Plasma Patterns of Tumor Necrosis Factor-α (TNF) and TNF Soluble Receptors During Acute Meningococcal Infections and the Effect of Plasma Exchange

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In 39 patients with acute meningococcal infections, the plasma concentrations of tumor necrosis factor-α (TNF) and its soluble receptors (sRs) TNFsR-p55 and TNFsR-p75 were measured from admission till recovery. At admission, patients with shock had significantly higher TNF, TNFsR-p55, and TNFsR-p75 values than patients without shock. In addition, during the first 24 hours, patients with shock had higher TNFsR-p75 to TNFsR-p55 ratios, indicating that in shock the increase of TNFsR-p75 exceeds that of TNFsR-p55. TNF measured more than 12 hours after admission failed to differentiate between shock and nonshock because TNF concentrations normalized within 12–24 hours. However, because concentrations of TNFsRs remained elevated for 5–6 days, at that time plasma TNFsRs still differentiated between shock and nonshock. Plasma exchange or whole blood exchange (PEBE), performed in 20 patients with shock, accelerated the decrease of plasma TNFsRs. However, because of a rebound after each PEBE session, the overall half-lives of both TNFsRs were not affected by PEBE.

Tumor necrosis factor-α (TNF) is a pivotal mediator in the pathogenesis of meningococcal infections [1, 2]. The biological activity of TNF is mediated via two TNF receptors on the target cell, named TNF-R1 (or TNFR-p55) and TNF-R2 (or TNFR-p75) [3, 4]. Cells can modulate their sensitivity to TNF by shedding the extracellular part of these receptors [5]. The resultant increased concentrations of TNF soluble receptors (TNFsRs) further impede TNF bioactivity by preventing the binding of TNF to cell-associated receptors [6, 7]. However, the presence of TNFsR may also prolong the activity of TNF, as the complexed TNF can dissociate later in the course of the disease [8]. Because of these TNF-modulating effects and because of the interference of TNFsRs in some TNF laboratory assays [9, 10], insight into the kinetic behavior of TNFsRs may be of help in understanding the biological role of TNF.

Experimental studies using bolus infusions of live bacteria [11], endotoxin [12, 13], various cytokines [14–17], or anti-CD3 antibodies [18] suggest different kinetics for TNF and TNFsR. Similarly, clinical observational studies showed persistently elevated TNFsR levels in patients with sepsis of various etiologies [19–22]. From these studies it appeared that the severity of the infectious insult determines the initial release of TNF and TNFsR. However, the kinetic behavior of TNFsR appeared to be determined principally by the renal function [23–25].

To date, only limited data are available on the kinetic behavior of TNFsRs in relation to that of TNF in patients with meningococcal infection and normal renal function. Therefore, we studied the kinetics of TNF, TNFsR-p55, and TNFsR-p75 from admission till recovery in patients with acute meningococcal infection, the prototype human disease of overwhelming endotoxin exposure. Because some of these patients were treated with plasma exchange or whole blood exchange (PEBE) [26], the influence of this treatment on the kinetics of TNFsRs was also studied.

Patients and Methods

Patients. The study group comprised 39 patients with life-threatening meningococcal infections admitted from November 1990 to August 1995 to the intensive care unit of the University Hospital Nijmegen (n = 34) and the Eemland Hospital, Amersfoort (n = 5). Patients were prospectively classified according to the absence (n = 15) or presence (n = 24) of shock. Shock was defined by the occurrence of hypotension refractory to fluid resuscitation and requiring inotropic or vasoactive support; hypotension was defined as a systolic blood pressure <100 mm Hg in adults, <85 mm Hg in children younger than 14 years, and <75 mm Hg in children younger than 4 years [2].

The patients without shock (nonshock patients/group) had a median Niklasson prognostic score [27] of 1 (range, 1–2), and the patients with shock (shock patients/group) had a median score of 4 (range, 2–6). Table 1 shows other prognostically relevant characteristics. All patients were treated with antibiotics, starting immediately after admission; 37 patients received steroids in a dosage equivalent to 0.6 mg/(kg·d) of dexamethasone (DXM) for 3 days. None of the patients participated in a treatment trial for sepsis.
Table 1. Demographic and disease characteristics of 39 patients with acute meningococcal infections.

<table>
<thead>
<tr>
<th>Characteristic of indicated patient group</th>
<th>Nonshock patients (n = 15)</th>
<th>Shock patients (n = 24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of females/males</td>
<td>7/8</td>
<td>15/9</td>
<td>.008</td>
</tr>
<tr>
<td>Age, y</td>
<td>15 (2–64)</td>
<td>5 (0–30)</td>
<td></td>
</tr>
<tr>
<td>Duration (h) of disease before hospital admission</td>
<td>22 (12–84)</td>
<td>12 (2.8–30)</td>
<td>.0001</td>
</tr>
<tr>
<td>No. of leukocytes in CSF (×10³/L)</td>
<td>13,800 (&gt;100–70,000)</td>
<td>95 (1–19,800)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>No. of peripheral leukocytes* (×10³/L)</td>
<td>19.3 (11.0–28.7)</td>
<td>4.3 (1.4–26.8)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>No. of platelets* (×10³/L)</td>
<td>155 (51–220)</td>
<td>62 (16–325)</td>
<td>.0007</td>
</tr>
<tr>
<td>Fibrinogen level* (mg/L)</td>
<td>4,840 (3,500–12,000)</td>
<td>1,680 (90–4,160)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Lactate level* (µmol/L)</td>
<td>2,600 (700–4,070)</td>
<td>5,120 (1,530–12,700)</td>
<td>.0005</td>
</tr>
<tr>
<td>Creatinine² (%)</td>
<td>128 (91–333)</td>
<td>217 (110–400)</td>
<td>.0005</td>
</tr>
<tr>
<td>No. of fatalities</td>
<td>0</td>
<td>5</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Nadir within 12 hours after hospital admission.

² Percentage of age- and gender-adjusted normal value (see [28]).

Twenty of the 24 shock patients (17 survivors and 3 nonsurvivors) were treated with additional PEBE, as reported previously [26]. In brief, in each session of plasma exchange, performed in patients >25 kg, 30–40 mL of plasma per kg was exchanged for fresh frozen plasma. In each session of blood exchange, performed in children <25 kg, 60–80 mL of whole blood per kg was exchanged for citrate anticoagulated whole blood.

PEBE was started as soon as possible after admission to the intensive care unit and was repeated after 12, 24, and 48 hours if the condition of the patient was still critical. Each PEBE session took 1–2 hours. The effect of PEBE on the endotoxin pattern in six patients in the present study was reported previously [26]. The admission data regarding some proinflammatory and anti-inflammatory cytokines of 30 patients have been published elsewhere [2], and the pattern of IL-1β and its modulating agents in 34 of the patients was reported recently [29].

All 15 nonshock patients survived; two had sensorineural deafness. Five shock patients died: two died after 2.5 and 10 hours, without PEBE, and two died 18 and 28 hours post-admission, after two PEBE sessions had been performed. One patient died after four PEBE sessions, when treatment was stopped 4 days after admission because of severely mutilating gangrene, acidosis, and anuria.

Blood sampling. Blood sampling for assays for plasma TNF, TNFsR-p55, and TNFsR-p75 started a median of 3.0 hours (range 0.0–11.1 hours) after hospital admission and stopped (in survivors) after a median of 156 hours (46–210 hours). Samples were drawn at frequent intervals during the first 2 days and once daily from day 3 to day 7. To quantitate the effect of PEBE on the kinetics of TNFsR-p55 and TNFsR-p75, 15 of the 20 patients treated with PEBE (12 survivors and 3 nonsurvivors) had blood sampled just before and after each PEBE session. After sampling, blood was immediately centrifuged twice and plasma or serum was stored at −20°C until assay.

Measurement of TNF, TNFsR-p55, TNFsR-p75, and creatinine. TNF, TNFsR-p55, and TNFsR-p75 were sought in platelet-poor EDTA-plasma; TNF was sought by RIA [2, 30]. The normal value of TNF, as measured in 143 healthy controls, was 94 ± 22 pg/mL (mean ± SD). The assay detects both free and bound TNF. TNFsR-p55 and TNFsR-p75 were measured by an ELISA (Hoffmann-La Roche, Basel, Switzerland). These assays were not influenced by the addition of human recombinant TNF. Normal values for TNFsR-p55 were 1,470 ± 190 pg/mL, and for TNFsR-p75, 2,520 ± 660 pg/mL. The median ratio of TNFsR-p75 to TNFsR-p55 in healthy controls was 1.7 (range, 1.2–2.2). Serum creatinine was measured by routine hospital laboratory procedures with a Hitachi 747 automatic analyzer (Hitachi, Tokyo). The creatinine values are expressed as a percentage of normal values adjusted to age and gender [28].

Calculations and statistics. The elimination of TNFsR-p55 and TNFsR-p75 was estimated with a log-linear model, described by the formula

\[
\ln \left( C_i(t) - C_{normal} \right) = a_0 + a_i + k t \quad (i = 1, \ldots, I-1)
\]

with

\[
C_i(t) = \text{plasma concentration of the } i\text{-th patient at time } t, \quad C_{normal} = \text{the upper normal reference range, expressed as mean } + 2 \text{ SD (1,850 pg/mL for TNFsR-p55 and 3,840 pg/mL for TNFsR-p75),}
\]

I = number of patients,

\[a_0, a_i, \text{ and } k\] = intercept parameters, and

\[k\] = slope parameter.

The parameters \(a_0, a_i,\) and \(k\) were calculated with multiple linear regression. The mean half-lives (i.e., \(ln 2/k\)) were compared with Student’s t-test. A Mann-Whitney U-test or Fisher’s exact test was used to compare nonshock and shock patients. \(P\) values of ≤.05 were considered significant.
Results

At admission, the median plasma TNF concentration in patients without shock was 101 pg/mL (range, 60–182 pg/mL). In patients with shock, the median TNF level was 323 pg/mL (range, 55–1,800 pg/mL) \((P = .0003)\) for the complete group and 262 pg/mL (range, 55–855 pg/mL) for survivors \((P = .002)\). Plasma TNF levels normalized within 1 day. TNF concentrations in nonshock and shock patients that were measured in later stages did not differ significantly (figure 1, upper panel). This contrasted to the plasma concentrations of TNFsR-p55 and TNFsR-p75, which were persistently and significantly more elevated in shock patients than in nonshock patients throughout the whole observation period (figure 1, lower panels).

Median TNFsR-p55 and TNFsR-p75 concentrations in nonshock patients at admission were 4,520 pg/mL (range, 2,330–8,940 pg/mL) and 9,410 pg/mL (range, 5,060–32,080 pg/mL), respectively. In surviving shock patients these values were 13,900 pg/mL (range, 2,630–29,300 pg/mL) and 32,930 pg/mL (range, 12,220–98,710 pg/mL) \((\text{both } P < .001)\). The median ratio of TNFsR-p75 to TNFsR-p55 at admission for nonshock patients was 2.2 (range, 1.4–4.0). This was slightly but significantly higher than for healthy controls \((P = .009)\). For shock patients, however, this ratio was much higher (median, 3.4; range, 1.8–6.2) \((P < .0001)\). As seen in figure 2, the ratio normalized gradually during recovery.

The serum creatinine concentration in surviving shock patients at admission was higher than in nonshock patients \((P = .002)\) but normalized rapidly. On day 2 the median creatinine value in shock patients was 99% (range, 45%–141%), which did not differ significantly from that in nonshock patients.

The course of plasma TNFsR levels showed a biphasic pattern, with a rapid decline initially and a slower decrease during further recovery. In spite of the rapid normalization of renal function, 50% of the nonshock patients and 82% of the surviving shock patients had increased TNFsR values on day 4. The mean half-lives of TNFsR-p55 and TNFsR-p75 over the complete observation period in patients not treated with PEBE were 61 ± 40 hours and 53 ± 32 hours, respectively. In patients treated with PEBE, the overall half-lives were not significantly different: 81 ± 26 hours and 70 ± 22 hours, respectively. However, detailed measurements just before and after PEBE sessions showed that PEBE influenced the TNFsR plasma pattern importantly, although this effect was only short-lasting (figure 3 and table 2).

Discussion

This study showed that, in patients with acute meningococcal infections, the plasma concentrations of TNF and its soluble receptors TNFsR-p55 and TNFsR-p75 follow different kinetics. At admission, patients with shock had significantly higher plasma TNF, TNFsR-p75, and TNFsR-p55 concentrations than nonshock patients. In addition, patients with shock had higher TNFsR-p75 to TNFsR-p55 ratios, indicating that their TNFsR-p75 levels were more elevated than their TNFsR-p55 levels. As plasma TNF normalized within 1 day, TNF concentrations measured more than 12 hours after admission failed to differentiate between nonshock and shock.

In contrast, plasma TNFsRs normalized only slowly, over 5–6 days, in spite of rapid restoration of the renal function.
Table 2. Half-lives of tumor necrosis factor soluble receptor-p55 (TNFsR-p55) and TNFsR-p75 before, during, and after subsequent plasma exchange or whole blood exchange procedures.

<table>
<thead>
<tr>
<th>Period</th>
<th>No. of observations</th>
<th>For TNFsR-p55</th>
<th>For TNFsR-p75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directly after admission</td>
<td>12</td>
<td>6.9 ± 5.1</td>
<td>7.7 ± 4.6</td>
</tr>
<tr>
<td>During 1st PEBE session</td>
<td>12</td>
<td>2.5 ± 1.3</td>
<td>2.4 ± 1.1</td>
</tr>
<tr>
<td>Between 1st and 2nd PEBE sessions</td>
<td>12</td>
<td>&gt;350</td>
<td>&gt;50</td>
</tr>
<tr>
<td>During 2nd PEBE session</td>
<td>12</td>
<td>3.9 ± 5.0</td>
<td>2.4 ± 2.1</td>
</tr>
<tr>
<td>Between 2nd and 3rd PEBE sessions</td>
<td>10</td>
<td>. . . *</td>
<td>. . . *</td>
</tr>
<tr>
<td>During 3rd PEBE session</td>
<td>10</td>
<td>2.3 ± 1.3</td>
<td>2.4 ± 1.7</td>
</tr>
</tbody>
</table>

NOTE. PEBE = plasma exchange or whole blood exchange.
* No real figure; because of an increase in TNFsR over this period, the mean half-lives turned out to be “negative.”

(within 2 days). Therefore, TNFsRs measured in the subacute stage still differentiated between nonshock and shock. PEBE influenced the plasma pattern of both TNFsRs by accelerating the decrease of plasma TNFsRs. However, as a rebound of TNFsR occurred after each PEBE session, PEBE did not significantly affect the overall half-life of TNFsR-p55 and TNFsR-p75.

The increased ratio of TNFsR-p75 to TNFsR-p55 in shock patients with extensive skin necrosis in the present study might reflect that the p55 receptor is less easily shed than the p75 receptor. This may be important for the toxic endotoxin effects such as shock, coagulation activation, and skin necrosis [31–36] that are mainly mediated via the p55 receptor. Another explanation for the increased TNFsR-p75 to TNFsR-p55 ratio might be found in the excessive activation of complement, granulocytes, and endothelium during the initial stage of meningococcal shock [37, 38].

Activated complement is believed to contribute to the shedding of TNFsR; in experimental sepsis, the shed TNFsRs appear in the circulation before the appearance of cytokines [39]. In addition, TNF-stimulated granulocytes shed selectively the p75 receptor [40], and endothelial cells express more TNFR-p75 than TNFR-p55 [41, 42]. Thus, the higher TNFsR-p75 to TNFsR-p55 ratio might be a reflection of these pathophysiological events in the early phase of meningococcal shock.

Support for these theories is found in our observation that in patients with less complement activation, intravascular neutrophil activation, and endothelial damage (i.e., patients with meningitis without shock), the increment in the ratio of TNFsR-p75 to TNFsR-p55 was less pronounced. In addition, DXM, a well-known modulator of the cytokine network, may have influenced the observed pattern. However, as DXM was pre-

Figure 2. The ratio of the plasma concentration of tumor necrosis factor soluble receptor-p75 (TNFsR-p75) to TNFsR-p55, during the first 7 days after admission, in patients with acute meningococcal infections. Panel A shows 144 ratios of 15 surviving patients without shock (open dots). Panel B shows 266 ratios of 24 patients with shock; black dots indicate data for surviving patients, and crosses are for fatalities. The dashed line and solid line indicate the best logarithmic fitting. The grey area indicates the normal range (mean ± 2 SD).

Figure 3. The course of the plasma concentrations of tumor necrosis factor soluble receptor-p55 (TNFsR-p55) (open circles and triangles) and TNFsR-p75 (black circles and triangles) in two representative patients (circles vs. triangles). Both patients were treated with additional plasma exchange sessions (thick lines).
scribed in similar dosages for 14 of the 15 nonshock patients and for 23 of the 24 shock patients, DXM cannot account for the difference between the two groups.

The present study underscores the importance of the timing of blood sampling for TNF, as its plasma concentration normalizes very rapidly. TNFσRs, in contrast, disappear more slowly and still differentiate between nonshock and shock when samples are drawn some days after admission. Thus, timing of blood sampling is less critical for TNFσR measurements, and TNFσRs are therefore a better marker for the prognostic evaluation of cytokine-mediated diseases [20, 21, 43]. The different kinetic behavior of TNF and its soluble receptors argues against use of the ratio of TNF to TNFσR as an indicator of proinflammatory imbalance [6].

Persistently elevated TNFσR plasma concentrations may be a reflection of impaired clearance or ongoing production. Several authors have suggested that in sepsis, the increased TNFσR concentrations are a reflection of impaired renal clearance [20, 21, 23, 24]. However, as we have shown, renal insufficiency is not the only explanation for the persistently elevated TNFσR levels, since renal function in nonshock patients and surviving shock patients both normalized within 2 days, whereas TNFσRs remained elevated for 5–6 days.

Recently, we argued that PEBE can accelerate the decrease of a circulating mediator only when the endogenous clearance of that mediator is low, i.e., when its half-life is long [44]. The overall half-life of both TNFσRs in the present study was 2–3 days, and as would be expected, PEBE clearly influenced the plasma pattern of these mediators. During PEBE sessions TNFσRs disappeared with half-lives of ~2.5 hours (table 2). However, after a PEBE session, a rebound of TNFσRs occurred. Because of this rebound, caused either by an increased release of TNFσRs from cell membranes or by their delivery from other deep compartments, the overall effect of PEBE on the pattern of TNFσR was negligible. Probably, for a persistent effect, more frequent PEBE sessions would have been required.

In previous studies we showed that PEBE had only minor or no effect on the plasma pattern of TNF, IL-1β, and IL-6 but that IL-1 soluble receptor type II and IL-6 soluble receptor were considerably affected [28, 44–46]. In the present study we showed that PEBE influences the concentration of TNFσRs but that this effect is only transient. Unfortunately, none of these observational studies allow conclusions on the clinical effect of PEBE. However, taken together, these results show that the kinetic behavior of various cytokines and their soluble receptors differs importantly [47]. As a consequence, PEBE has a complex impact on the cytokine network. The biological effects of this impact are still unsolved.

References


