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Correlation between Proinflammatory Cytokines and Antiinflammatory Mediators and the Severity of Disease in Meningococcal Infections

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Pro- and antiinflammatory cytokines and mediators were measured in 39 patients with acute life-threatening meningococcal infections classified into 3 groups: A, meningitis without shock ($n = 20$); B, meningitis with shock ($n = 9$); and C, shock without meningitis ($n = 10$). The plasma concentrations of proinflammatory endotoxin, tumor necrosis factor- α (TNF- α), interleukin (IL)-6, and IL-8 and antiinflammatory cytokines and mediators IL-1 receptor antagonist, IL-10, and soluble TNF receptors p55 and p75 were strongly associated with this classification; the highest concentrations were in group C. IL-4 was not measurable. IL-1 β was increased only in rapidly fatal cases. In addition, cerebrospinal fluid (CSF) was analyzed in 21 patients for TNF- α and its soluble receptors. In CSF, these compounds were mainly increased in group A, reflecting an intrathecal compartmentalized cytokine production. It is concluded that both pro- and antiinflammatory mediators are simultaneously increased and are strongly associated with a classification based on simple clinical parameters.

Acute infections with the gram-negative bacterium *Neisseria meningitidis* are characterized by an overwhelming course. Characteristically, within a few hours and up to a day, a child or young adult is struck down from full health to a perilous condition [1]. In its septic form, this infection clinically resembles the experimental model in which endotoxin or lipopolysaccharide (LPS) is infused. After LPS infusion, serum concentrations of so-called proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6, and IL-8, sharply increase, and a septic shock-like state develops [2–7]. The short peak of proinflammatory cytokines is directly followed by an increase of IL-1 receptor antagonist (IL-1Ra), IL-10, and soluble TNF receptors (sTNFRs) [8–10]. These mediators are considered antiinflammatory because they reduce mortality in experimental endotoxemia [11–13].

In patients with meningococcal infections, serum concentrations of the proinflammatory cytokines reportedly correlate with the degree of systemic involvement and severity of disease, and these cytokines are believed to play a pivotal role in the pathogenesis of meningococcal disease [14–17]. sTNFRs are also increased, and with a small series of patients, we reported high concentrations of the antiinflammatory IL-1Ra [18, 19]. As published recently, the antiinflammatory IL-10 behaves similarly: high concentrations at admission in severely

ill patients [20]. No data are available about the concentrations of IL-4 in meningococcal infections.

The principal aim of this study was to compare the concentrations of the above-mentioned pro- and antiinflammatory mediators in plasma and cerebrospinal fluid (CSF) in patients with life-threatening meningococcal infections with clinical findings and severity of disease.

Materials and Methods

Patients. During November 1990 to June 1994, 45 patients with acute life-threatening bacteriologically proven meningococcal infections were admitted to the intensive care unit (ICU) of University Hospital Nijmegen (37 patients) or Eemland Hospital Amersfoort (8). In all patients, diagnosis was verified by a positive culture of CSF, blood, or skin biopsy or by direct detection of gram-negative diplococci in a CSF or skin biopsy smear (or both) [21]. From 39 of these patients, a plasma or serum sample was available for endotoxin or cytokine (or both) measurements. Leukocytes in CSF were counted in all patients; in 21 patients, a CSF sample was available for cytokine measurement.

Patients were classified according to clinical findings and severity of systemic involvement [22]. Group A ($n = 20$) had meningitis without shock, group B ($n = 9$) had meningitis with shock, and group C ($n = 10$) had shock without meningitis. Meningitis was defined as $>100 \times 10^6$ leukocytes/L in CSF. Shock was defined as a systolic blood pressure in the first hours after admission of <100 mm Hg in adults, <85 mm Hg in children 4–14 years old, and <75 mm Hg in children <4 years old [22–24]. Patients with shock were refractory to a fluid bolus and needed inotropic and vasoactive support. All patients received antibiotics immediately after admission; 33 patients were also treated with steroids. Fourteen patients with shock underwent additional plasma exchange or whole blood exchange [25].

Table 1 summarizes the demographic characteristics and prognostic parameters, such as duration of disease before first hospital

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Table 1. Characteristics of 39 patients with severe meningococcal infections.

	Group A: meningitis without shock	Group B: meningitis and shock	Group C: shock without meningitis
No. of patients	20	9	10
No. female/no. male	11/9	5/4	2/8
Age, years	14.5 (2-64)	14 (0-21)	4* (1-18)
Disease period before first hospital admission, h	23 (11.5-84)	10.5 [†] (3-30)	11.75* (2.75-16.5)
Leukocytes in cerebrospinal fluid, $\times 10^6/L$	13,650 (3900-70,000)	417* (107-6000)	15 (1-98)
Peripheral leukocytes (at first hospital admission), $\times 10^9/L$	20.9 (12.8-30.9)	11.8* (2.8-26.8)	4.1* (2.0-13.6)
Bicarbonate (nadir within 12 h after first hospital admission), mmol/L	21.1 (15-28.4)	14.0* (11.5-21.2)	13.9* (2.9-18.3)
Platelets (nadir within 12 h after first hospital admission), $\times 10^9/L$	165 (51-388)	66* (42-325)	40* (11-155)
Fibrinogen (nadir within 12 h after first hospital admission), mg/L	5018 (3500-12,000)	2400* (230-4115)	1164* (90-3732)
No. of deaths (%)	2 (10)	1 (11)	4 (40)

NOTE. Data are median (range) unless stated otherwise. Significantly different vs. group A: $P \leq .005$, [†].05.

admission, the number of leukocytes in CSF and in peripheral blood, the bicarbonate concentration, the platelet count, and the fibrinogen concentration [23-27].

Blood sampling and cytokine measurements. Blood samples for cytokine assay were drawn at a median of 1 h (range, 0-9.5) after ICU admission. In patients treated with plasma or whole blood exchange, blood was drawn before the start of the first exchange session. Since most patients ($n = 28$) were referred by other hospitals, the delay between first hospital admission and first

blood sampling was a median of 4 h (range, 0-17). Consequently, most blood samples (37/39) were drawn after the start of antibiotic and steroid treatment.

Endotoxin level was determined in platelet-rich heparin plasma by a chromogenic limulus amoebocyte lysate assay (KabiVitrum, Stockholm); the detection limit was 12.5 pg/mL [25]. Cytokines and sTNFRs were measured in aprotinin-containing platelet-poor EDTA plasma as described [19]. In some instances, when no EDTA plasma was available, citrate plasma or serum was used. Due to the limited amount of sample, in CSF only TNF- α and sTNFRs were measured.

TNF- α , IL-1 β , and IL-1Ra were determined by RIA using polyclonal rabbit antibodies, according to methods of Granowitz et al. [8], van der Meer et al. [28], and Lisi et al. [29] with some minor modifications. With these modifications, the lower detection limit decreased to 20 pg/mL for TNF- α and IL-1 β . TNF- α values in serum were reproducibly higher than values in plasma. Therefore, TNF- α data obtained in serum were corrected according to the following formula: plasma value = $0.584 \times$ serum value + 19.5 pg/mL (formula calculated from 128 paired serum and plasma samples [$r = .958$]).

The IL-1Ra assay appeared to lack parallelism, so all samples were diluted at a fixed ratio (10 μ L of sample and 90 μ L of RIA buffer solution) that gave a detection range of 600-50,000 pg/mL. IL-4 was measured by ELISA (Genzyme, Cambridge, MA). IL-6 was also measured by ELISA; this assay was not influenced by the presence of soluble IL-6 receptors [30]. For measurement of IL-8, a sandwich EIA was used (Quantikine; R & D Systems, Minneapolis). IL-10 was measured by sandwich ELISA (Medgenix, Fleurus, Belgium).

sTNFRs p55 and p75 were measured by an enzyme-linked immunobinding assay (ELIBA; Hoffmann-La Roche, Basel, Switzerland). Normal values (\pm SD) from 19 healthy volunteers were 1470 ± 190 and 2520 ± 660 pg/mL for sTNFR p55 and p75, respectively. The TNF- α and sTNFR assays were not influenced by the addition of recombinant sTNFR (up to 10 ng/mL) or TNF- α (up to 2.5 ng/mL).

Statistical methods. The principal aim of the study was to relate the concentrations of the measured mediators in plasma or

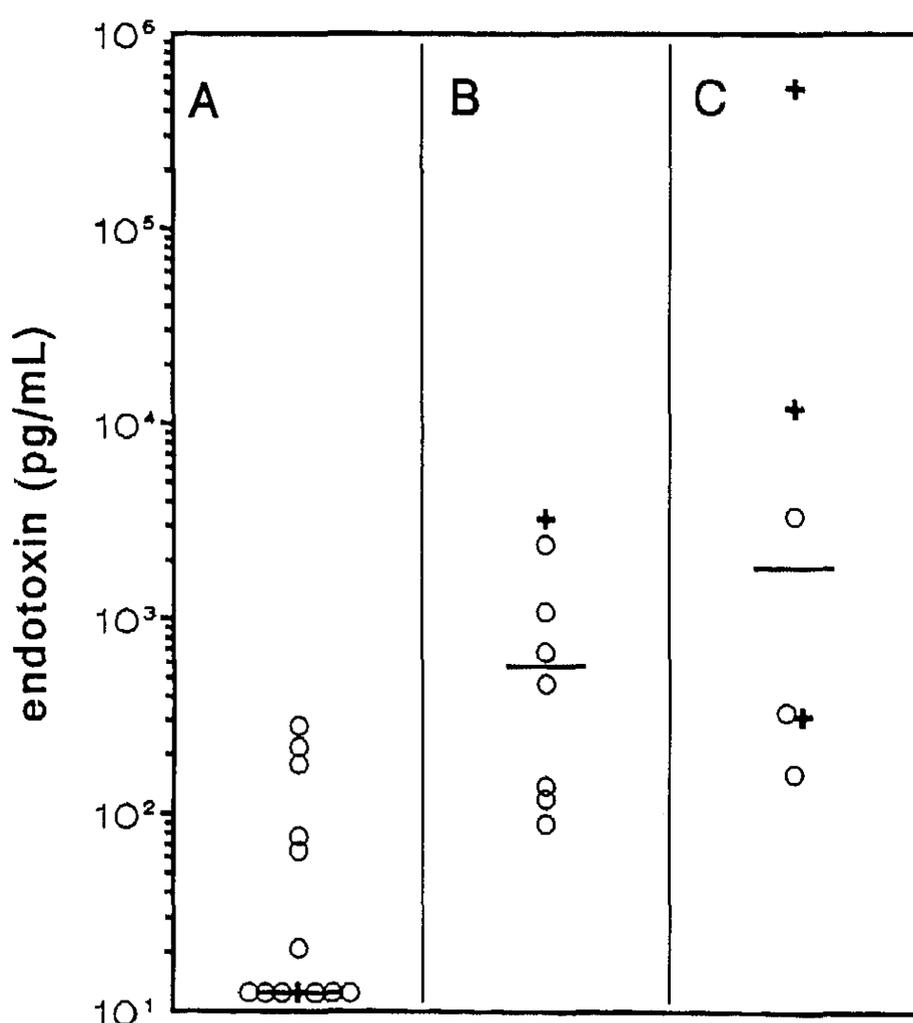


Figure 1. Plasma concentrations of endotoxin on admission to intensive care unit in patients with meningitis (group A), meningitis with shock (group B), or shock without meningitis (group C). Patients who died are indicated with +. Horizontal lines indicate medians.

Table 2. Median plasma or serum concentrations of endotoxin, proinflammatory cytokines, and antiinflammatory cytokines and receptors on admission to the intensive care unit.

Mediator	No. of evaluable patients	Group A: meningitis without shock	Group B: meningitis and shock	Group C: shock without meningitis
Endotoxin	27	<12.5 (<12.5–220)	575* (90–3300)	1859* (162–530,000)
TNF- α	36	101 (60–182)	138 (75–220)	320* [†] (102–1800)
IL-1 β	35	55 (25–110)	40* (30–50)	110* ^{††} (30–2500)
IL-6	29	795 (46–2550)	5716 [‡] (341–287,500)	36,040* [†] (3240–10.8 \times 10 ⁶)
IL-8	19	52 (<20–250)	2462* (325–3050)	6000* (577–>60,000)
IL-1Ra	35	5750 (635–12,000)	8500 [‡] (4400–24,250)	26,250* [†] (6475–>50,000)
IL-10	33	32 (<11–369)	869 [‡] (13–6177)	6396* [†] (576–15,711)
sTNFR p55	39	4505 (2685–33,820)	13,390 [‡] (2630–28,490)	17,490* (6310–40,344)
sTNFR p75	39	10,798 (5500–32,080)	29,290* (12,220–59,140)	67,961* [†] (21,175–94,756)

NOTE. Tumor necrosis factor- α (TNF- α), interleukin (IL), IL-1 receptor antagonist (IL-1Ra), soluble TNF receptor (sTNFR). Data are pg/mL (range). Significantly different vs. group A: $P \leq .005$, [†].05. Significantly different vs. group B: ^{††} $P \leq .05$.

CSF with the severity of disease as expressed by the classification system of Halstensen et al. [22]. Therefore, the study parameters were compared with the Kruskal-Wallis test. To allow for multiple comparisons, a Bonferroni correction was applied. Thus, $P \leq .0042$ was considered significant. In this case, groups A–C were mutually compared using the Mann-Whitney U test. Spearman's rank correlation coefficient was calculated to quantify the correlation between study parameters.

Results

Patient groups and outcome. All group A patients had a positive culture or Gram's stain of CSF, whereas only 5 of them had meningococci demonstrated in skin biopsy or blood culture. In groups B and C, CSF examination showed meningococci in 17 of 19 patients; in these groups, skin biopsies or blood cultures were positive in 18 patients, indicating a more systemic involvement of the infection.

In total, 7 patients died. In group A, 2 patients died of cerebral herniation directly after admission. In group B, 1 patient died after 4 days when treatment was terminated because of severely mutilating distal gangrene and progressive metabolic acidosis. In group C, 4 patients died from intractable shock within 16 h after admission. Among the 32 survivors, 4 had serious sequelae. One group A patient had sensorineural deafness, 2 patients (1 each, groups B and C) needed plastic surgery for necrotic skin areas, and 1 group C patient had both legs amputated because of distal gangrene.

Concentrations of cytokines. The plasma concentration of endotoxin was elevated in several samples and corresponded well with the classification of patients into groups (figure 1). Proinflammatory cytokines TNF- α , IL-1 β , IL-6, and IL-8 were also increased in several samples (table 2). Very high values were found in septic patients with fatal disease. In these patients, TNF- α was (median) 495 pg/mL (range, 165–1800), IL-1 β was 145 pg/mL (range, 50–2500), IL-6 was 1.8×10^6 pg/

mL (range, 2.87×10^5 – 10.8×10^6), and IL-8 (measured in only 2 patients) was >33,000 pg/mL. In a group A patient dying of cerebral herniation, concentrations of these cytokines were only minimally increased. The levels of TNF- α , IL-8, and IL-6 reflected the degree of systemic involvement and severity of disease as expressed by the classification into groups A–C. IL-1 β showed a different pattern; it was increased only in rapidly fatal cases with septic shock. All 4 patients with shock and IL-1 β >115 pg/mL died within 16 h.

Antiinflammatory mediators IL-10, IL-1Ra, and sTNFRs p55 and p75 showed a similar pattern and also corresponded well with the categories (A–C) of severity of disease (table 2, figure 2). Circulating IL-4 was not detectable in any plasma sample.

As shown in table 3, the correlation between the plasma concentrations of the various proinflammatory and antiinflammatory mediators was very high ($P \leq .002$), except for IL-1 β , which did not correlate at all with the other mediators.

The plasma concentration of TNF- α in meningitis patients without shock (group A) was in the normal range (comparing values from controls). However, CSF concentrations of TNF- α and sTNFRs of these patients were definitely elevated; medians were TNF- α , 5585 pg/mL (range, 555 to >6000); sTNFR p55, 6700 pg/mL (range, 4260–48,500); and sTNFR p75, 16,730 pg/mL (range, 2165–94,680) (figure 3). These values differed significantly from those in group C: TNF- α , 160 pg/mL (range, <160–600; $P = .002$); sTNFR p55, 4320 pg/mL (range, 1630–6440; $P = .016$); and sTNFR p75, 1970 pg/mL (range, <300–5670; $P = .001$). Patients of group C who rapidly died tended to have higher CSF concentrations (figure 3C).

Discussion

In this study during the acute stage of meningococcal infection, circulating concentrations of both pro- and antiinflammatory cytokines and mediators were increased and corresponded

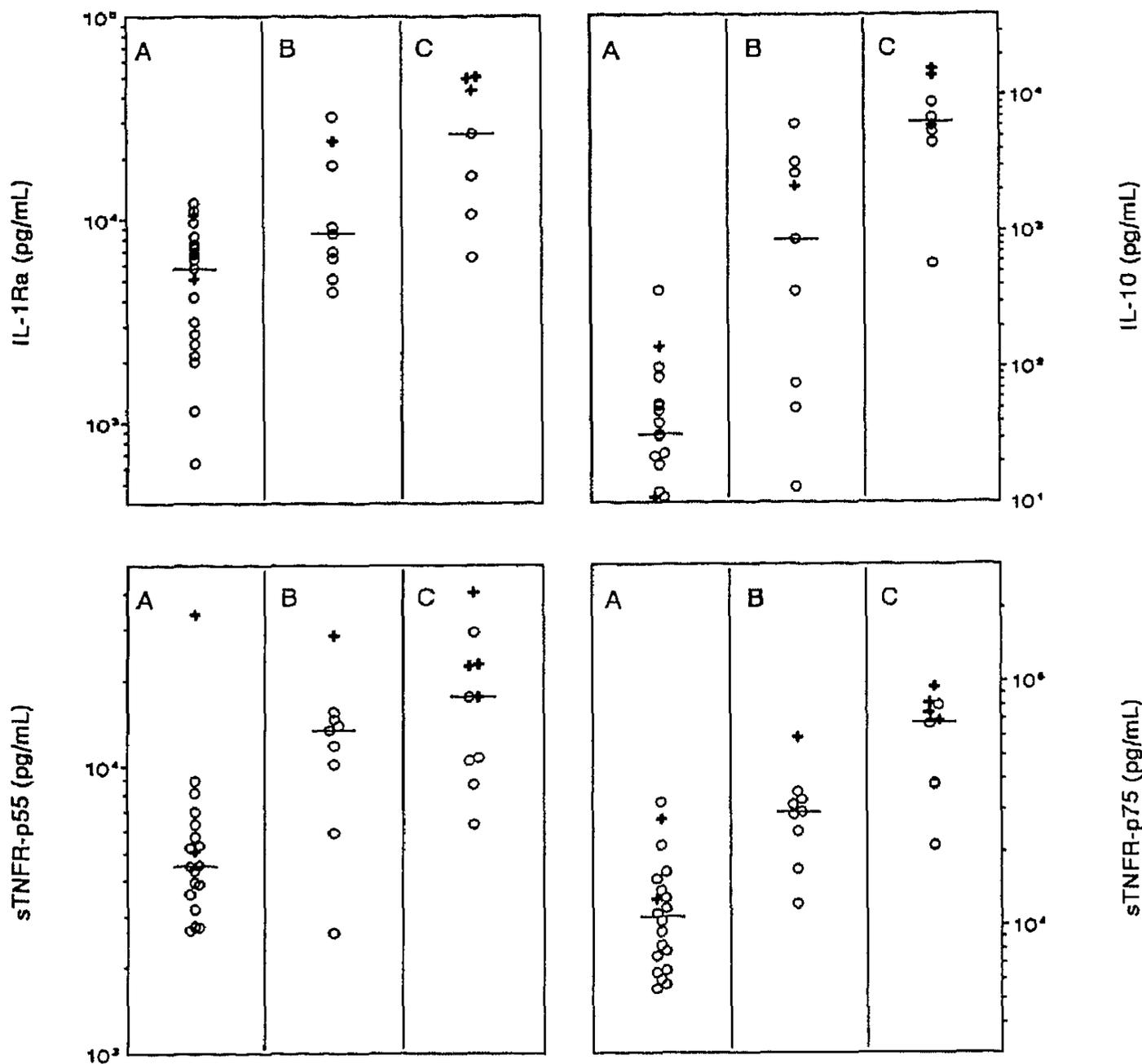


Figure 2. Plasma concentrations of antiinflammatory cytokines interleukin (IL)-10, IL-1 receptor antagonist and soluble TNF receptors p55 and p75 on admission to intensive care unit in patients with meningitis (group A), meningitis with shock (group B), or shock without meningitis (group C). Patients who died are indicated with +. Horizontal lines indicate medians.

with a clinical classification based on the degree of systemic involvement and severity of disease.

In 1987, Halstensen et al. [22] classified patients with meningococcal infections into 4 subgroups: a, meningitis without hypotension; b, septicemia with hypotension and meningitis; c, septicemia with hypotension but without meningitis; and d, septicemia without hypotension or meningitis. Outcome clearly differed among these groups, with mortality rates of 1.3%, 7.4%, and 28.6% in groups a–c, respectively; all group d patients survived. More refined and complex prognostic scoring systems using laboratory data such as leukopenia, acidosis, and diffuse intravascular coagulation are also available (reviewed in [31]). The classification system of Halstensen et al. [22] is tightly coupled to these prognostic parameters (table 1). In addition, we have shown that the concentrations of endotoxin and proinflammatory cytokines TNF- α , IL-6, and IL-8 correspond with this classification system, which confirms earlier findings [16, 17, 32, 33].

The pattern for IL-1 β was different. Similar to findings of Waage et al. [16], we found increased IL-1 β concentrations only in group C patients with a rapidly fatal course. Remarkably, the IL-1 β concentration in patients with shock who survived ($n = 12$) was significantly lower than in survivors without

shock ($P = .05$). This observation may explain the conflicting data about the role of IL-1 β in sepsis of different etiologies. Some studies report a positive correlation between IL-1 β and the severity of disease, whereas others report the opposite [4, 34, 35].

The exact role of IL-6 in the genesis of sepsis, having both pro- and antiinflammatory effects, has still to be established. In an experimental setting, IL-6 has been infused without lethality, and in vitro experiments have shown that IL-6 can inhibit TNF- α and IL-1 β production and stimulate IL-1Ra production either directly or by IL-6–induced acute-phase proteins [36–38]. Yet, in clinical studies of patients with sepsis of different etiologies, IL-6 correlated well with the severity of disease [39]. Our results confirm this correlation for patients with meningococcal infections, indicating that IL-6 is a good indicator of activation of the cytokine network.

IL-1Ra and IL-10 are truly antiinflammatory cytokines. Both agents improve survival in experimental endotoxemia [11, 12]. IL-1Ra blocks the proinflammatory action of IL-1 by competitive binding to the IL-1 receptor [40]. IL-10 inhibits, as does IL-4, the production of TNF- α , IL-1 β , IL-6, and IL-8 and stimulates the production of IL-1Ra [12, 41–44]. In our study, IL-1Ra and IL-10 were produced at an early stage of meningo-

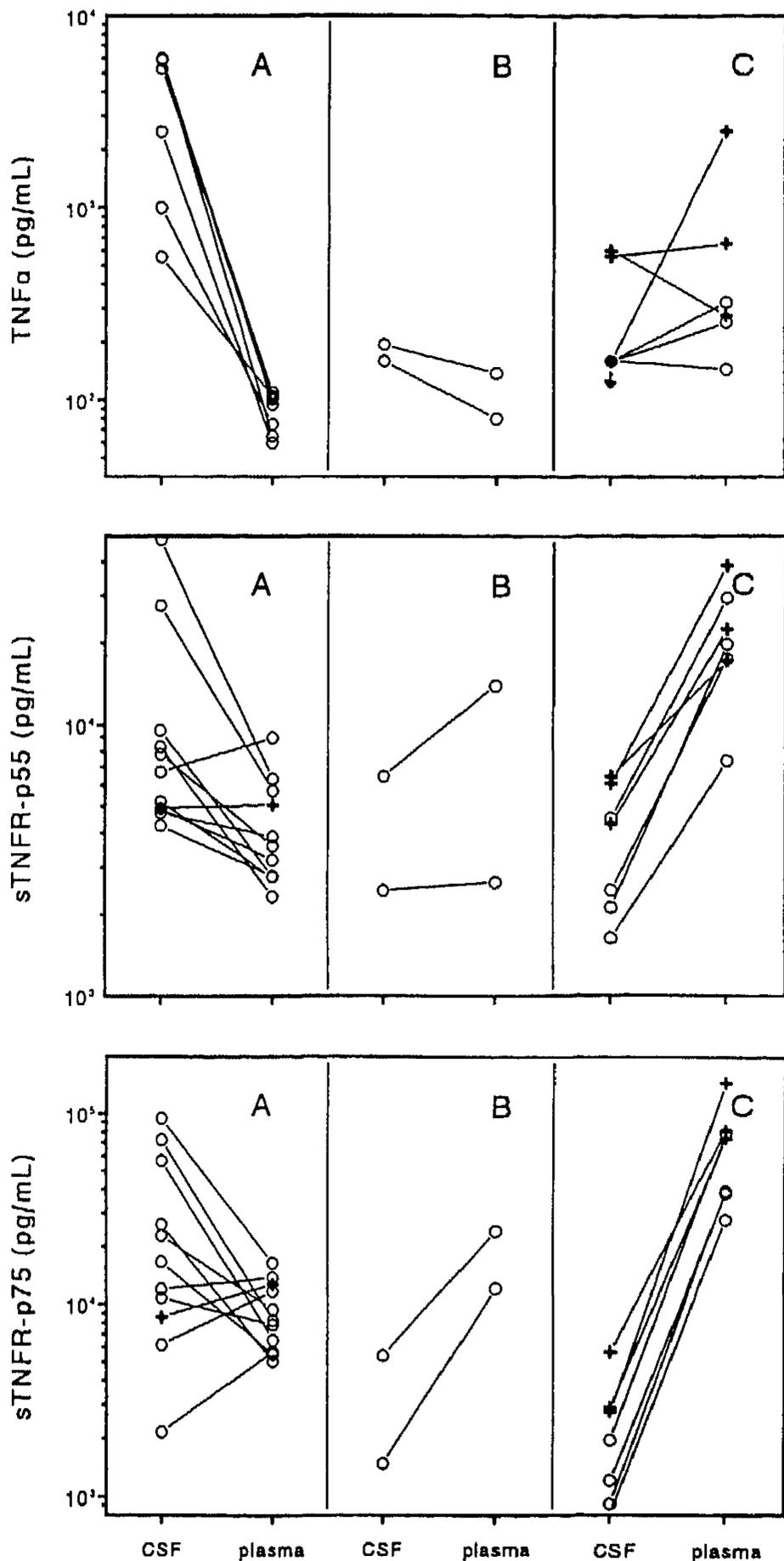


Figure 3. Paired concentrations of tumor necrosis factor- α (TNF- α) and soluble TNF receptors (sTNFRs) p55 and p75 in cerebrospinal fluid and plasma from patients with meningitis (group A), meningitis with shock (group B), or shock without meningitis (group C). Patients who died are indicated with +.

coccal infections and the plasma concentrations corresponded with the severity of disease, similar to the pattern observed for the proinflammatory cytokines.

The role of endogenous sTNFR during infections is complex. It is believed that sTNFR is released into the circulation after binding of TNF- α on the target cell. As such, this shedding may protect the cell against ongoing stimulation [45]. Shed

TNFRs also may be able to capture TNF- α , inhibiting its biologic effects. Thus, sTNFR may have antiinflammatory properties.

However, sTNFRs may also be proinflammatory, since they increase the half-life of TNF- α and therefore prolong its action [46]. In sepsis and meningococcal infections, sTNFRs p55 and p75 are increased [13, 18, 19]. Girardin et al. [18] reported higher TNF- α -to-sTNFR ratios in more severely ill patients and concluded that sTNFRs may detoxify TNF- α . The maximal sTNFR concentrations in our study were nearly identical to those of Girardin et al. (~50 ng/mL for p55 and 100 ng/mL for p75). This suggests that the human body contains a given number of TNFRs that can be shed on maximal stimulation. Consequently, during disease with very high TNF- α plasma concentrations, the TNF- α -to-sTNFR ratio will automatically increase.

The interpretation of sTNFR concentrations in patients with septic shock is even more difficult, since sTNFR is mainly cleared by the kidneys, and patients with shock and renal insufficiency will have higher sTNFR levels [47, 48]. In our study, TNF- α -to-sTNFR ratios did not differ significantly among groups A-C. In addition, 3 of 5 septic patients who died had low TNF- α -to-sTNFR ratios. Overall, our results do not support a purely antiinflammatory role for endogenous sTNFR but rather suggest that sTNFR concentrations reflect preceding or ongoing TNF- α activity.

sTNFRs p55 and p75 differ with respect to their distribution on different cells [45]. In our study, the median ratio of plasma p75 to p55 in patients with meningitis (group A) was 2.27 (range, 0.81-4.02), whereas in patients with shock without meningitis (group C), this was 3.18 (range, 2.19-6.24; $P = .018$). For comparison, in 19 healthy volunteers, this ratio was 1.67 (range, 1.23-2.18). The p75-to-p55 ratio in CSF also differed: 3.21 (range, 0.51-7.26) in group A and 0.46 (range, <0.18-0.88) in group C ($P < .001$). This suggests either a disproportional release or differential clearance of these compounds in both types of meningococcal infections.

Our observation of very high TNF- α concentrations in CSF of patients with meningitis without shock (group A) and concomitant low plasma concentrations is in line with that of Halstensen et al. [17], Brandtzaeg et al. [49], and Waage and colleagues [50, 51]. Those investigators reported on the compartmentalization of cytokine production in meningococcal infections. In our study, sTNFR showed a similar pattern of high concentration in CSF and low concentration in plasma in group A. Although the presence of TNF- α up to 2500 pg/mL did not influence the sTNFR assay, interaction at much higher levels of TNF- α (as observed in some CSF samples) cannot be totally excluded. Remarkably, we also found increased TNF- α and sTNFR concentrations in CSF of patients without meningitis who died rapidly of shock (group C). Possibly in these cases the increase in TNF- α and sTNFR in CSF is of a different origin, since it is known that a high systemic TNF- α or IL-1 β concentration can permeate the blood-brain barrier [52]. The

Table 3. Spearman's correlation coefficients between plasma concentrations of measured mediators (cytokines) on admission to the intensive care unit.

	Endotoxin	TNF- α	IL-1 β	IL-6	IL-8	IL-1Ra	IL-10	sTNFR p55	sTNFR p75
Endotoxin	1.00								
TNF- α	0.71	1.00							
IL-1 β	-0.08	0.19	1.00						
IL-6	0.86	0.79	0.07	1.00					
IL-8	0.91	0.81	0.00	0.75	1.00				
IL-1Ra	0.66	0.52	0.28	0.72	0.72	1.00			
IL-10	0.71	0.66	0.09	0.71	0.85	0.80	1.00		
sTNFR p55	0.77	0.65	0.28	0.70	0.89	0.57	0.72	1.00	
sTNFR p75	0.80	0.77	0.14	0.72	0.88	0.65	0.80	0.90	1.00

NOTE. Tumor necrosis factor- α (TNF- α), interleukin (IL), IL-1 receptor antagonist (IL-1Ra), soluble TNF receptor (sTNFR).

different sTNFR p75-to-p55 ratios found in CSF of patients with (group A) and without (group C) meningitis may support this theory. Thus, cytokine production in meningococcal infections is compartmentalized but probably not completely segregated, and mutual influences may complicate the final cytokine pattern.

In conclusion, in our patients with life-threatening meningococcal infections, the degree of cytokine activation corresponded well with a classification based on easily perceptible and simple clinical parameters. During the early stage of the disease both pro- and anti-inflammatory cytokines were increased and probably represented a generalized activation of the cytokine network. This activation was compartmentalized but not segregated.

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