The Relationship between Plasminogen Activator Inhibitor-1 and Proinflammatory and Counterinflammatory Mediators in Children with Meningococcal Septic Shock

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Proinflammatory cytokines (tumor necrosis factor [TNF]-α and interleukin [IL]-6 and -8), counterinflammatory compounds (IL-10 and soluble TNF receptors p55 and p75 [sTNFR-55 and -75]), and hemostatic parameters were determined in 38 patients with meningococcal septic shock. Eleven patients (29%) died. Serum levels of pro- and counterinflammatory compounds and plasma levels of plasminogen activator inhibitor (PAI)-1 were significantly higher in nonsurvivors. The interval between appearance of petechiae and blood sampling was shorter in nonsurvivors than in survivors (3.6 ± 2.4 vs. 6.1 ± 3.3 h; $P = .04$). This interval correlated strongly with the levels of TNF-α, IL-6, -8, and -10, sTNFR-55 and -75, and PAI-1. However, with the exception of PAI-1, differences between concentrations of these mediators disappeared after adjustment for the interval. PAI-1 levels correlated with TNF-α concentrations ($r = .75$; $P < .001$) and were 1.9 ($P = .01$) times higher in nonsurvivors at a similar TNF-α concentration. Thus, an increased PAI-1 response to TNF-α may be associated with fatality, probably because of polymorphism of the PAI-1 gene.

Septic shock with purpura is a serious life-threatening disease in otherwise healthy children and young adults. The syndrome is most frequently due to *Neisseria meningitidis,* although occasionally *Haemophilus influenzae* type b is involved [1–6].

Lipopolysaccharide (endotoxin), a component of the outer membrane of gram-negative bacteria, induces the release of proinflammatory cytokines (tumor necrosis factor [TNF]-α and interleukin [IL]-1β, -6, and -8) in patients with sepsis. Subsequently, endotoxins and cytokines stimulate the production of a wide range of additional inflammatory mediators (i.e., arachidonic acid metabolites, complement, platelet-activating factor), influence the function of leukocytes and endothelial cells, and activate hemostasis [7–11]. The production of proinflammatory cytokines and the extent of the inflammatory response is downregulated by counterinflammatory compounds, such as IL-10, and naturally occurring antagonists of TNF-α, including the soluble (s) extracellular domains of the 55- and 75-kDa membrane-bound TNF receptors (sTNFR-55 and -75).

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The Medical Ethics Committee, University Hospital Rotterdam, approved the study protocol. Written informed consent was obtained from the parents or legal representatives of all subjects.

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IL-10 is produced by activated monocytes and suppresses the endotoxin-induced production of TNF-α and IL-1β, -6, and -8 [12]. The biologic activity of TNF-α is also neutralized by sTNFR-55 and -75 [13–17]. sTNFR is shed from the cell surface of, for example, polymorphonuclear cells in response to many of the same inflammatory stimuli that induce TNF-α [18].

Endothelial cells are among the principal targets for the action of endotoxin, TNF-α, and IL-1β. These cells change to a procoagulant state and can modulate the fibrinolytic system by secretion of tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor (PAI)-1, which respectively activate and inhibit fibrinolysis. The activation of coagulation together with the inhibition of the fibrinolysis are responsible for the development of a hypercoagulable state, fibrin deposition, and microthrombi [19]. Fibrin deposition and complement activation cause extensive endothelial damage and are associated with multiple organ failure [20–22].

Inflammatory mediators and coagulation disorders are involved in the pathophysiology of septic shock and should be associated with disease severity. Thus, we investigated the balance between proinflammatory cytokines (TNF-α, IL-6, IL-8) and counterinflammatory compounds (IL-10, sTNFR-55, sTNFR-75) in admission serum samples of 38 consecutive children with meningococcal septic shock (MSS) and studied their relationship with indicators of hemostasis.

Methods

Patients

We prospectively recruited patients between ages 3 months and 18 years with septic shock and petechiae or purpura. Primary or

secondary referrals were admitted to the pediatric intensive care unit (PICU), Sophia Children's Hospital, between October 1991 and September 1994. Patients were eligible for inclusion if they met the following criteria: presence of petechiae or purpura for <12 h and presence of shock (systolic blood pressure <75, <80, <85, or <100 mm Hg in children ages 3 months to 1 year or 1–5, 6–12, and >12 years old, respectively). Children were also included when poor end-organ perfusion was present (defined as occurrence of at least two of the following criteria): (1) unexplained metabolic acidosis (pH ≤7.3), base excess −5 mmol/L or lower, or arterial plasma lactate levels >2.0 mmol/L; (2) arterial hypoxia (Po_2 ≤75 mm Hg, Po_2-to-Fio_2 ratio <250, or Tco_2 ≤96% in patients without overt cardiopulmonary disease); (3) acute renal failure (oliguria with urine output <0.5 mL/kg/h for >1 h despite acute volume loading or evidence of adequate intravascular volume and without preexistent renal disease); or (4) sudden deterioration of the patient's mental status. Most of the patients were enrolled in a randomized, placebo-controlled trial to study the efficacy of HA-1A human monoclonal antibody (Centoxin; Centocor, Malvern, PA) in MSS. Initial blood samples were obtained before administration of HA-1A or placebo. There was no selection in the HA-1A trial to bias the present study.

On PICU admission, the severity of illness was assessed using the pediatric risk for mortality (PRISM) score, a severity-of-illness index [23]. Parents were asked to indicate the onset of symptoms as precisely as possible. The time of onset of petechiae was defined as the mean time between observation of the child with and without petechiae. The times of initiation of antibiotic treatment, PICU admission, and blood sampling were carefully registered by the investigators. Decisions regarding the use of antibiotics, intravenous fluids, inotropic and vasopressor support, and initiation of mechanical ventilation were made by the patient's attending physician.

Laboratory Studies

Cerebrospinal fluid and blood specimens were routinely cultured. Blood samples for the determination of biochemical parameters, TNF-α, IL-6, -8, and -10, and sTNFR-55 and -75 were obtained from an arterial catheter and collected into sterilized siliconized glass tubes (Vacutainer; Becton Dickinson, Meylan, France) and allowed to clot at room temperature. The samples were centrifuged at 1600 *g* for 10 min at 4°C. Aliquots were stored at −80°C until assayed. Blood for the platelet and leukocyte counts was collected in a Microtainer containing EDTA(K_2). Blood for the coagulation and fibrinolysis assays was collected in 0.109 *M* trisodium citrate (anticoagulant to blood 1:9 vol/vol) and in a 0.109 *M* trisodium citrate mixture (theophylline, adenosine, dipyridamole; Diatube H, Diagnostica Stago, Asnières-sur-Seine, France). These samples were immediately chilled on ice and centrifuged at 2800 *g* for 15 min and then at 45,000 *g* for 30 min at 4°C. Platelet and white blood cell counts were determined by flow cytometer (H1 system; Technicon Instruments, Tarrytown, NY); C-reactive protein (CRP) was measured by immunonephelometric assay [24].

Cytokines and inhibitors. Levels of TNF-α, IL-6, -8, and -10, and sTNFR-55 and -75 in serum were determined by ELISA (Medgenix, Fleurus, Belgium). Mediators were determined according to the manufacturer's instructions with the following detection limits (lowest positive standard): TNF-α, 15 pg/mL; IL-6, -8, and -10, respectively, 30, 7, and 11 pg/mL; and sTNF-55 and -75, respectively, 0.4 and 1.0 ng/mL.

Parameters of coagulation and fibrinolysis. All assays were done with commercially available reagents and methods. Clotting assays were used for the determination of the activated partial thromboplastin time (aPTT). Factor V was determined with a one-stage assay using factor V–deficient plasma and fibrinogen according to the Clauss method [25] (Behringwerke, Marburg, Germany). Antithrombin III (ATIII) activity and protein C activity were determined by chromogenic substrate assays (Behringwerke). Total protein S was measured by ELISA (Diagnostica Stago). Plasminogen was determined spectrophotometrically using a chromogenic synthetic substrate (Behringwerke). Plasma tPA antigen concentration was measured by ELISA as described [26] as was PAI-1 (Diagnostica Stago). A semiquantification of fibrin-fibrinogen degradation products (FDP) in plasma was done by latex agglutination (Diagnostica Stago).

Disseminated intravascular coagulation (DIC) was defined by the combination of three of the following features: platelet count $<150 \times 10^9$/L, fibrinogen <2 g/L, factor V <60%, and the presence of FDP [27].

Statistical Analysis

Results are expressed as mean ± SD unless stated otherwise. Differences between groups of variables were tested by the Mann-Whitney test. Frequencies of various findings between groups were compared by Fisher's exact test. Pearson's correlation coefficient was used to evaluate the relation between specific variables. Multiple regression analysis was done to evaluate factors that might affect the difference in levels of mediators between survivors and nonsurvivors. Two-tailed *P* values ≤.05 were considered statistically significant.

Results

During the 3-year study period, 43 patients with septic shock and purpura were admitted to the PICU. Five patients did not fulfill the entry criteria: 3 had purpura for >12 h before admission, 1 was <3 months old, and informed consent was not obtained for 1 child.

Patient characteristics. Thirty-eight patients (23 boys, 15 girls) entered the study. Of these, 36 participated in the clinical trial to study the efficacy of HA-1A human monoclonal antibody. The median age was 4.1 years (range, 0.7–17.9). Twenty-nine patients were referred from another hospital. The PRISM scores at PICU admission ranged from 1 to 25. Cultures of blood, cerebrospinal fluid, or skin biopsies from 34 children grew *N. meningitidis.* In 4 cases with sterile cultures, the diagnosis was made on the basis of typical clinical findings. None of the patients received antibiotic treatment before or during transport to the hospital. The duration (mean ± SD) of symptoms and the interval between the appearance of petechiae and admission to Sophia Children's Hospital were 17.4 ± 7.2 and 5.4 ± 3.3 h, respectively. All patients needed inotropic and

Table 1. Characteristics of patients with meningococcal septic shock.

Characteristic	Survivors (n = 27)	Nonsurvivors (n = 11)	P
Age (years)	7.3 ± 5.7	5.1 ± 3.9	.43
Sex (% male)	16 (59)	7 (64)	1.0
Transferrals*	22 (82)	7 (64)	.40
Interval (h) from			
Onset of symptoms to PICU admission	18.5 ± 7.7	14.4 ± 4.8	.09
Appearance to petechiae to PICU admission	6.1 ± 3.3	3.6 ± 2.4	.04
Duration of antibiotic treatment (h)	4.7 ± 1.8	2.8 ± 1.8	.10
PRISM score	8.6 ± 5.4	18.6 ± 5.5	<.001
Clinical hematology, white blood cells ($\times 10^9$/L)	15.1 ± 10.3	5.4 ± 3.2	.004
Clinical chemistry			
Creatinine (μmol/L)	102 ± 68	135 ± 65	.08
C-reactive protein (mg/L)	131 ± 60	81 ± 43	.02
Microbiology			
N. meningitidis	25 (93)	9 (82)	.56
No growth	2 (7)	2 (18)	

NOTE. Data are mean ± SD or no. (%). PICU, pediatric intensive care unit; PRISM, pediatric risk for mortality score.

* Patients transferred from first institution to PICU, Sophia Children's Hospital.

vasopressor support. Twenty-five of the 38 patients required mechanical ventilation. The overall fatality rate was 29%.

Table 1 shows the demographic and clinical characteristics of the 27 survivors and 11 fatalities. Survivors and nonsurvivors were evenly distributed in regard to time of onset of petechiae and time of hospitalization. The interval between onset of petechiae and PICU admission was significantly shorter in nonsurvivors. Serum CRP levels were also significantly lower in nonsurvivors than in survivors and were highly correlated with the interval between the onset of symptoms and petechiae and the moment of blood sampling (r = .56, P < .001 and r = .45, P = .005, respectively).

The parameters of coagulation and fibrinolysis are summarized in table 2. DIC was observed in all nonsurvivors and in 13 (48%) of the 27 survivors (P = .003). The aPTT was significantly more prolonged in those who did not survive. The inhibitors of coagulation (ATIII, protein C, and protein S) were generally decreased, but more so in the nonsurvivors. Plasminogen levels were similar in survivors and nonsurvivors. The tPA, PAI-1 antigen, and FDP levels were higher in nonsurvivors than in survivors.

Proinflammatory cytokines and counterinflammatory compound levels at admission. At admission, serum levels of proinflammatory cytokines and counterinflammatory compounds were significantly higher in the patients who subsequently died (table 3). Highly significant positive correlations were observed between all of these mediators (table 4). In addition, serum cytokine levels were negatively correlated with the interval between the appearance of petechiae and blood sampling (P < .001 for all; TNF-α: r = −.55; IL-6: r = −.57; IL-8: r = −.58; IL-10: r = −.59; figure 1). Multiple regression analysis for the relation between serum cytokine levels and survival and duration of skin lesions showed that the time-adjusted concentrations of the cytokines TNF-α and IL-6, -8, and -10 were not significantly higher in children who died versus survivors. sTNFR-55 and -75 were significantly higher in nonsurvivors and also correlated with the interval between the onset of petechiae and initial serum measurements (sTNFR-55: r = −.36, P = .03; sTNFR-75: r = −.61, P < .001). Both sTNFRs remained markedly elevated during the first 24 h after hospitalization (data not shown).

Table 2. Coagulation and fibrinolysis data in patients with meningococcal septic shock.

Characteristic	Reference range	Survivors (n = 27)	Nonsurvivors (n = 11)	P
Coagulation				
Platelets ($\times 10^9$/L)	150–450	120 ± 45	65 ± 37	.002
aPTT* (s)	28–40	52 (33–>200)	92 (58–200)	<.001
Factor V (%)	70–140	43 ± 23	22 ± 12	.007
ATIII activity (%)	80–120	66 ± 14	51 ± 12	.01
Protein C activity (%)	70–140	21 ± 11	17 ± 8	.08
Protein S total (%)	65–108	57 ± 18	41 ± 10	.006
Fibrinogen* (g/L)	1.8–3.5	2.6 (<0.4–5.3)	1.2 (<0.4–2.5)	.005
Fibrinolysis				
Plasminogen (%)	75–140	62 ± 13	53 ± 14	.10
TPA antigen (ng/mL)	<10	25 ± 14	35 ± 19	.13
PAI-1 antigen (ng/mL)	4–40	971 ± 848	2500 ± 1390	<.001
FDP* (mg/L)	<5	70 (20–>300)	120 (100–220)	.02

NOTE. Data are mean ± SD unless specified otherwise. aPTT, activated partial thromboplastin time; ATII, antithrombin III; TPA, tissue-type plasminogen activator; PAI, plasminogen activator inhibitor; FDP = fibrin-fibrinogen degradation products.

* Median (range).

Table 3. Serum levels of cytokines and soluble (s) tumor necrosis factor (TNF) receptors (R) in patients with meningococcal septic shock.

	Survivors (n = 27)	Nonsurvivors (n = 11)	P
TNF-α (pg/mL)	144 (35–3130)	450 (74–2680)	.03
IL-6 (pg/mL)	107,600 (2990–4,515,000)	1,081,000 (25,310–5,758,000)	.005
IL-8 (pg/mL)	746 (31–113,100)	30,760 (599–118,500)	.005
IL-10 (pg/mL)	1479 (68–20,440)	14,780 (636–28,070)	.01
sTNFR-55 (ng/mL)	15.5 (6.2–32.3)	27.2 (8.5–36.6)	.05
sTNFR-75 (ng/mL)	51.2 (22.5–149.4)	79.6 (10.0–119.7)	.04

NOTE. Data are median (range). IL, interleukin.

Serum cytokine levels were also associated with the duration of antibiotic treatment (TNF-α: $r = -.40$, $P = .02$; IL-6: $r = -.37$, $P = .03$; IL-8: $r = -.33$, $P = .05$; IL-10: $r = -.39$, $P = .02$). However, these associations were weaker than those with the duration of petechiae. The duration of petechiae and the duration of antibiotic treatment were significantly correlated ($r = .50$, $P = .002$). When these time intervals were simultaneously related by multiple regression analysis to the levels of mediators, the duration of petechiae was most predictive ($P < .005$ for TNF-α, IL-6, IL-8, IL-10) while an additional significant predictive value was not observed for the duration of antibiotic treatment.

Correlation between inflammatory cytokines and clinical features. The relationship between cytokines and certain hematologic parameters was assessed. The peripheral white blood cell count and the CRP level were negatively correlated with levels of TNF-α ($r = -.59$, $P < .001$ and $r = -.46$, $P = .004$), IL-6 ($r = -.67$, $P < .001$ and $r = -.62$, $P < .001$), and IL-8 ($r = -.68$, $P < .001$ and $r = -.62$, $P < .001$) and with the interval between the onset of petechiae and blood sampling ($r = .54$, $P < .001$ and $r = -.45$, $P = .005$). Initial serum TNF-α levels correlated significantly with the aPTT ($r = .47$, $P = .003$) and the concentrations of factor V ($r = -.51$, $P < .001$), tPA ($r = .63$, $P < .001$), and PAI-1 ($r = .75$, $P < .001$). PAI-1 levels were significantly higher in nonsurvivors than in survivors (2500 ± 1390 vs. 971 ± 848 ng/mL; $P < .001$), even when adjusted for duration of skin lesions before blood sampling ($P = .02$; figure 2). Of interest, PAI-1 concentrations were 1.9 times higher in nonsurvivors ($P = .01$) than in survivors at similar TNF-α serum levels as shown by analysis of covariance (figure 3). This relationship between the levels of PAI-1 and TNF-α was not affected by their association with the time interval (partial $r = .60$, $P < .001$).

Table 4. Correlation between serum levels of cytokines and soluble (s) receptors (R) for tumor necrosis factor (TNF) in patients with meningococcal septic shock.

	TNF-α	IL-6	IL-8	IL-10	sTNFR-55
IL-6	.90	—			
IL-8	.90	.92	—		
IL-10	.79	.85	.89	—	
sTNFR-55	.82	.84	.80	.82	—
sTNFR-75	.87	.82	.78	.75	.77

NOTE. Probabilities for all correlations were <.001. IL, interleukin.

Discussion

Systemic meningococcal disease has a wide spectrum of severity, ranging from benign meningococcemia to fulminant septic shock with multiple organ failure and death. TNF-α and IL-1β are thought to play a central role in the pathophysiology of this disease. These cytokines are involved in the induction of other proinflammatory cytokines, such as IL-6 and -8, and are involved in the activation of the coagulation and fibrinolysis. Our study confirms the findings of other investigators that disease severity and outcome are related to concentrations of TNF-α, IL-6, -8, and -10, and sTNFR-55 and -75 [4, 28–33].

A wide interindividual variability in TNF-α release after stimulation by endotoxin has been demonstrated in vitro in whole blood samples and peripheral blood mononuclear cells isolated from healthy volunteers [34, 35]. The high initial TNF-α levels in those who do not survive MSS have been interpreted as the result of an exaggerated response to circulating endotoxin [36]. This production of inappropriately large quantities of TNF-α may be due to the presence of a genetic variant in the promotor region of the TNF gene (*TNF2* allele) as previously observed in patients with cerebral malaria [37]. The *TNF2* allele has been associated with higher constitutive expression and greater secretion of TNF-α after induction [38].

The initial concentrations of TNF-α in this and previous studies were significantly higher in MSS nonsurvivors than in survivors. However, the magnitude of TNF-α serum levels and of other proinflammatory cytokines (e.g., IL-6 and -8) is also determined by the duration of disease when blood samples are obtained, perhaps because these cytokines rapidly disappear during the acute phase of septic shock. [29, 39–41]. Accordingly, we found a strong negative correlation between initial cytokine levels and the interval between onset of purpuric skin lesions and blood sampling. This association is in contrast to previous reports [4, 32, 39]. In addition, in the present study,

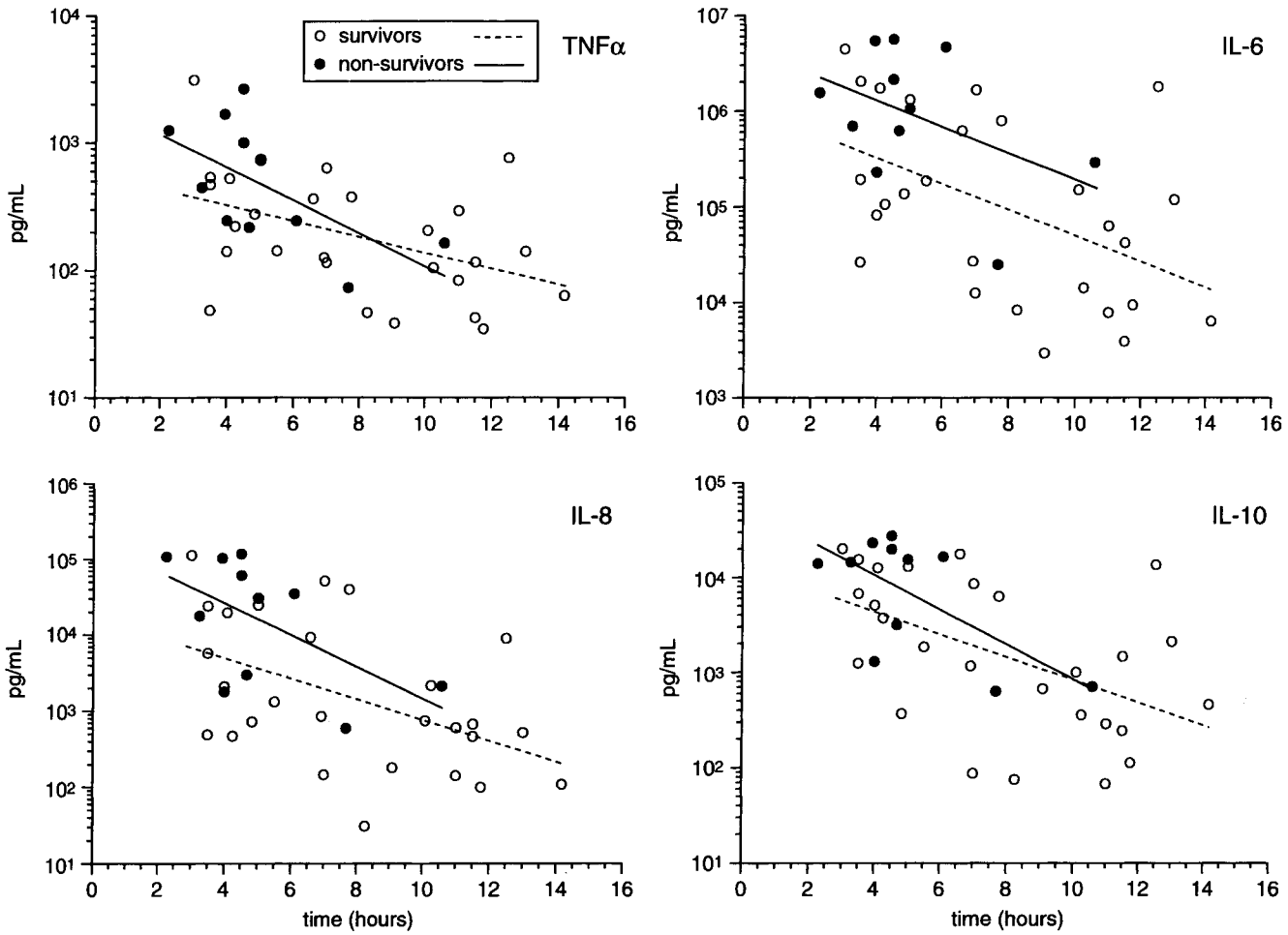

Figure 1. Relation between initial serum concentrations of inflammatory compounds (tumor necrosis factor [TNF]-α, interleukin [IL]-6, -8, -10) and interval between onset of petechiae and blood sampling in 38 children with meningococcal septic shock. Solid and dotted lines indicate regression through values for each parameter. Slopes between regression lines of survivors and nonsurvivors did not significantly deviate from parallelism for each parameter.

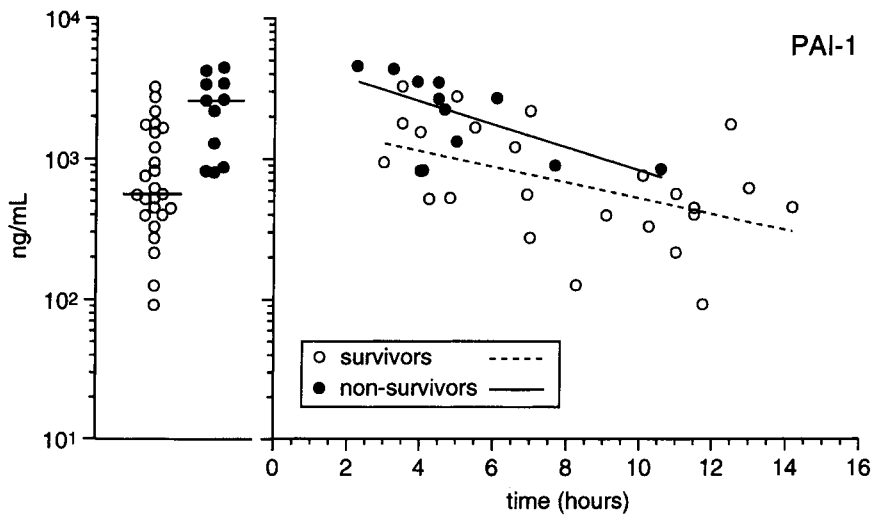

Figure 2. Initial plasma levels of plasminogen activator inhibitor (PAI-1; n = 36). Left, relation between PAI-1 levels and survival. Right, initial PAI-1 plasma levels in relation to interval between onset of petechiae and blood sampling. Slope between survivors and nonsurvivors did not significantly deviate from parallelism. Time-adjusted PAI-1 levels were 2.1 times higher in nonsurvivors (P = .02).

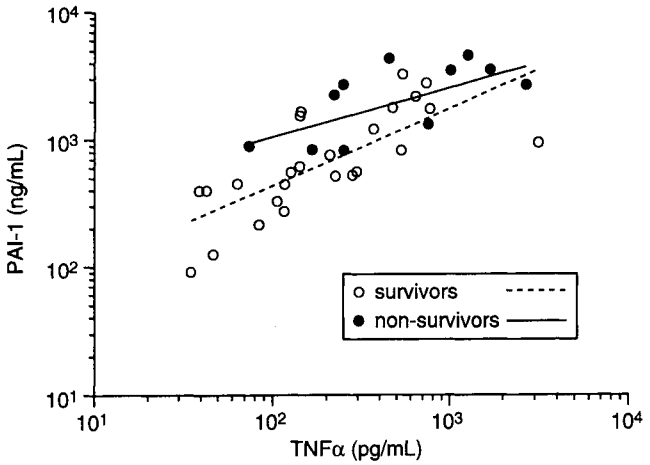

Figure 3. Relation between initial levels of tumor necrosis factor (TNF)-α and plasminogen activator inhibitor-1 (PAI-1) in 36 patients with meningococcal septic shock. Slope between regression lines of survivors and nonsurvivors did not significantly deviate from parallelism (P = .31).

CRP levels, which indirectly reflect the duration of illness [42], were significantly correlated with the interval between the onset of petechiae and blood sampling. This significantly shorter interval and the lower level of CRP in nonsurvivors suggest a shorter disease course and may therefore explain the higher levels of cytokines. The earlier PICU admission of nonsurvivors may indicate that persons who do not survive accumulate more native lipopolysaccharide in a shorter time, trigger all mediator systems more intensively, and are recognized as more severely ill earlier in the course of disease. Alternatively, nonsurvivors may have been admitted earlier because of a more rapid deterioration due to greater responsiveness to lipopolysaccharides or proinflammatory cytokines.

The clinical features of patients with MSS show similarities to those observed in experimental endotoxemia in humans. A challenge with endotoxin in healthy volunteers results in a transient occurrence of a sepsis-like syndrome. The peak levels of cytokines correspond with the transient leukopenia that occurs shortly after the endotoxin challenge [10, 43]. In experiments in baboons infused continuously with *Escherichia coli,* peak levels of TNF-α also occurred very early in the course of disease [44, 45]. Similarly, high levels of TNF-α, IL-6 and -8, low white blood cell counts, and low CRP levels were observed in patients from whom blood was obtained shortly after the onset of petechiae. We therefore assume that the onset of petechiae is a useful set point during the course of invasive meningococcal disease. This assumption is supported by multiple regression analysis, which indicated that serum levels of cytokines are dependent on the duration of petechiae and not on the duration of antibiotic treatment.

Of interest, the differences in the concentrations of mediators between survivors and nonsurvivors disappeared after adjustment for the time between onset of petechiae and blood sampling. These data suggest that the survivors and nonsurvivors in the present study may have had similar releases of inflammatory cytokines (TNF-α, IL-6, IL-8).

The proinflammatory cytokines are counteracted by counter-inflammatory compounds. We observed significantly higher concentrations of IL-10 in nonsurvivors with MSS. Lehmann et al. [29] recently reported that high IL-10 levels in patients with meningococcal disease are associated with fatality. In contrast, Derkx et al. [39] did not confirm this observation in patients with MSS, which may be explained by the relatively small number of patients evaluated. However, the time-adjusted IL-10 levels in the present study were similar between survivors and nonsurvivors. IL-10 acts as a potent inhibitor of the release of the proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8 from T cells [46], polymorphonuclear leukocytes [47], and monocytes and macrophages [12, 48, 49]. In animal models of sepsis, IL-10, given before or soon after challenge with gram-negative bacterial endotoxin or staphylococcal enterotoxin B, reduced TNF-α production and mortality [50–52]. Chernoff et al. [53] showed that a single intravenous injection of IL-10 in humans reduces mitogen-induced T cell proliferation and suppresses TNF-α and IL-1β production from whole blood stimulated ex vivo with endotoxin. In the present study, the strong correlation between IL-10 and the proinflammatory cytokines TNF-α, IL-6, and IL-8 in survivors and nonsurvivors suggests the presence of an adequate IL-10 response to down-regulate the production of proinflammatory cytokines.

In addition, sTNFR-55 and -75 can neutralize the biologic activity of TNF-α. Girardin et al. [31] found that high levels of the sTNFRs are associated with an increased likelihood of fatality, and Froon et al. [54] suggest that increased serum levels of sTNFRs in patients with sepsis syndrome are merely the result of renal failure. Normally, most sTNFRs are removed from the circulation by the kidneys, although the liver and lungs are probably also involved [55]. The higher serum creatinine levels of nonsurvivors (P = .08) in the present study may at least partly explain the differences in sTNFR concentrations.

Abnormalities of coagulation and fibrinolysis play an important role in the pathophysiology of MSS. It has been known for some time that endotoxin, TNF-α, and IL-1β contribute to the activation of the coagulation and fibrinolysis [56, 57]. Normally the endothelial cell provides a blood vessel lining that reduces the coagulability of blood. TNF-α causes endothelial cells to have procoagulant activity by enhancing the expression of tissue factor and by suppressing cofactor activity for the anticoagulant protein C [57–61]. The prolonged aPTT in patients in the present study indicates a massive consumption of coagulation clotting factors, leading to a bleeding tendency. In addition, the plasma levels of the natural inhibitor of coagulation, ATIII, and of protein C and protein S were markedly depressed, resulting in a procoagulant state. The hypercoagulability that occurs during DIC results in the generation and deposition of fibrin, leading to the formation of microvascular thrombosis in various organs and perhaps to multiple organ

failure and ultimately death. In the present study, DIC occurred in 13 of the 27 survivors and in all of the nonsurvivors (P = .003).

Endotoxin, TNF-α, and IL-1β modulate the fibrinolytic system to secrete both TPA and PAI-1, which respectively activate and inhibit fibrinolysis [62–68]. Moreover, fibrin and thrombin formed during coagulation are also potent inducers for the release of tPA and PAI-1 [69, 70]. Fibrinolysis can be initiated by the release of tPA from vascular endothelium that converts plasminogen into the active enzyme plasmin that degrades fibrin in the thrombi. The activity of tPA in patients is counterregulated by PAI-1 that binds to and thereby inhibits tPA. Protein C can inhibit PAI-1 activity. In the present study, the levels of PAI-1 antigen were significantly higher in nonsurvivors. This finding together with decreased protein C activity result in insufficient fibrinolytic activity during a markedly procoagulant state that is associated with vital organ microembolization [19–22]. Administration of recombinant tPA may therefore be considered an adjuvant therapeutic option in patients with fulminant meningococcemia as suggested by Zenz et al. [71].

In previous studies, PAI-1 levels rapidly decreased after hospitalization [72, 73], and PAI-1 and TNF-α levels were strongly associated [74, 75]. Further analysis of our findings showed that PAI-1 levels were significantly dependent on the interval between onset of petechiae and blood sampling and survival. Of interest, PAI-1 concentrations in this study were significantly higher in nonsurvivors at a similar TNF-α concentration. We questioned whether interindividual differences in responsiveness to TNF-α, for example, may contribute to outcome in patients with MSS. The presence of a single base pair insertion/deletion (allele frequency 0.53/0.47) polymorphism in the promotor of the PAI-1 gene has been associated with differences in release of PAI-1 in postoperative patients [76] and in patients with an increased risk of recurrent myocardial infarction [77].

The promotor containing the deletion allele produced six times more mRNA than the insertion allele in response to IL-1β [78]. The insertion/deletion polymorphism in the PAI-1 promotor is of functional importance in the regulation of the expression of the PAI-1 gene [78]. These data support the hypothesis that individuals homozygous for the *del* allele may respond with increased PAI-1 expression in the acute phase of MSS. The possible presence of PAI-1 gene polymorphism in patients with MSS is strengthened by the relatively low PAI-1 response (945 ng/mL) in 1 patient with an extremely high TNF-α level (3130 pg/mL) who survived MSS.

The possible beneficial effects of HA-1A on the outcome of children with MSS is not yet known. However, a recent study did not find a reduction in the 14-day mortality rate in patients with gram-negative bacteremia and septic shock [79].

We conclude that high levels of proinflammatory cytokines and counterinflammatory compounds are associated with fatality. After the levels of inflammatory mediators are adjusted for time after the onset of petechiae, the differences between survivors and nonsurvivors disappeared. We therefore propose that the outcome in patients with MSS is probably not related to TNF gene polymorphism. However, the increased PAI-1 response to, for example, TNF-α in the fatal cases suggests that the presence of polymorphism in the expression of the PAI-1 gene may contribute to the outcome of MSS.

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