1989. Antibody, macrophages, dengue virus infection, shock and hemorrhage: a pathogenetic cascade. *Rev Infect Dis 11* (*suppl 4)*: S830–S839.
8. Pang T, 1989. Pathogenesis of dengue haemorrhagic fever: current perspectives. *Adv Exp Med Biol 257:* 155–168.
9. Halstead SB, 1988. Pathogenesis of dengue: challenges to molecular biology. *Science 239:* 476–481.
10. Bhamarapravati N, 1989. Hemostatic defects in dengue hemorrhagic fever. *Rev Infect Dis 11* (suppl 4)*:* 826–829.
11. Kurane I, Ennis FA, 1992. Immunity and immunopathology in dengue virus infections. *Sem Immunol 4:* 121–127.
12. Hayes EB, Gubler DJ, 1992. Dengue and dengue hemorrhagic fever. *Pediatr Infect Dis J 11:* 311–317.
13. Cosgriff M, 1991. Mechanisms of disease in hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. *Rev Infect Dis 13:* 97–107.
14. Nathan CF, 1987. Secretory products of macrophages. *Clin Invest 79:* 319–323.

15. Hack CE, de Groot ER, Felt-Bersma RJF, Nuijens JH, Strack van Schijndel RJM, Eerenberg-Belmer AJM, Thijs LG, Aarden LA, 1989. Increased plasma levels of interleukin-6 in sepsis. *Blood 74:* 1704–1710.
16. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T, 1989. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. *J Exp Med 169:* 333–338.
17. Helfgott DC, Tatter SB, Santhanam U, Clarick RH, Bhardwaj N, May LT, Sehgal PB, 1989. Multiple forms of IFN-B2/IL-6 in serum and body fluids during acute bacterial infection. *J Immunol 142:* 948–953.
18. Wolbink GJ, Schalkwijk C, Baars JW, Wagstaff J, Van de Bosch H, Hack CE, 1995. Therapy with interleukin-2 induces the systemic release of phospholipase-A2. *Cancer Immunol Immunother 41:* 287–292.
19. Hober D, Poli L, Roblin B, Gestas P, Chungue E, Granic G, Imbert P, Pecarere JL, Vergez-Pascal R, Wattre P, 1993. Serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) in dengue-infected patients. *Am J Trop Med Hyg 48:* 324–331.
20. Kuno G, Bailey RE, 1994. Cytokine responses to dengue infection among Puerto Rican patients. *Mem Inst Oswaldo Cruz 89:* 179–182.
21. Iyngkaran N, Yadav M, Sinniah M, 1995. Augmented inflammatory cytokines in primary dengue infection progressing to shock. *Singapore Med J 36:* 218–221.
22. Bethell DB, Flobbe K, Cao XT, Day NP, Pham TP, Buurman WA, Cardosa MJ, White NJ, Kwiatkowski D, 1998. Pathophysiologic and prognostic role of cytokines in dengue hemorrhagic fever. *J Infect Dis 177:* 778–782.
23. Pinto LM, Oliveira SA, Braga EL, Nogueira RM, Kubelka CF, 1999. Increased pro-inflammatory cytokines (TNF-alpha and IL-6) and anti-inflammatory compounds (sTNFRp55 and sTNFRp75) in Brazilian patients during exanthematic dengue fever. *Mem Inst Oswaldo Cruz 94:* 387–394.
24. Weber J, Gunn H, Yang J, Parkinson D, Topalian S, Schwartzentruber D, Ettinghausen S, Levitt D, Rosenberg SA, 1994. A phase I trial of intravenous Interleukin-6 in patients with advanced cancer. *J Immunother 15:* 292–302.
25. Vigushin DM, Pepys MB, Hawkins PN, 1993. Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. *J Clin Invest 91:* 1351–1357.
26. Vadas P, Pruzanski W, Farewell V, 1991. A predictive model for the clearance of soluble phospholipase A2 during septic shock. *J Lab Clin Med 118:* 471–475.
27. Nevalainen TJ, Losacker W, 1997. Serum phospholipase A2 in dengue. *J Infect 35:* 251–252.
28. Velzing J, Groen J, Drouet MT, Van Amerongen G, Copra C, Osterhaus AD, Denbel V, 1999. Induction of protective immunity against dengue virus type 2: comparison of candidate live attenuated and recombinant vaccines. *Vaccine 17:* 1312–1320.
29. Wolbink GJ, Bossink AW, Groeneveld ABJ, de Groot MC, Thijs LG, Hack CE, 1998. Complement activation in patients with sepsis is in part mediated by C-reactive protein. *J Infect Dis 177:* 81–87.
30. Hack CE, Aarden LA, Thijs LG, 1997. Role of cytokines in sepsis. *Adv Immunol 66:* 101–195.
31. Mold C, Du Clos TW, Nkayama S, Edwards KM, Gewurz H, 1982. C-reactive protein: reactivity with complement and effects on phagocytosis. *Ann NY Acad Sci 389:* 251–259.
32. Hack CE, Wolbink GJ, Schalkwijk C, Speijer H, Hermens WT, Van de Bosch H, 1997. A role for secretory phospholipase A2 and C-reactive protein in the removal of injured cells. *Immun. Today 18:* 111–115.
33. Vadas P, Pruzanski W, 1993. Induction of group II phospholipase A2 expression and pathogenesis of the sepsis syndrome. *Circ Shock 39:* 160–167.
34. Vadas P, Pruzanski W, Stefanski E, Sternby B, Mustard R, Bohnen J, Fraser I, Farewell V, Bombardier C, 1988. Pathogenesis of hypotension in septic shock: correlation of circulating phospholipase A_2 levels with circulatory collapse. *Crit Care Med 16:* 1–7.
35. Green S, Vaughn DW, Kalayanarooj S, Nimmannitya S, Suntayakorn S, Nisalak A, Lew R, Innis BL, Kurane I, Rothman AL, Ennis FA, 1999. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. *J Infec Dis 179:* 755–762.
36. Halstead SB, 1970. Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypothesis and discussion. *Yale J Biol Med 42:* 350–362.
37. Kliks SC, Nisalak A, Brandt WE, Wahl L, Burke DC, 1989. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *Am J Trop Med Hyg 40(4):* 444–451.
38. Sangkawibha N, Rojanasuphot S, Ahandrik S, Viriyapongse S, Jatanasen S, Salitul V, Phanthumachinda B, Halstead SB, 1984. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand I. The 1980 outbreak. *Am J Epidemiol 120:* 653–669.
39. Gubler DJ, Reed D, Rosen L, Hitchcock JC, 1978. Epidemiologic, clinical, and virologic observations on dengue in the Kingdom of Tonga. *Am J Trop Med Hyg 27:* 581–589.
40. Morens DM, Sather GE, Gubler DJ, Rammohan M, Woodall JP, 1987. Dengue shock syndrome in an American traveler with primary dengue 3 infection. *Am J Trop Med Hyg 36(2):* 424–426.
41. Scott RM, Nimmannitya S, Bancroft WH, Mansuwan P, 1976. Shock syndrome in primary dengue infections. *Am J Trop Med Hyg 25(6):* 866–874.
42. van der Poll T, Levi M, Hack CE, ten Cate H, van Deventer SJ, Eerenberg AJ, de Groot ER, Jansen J, Gallati H, Buller HR, 1994. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. *J Exp Med 179:* 1253–1259.
43. Balsinde J, Balboa MA, Insel PA, Dennis EA, 1999. Regulation and inhibition of phospholipase A_2. *Annu Rev Pharmacol Toxicol 39:* 175–189.
44. Murakami M, Nakatani Y, Atsumi GI, Inoue K, 1997. Regulatory function of phospholipase A_2. *Crit Rev Immunol 17:* 225–283.
45. Romaschin AD, DeMsjo WC, Winton T, Costa M, Chang G, Rubin B, Gamliel Z, Walker PM, 1992. Systemic phospholipase A_2 and cachectin levels in adult respiratory distress and multiple organ failure. *Clin Biochem 25:* 55–60.
46. Vadas P, Scott K, Smith G, Rajkovic E, Stefanski E, Schouten BD, Singh R, Prucanski W, 1992. Serum phospholipase A_2 enzyme activity and immunoreactivity in a prospective analysis of patients with septic shock. *Life Sci 50:* 807–811.
47. Santos AA, Browning JL, Scheltinga MR, 1994. Are events after endotoxemia related to circulating phospholipase A_2? *Ann Surg 219:* 183–192.
48. Nyman KM, Uhl W, Forssman J, 1996. Serum phospholipase A_2 in patients with multiple organ failure. *J Surg Res 60:* 7–14.
49. Edelson JD, Vadas P, Villar J, Mullen JBM, Pruzanski W, 1991. Acute lung injury induced by phospholipase A2. *Am Rev Respir Dis 143:* 1102–1109.
50. Zhou W, McCollum MO, Levine BA, Olson MS, 1992. Role of PAF in pancreatitis-associated acute lung injury in the rat. *Am J Pathol 140:* 971–979.
51. Baek SH, Yun SS, Kwon TK, Kim JR, Chang HW, Kwak JY, Kim JH, Kwun KB, 1999. The effects of two new antagonists of secretory PLA_2 on TNF, iNOS, and COX-2 expression in activated macrophages. *Shock 12:* 473–478.