Circulating Interleukin-6 Receptor in Patients with Sepsis Syndrome

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Concentrations of interleukin (IL)-6, soluble IL-6 receptor (sIL-6R), and soluble tumor necrosis factor receptor (sTNFRs) p55 and p75 were measured in 25 patients with sepsis syndrome. Sequential blood samples were drawn from patients during a 7-h period. IL-6 concentrations were 34–763,000 pg/mL; they were higher in nonsurvivors than survivors, but the difference was not statistically significant. In septic patients, the median sIL-6R concentration was significantly lower than in 19 healthy volunteers (43 vs. 80 ng/mL). sIL-6R concentrations in survivors were not significantly different than those in nonsurvivors. There was a negative correlation between IL-6 and sIL-6R in septic patients (r = −.72). In patients with moderately impaired renal function, sIL-6R levels were not affected, but the concentrations of sTNFRs were significantly higher.

Cytokines play a pivotal role in the generation of sepsis syndrome. The presence of high plasma levels of cytokines such as interleukin (IL)-1, tumor necrosis factor (TNF), and IL-6 is particularly associated with disease severity and prognosis. Recently, however, it has become evident that not only the cytokine but also circulating cytokine receptors and cytokine–cytokine receptor complexes are involved in the effects of cytokines in sepsis.

Several cytokines, including IL-6 [1] and TNF [2], have circulating soluble receptors, which have a modulating effect on their activity. For example, binding of TNF to its soluble receptor inhibits its biologic activity, thereby protecting the organism from the harmful effects of excessive TNF [2]. However, low concentrations of soluble TNF receptors (sTNFRs) can augment the effects of the cytokine by increasing its half-life [3]. In contrast, the soluble IL-6 receptor (sIL-6R) seems to stimulate the biologic activity of IL-6. In the presence of sIL-6R, the IL-6–stimulated production of acute-phase proteins in hepatic cell cultures is enhanced [4] and IL-6-dependent myeloma cell lines show increased growth [5].

sIL-6R is a 50- to 55-kDa ligand-binding protein that binds IL-6. It is the extracellular part of the gp80 subunit from the membrane-anchored IL-6 receptor. When IL-6 is bound to membrane-anchored gp80, the complex associates with a signal transducing subunit, gp130, that generates a signal into the cell [6].

In septic patients, levels of circulating IL-6 correlate well with the severity of disease and patient mortality [7]. Since sIL-6R may strongly modulate the effects of IL-6, we studied the circulating levels of IL-6 and sIL-6R in a group of septic patients. We also determined both soluble TNF receptors (sTNFRs; p55 and p75) to compare their patterns with those of sIL-6R. Different patterns were expected because of the assumed differences in biologic function.

Materials and Methods

Patients. Sixteen men and 9 women with a mean age of 58 years (range, 18–86) participated in the study. They were selected from patients in the intensive care unit of the University Hospital Nijmegen. All study patients had sepsis syndrome according to the criteria of Bone [8]. In brief, patients had clinical evidence of infection, tachycardia, fever or hypothermia, and tachypnea. accompanied by at least one of the following manifestations of inadequate organ function or perfusion: alteration of mental status, hypoxemia, metabolic acidosis, oliguria, or disseminated intravascular coagulation. Exclusion criteria were the use of >20 mg/day of glucocorticosteroids and a creatinine clearance of <10 mL/min.

Nineteen of the 25 patients were in shock, which was defined as a sustained decrease of systolic blood pressure to <90 mm Hg or a drop of 40 mm Hg from baseline or the presence of any vasopressors. APACHE II scores were calculated over the 24-h period before study inclusion (mean ± SD, 20.0 ± 5.6; range, 9–30). For calculation of the scores, the Glasgow coma score (15) was estimated to be normal because most patients were sedated at testing.

At study entry, 6 patients had bacteremia: Blood cultures revealed gram-negative rods in 3 patients, gram-positive cocci in 1, and mixed flora in 2. The infectious diagnoses of the nonbacteremic patients included peritonitis (6), pneumonia (3), pyelonephritis (1), catheter-site infection (2), soft tissue infection...
(2), and mediastinitis (1). In 4 patients, no definite infectious diagnosis could be made. Nine patients (36%) died within 30 days after inclusion. The estimated mortality rate for the patient group, according to the calculated APACHE II score, was 30–40%.

**Patient samples.** Starting 30 min before the first dose of antibiotic and then 0, 1, 2, 4, and 6 h later, blood samples were drawn from each participant. Serum samples were allowed to clot at room temperature, and sera were collected after centrifugation. Plasma was collected in 4-mL tubes (Vacutainer System; Becton Dickinson, Rutherford, NJ) containing 48 μL of 15% EDTA(K3) and 250 μL of aprotinin (Bayer, Leverkusen, Germany). Sera and plasma were stored at −20°C until use.

**Control samples.** As controls, 1 blood sample was drawn from each of 19 healthy hospital employees. Data on age and sex were not collected.

**IL-6 and sIL-6R ELISA.** IL-6 and sIL-6R ELISAs were used as described [6]. The detection ranges of the ELISAs were 20–800 pg/mL (IL-6) and 0.4–25.0 ng/mL (sIL-6R). Neither the addition of IL-6 to sIL-6R nor the addition of sIL-6R to IL-6 influenced the detection of the cytokine or its soluble receptor [6]. IL-6 and sIL-6R were determined in serum.

**sTNFR p55 and p75 determinations.** sTNFRs were determined in plasma using an enzyme-linked immunobinding assay provided by H. Gallati (Hoffmann-La Roche, Basel, Switzerland). Detection levels were 80 pg/mL for p55 and 300 pg/mL for p75. In healthy volunteers, normal values (median ± SD) are 1470 ± 190 pg/mL for p55 and 2520 ± 660 pg/mL for p75 [9].

**Statistical analysis.** Spearman’s correlation coefficients were used to calculate the relationship between IL-6 and sIL-6R levels and APACHE II scores. Differences between groups were tested using the Wilcoxon rank sum test: P < .05 was considered significant, two-tailed test.

**Results**

Over the 7-h study period, which began 30 min before administration of antibiotic, the mean coefficient of variation for sIL-6R and IL-6 in the septic patients was 16% and 36%, respectively; thus, there were only minor variations. Figure 1 shows the median values for each patient.

IL-6 concentrations were elevated in all patients (range, 34–736,000 pg/mL). Although not statistically significant (P = .4), median IL-6 concentrations in the nonsurvivor group (3060 pg/mL) were higher than in the survivor group (988 pg/mL). IL-6 concentrations in the healthy volunteers were all below the detection limit (<20 pg/mL) and statistically different from the patients (P < .0001).

The median level of circulating sIL-6R in the control group was 80 ng/mL (range, 46–115). In septic patients, however, sIL-6R levels were significantly reduced (median, 43 ng/mL; range, 10–165) compared with the healthy controls (P = .0001).

There was a strong negative correlation between median levels of IL-6 and the accompanying sIL-6R in septic patients (r = −.72; P = .0001). The median levels of IL-6 for each patient correlate well with the APACHE II score (r = .50; P = .01). For the median sIL-6R levels no significant correlation was found with the APACHE II score. When the IL-6:sIL-6R ratio was calculated and related to APACHE II scores, the Spearman correlation coefficient remained the same as for IL-6 alone (r = .50, P = .01).

sTNFR p55 and p75 concentrations were also determined in the septic patients. The mean coefficient of variation for sTNFR p55 and p75 were 8% and 10%, respectively, indicat-
Concise Communications

Effect on shedding of the gp80 ligand-binding protein [13].

of an sIL-6R form through transcription of a specific mRNA.

sIL-6R is different from the one in sepsis; however, to our

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to the findings reported in HIV-seropositive pa­

patients with clonal gammopathy, in whom levels of sIL-6R were elevated compared with controls [1, 5]. These data contrast with the findings reported in HIV-seropositive pa­

patients and patients with monoclonal gammopathy, in whom levels of sIL-6R were elevated compared with controls [1, 5]. These diseases are obviously fundamentally different from sepsis, but increased IL-6 concentrations have been de­

scribed in patients with all three diseases [10, 11]. Appar­

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clonal gammopathy, the relationship between IL-6 and

sIL-6R is different from the one in sepsis; however, to our

knowledge, a study measuring both IL-6 and sIL-6R in these

patients has not been published.

How sIL-6R concentrations are regulated is not clear. Possi­

bly the IL-6/sIL-6R complex is internalized by the effector

cells, resulting in a decreased expression of gp80 on the cell

surface [12]. Assuming that membrane-anchored IL-6R is the

main source of sIL-6R, the increased turnover of mem­

brane-anchored IL-6R in the presence of high levels of IL-6

might be responsible for the decreased levels of sIL-6R.

It is known that sIL-6R is generated either by shedding of

the membrane-anchored gp80 [13] or by direct production of

an sIL-6R form through transcription of a specific mRNA

[14]. How this production is regulated has not been deter­
mined. Protein kinase C (PKC) seems to play an important

role in shedding. Inhibitors of PKC might have a down-regu­
lating effect on sIL-6R production. IL-6 seems to have no

effect on shedding of the gp80 ligand-binding protein [13].

Snyers and Content [15] showed that the expression of the

gp130 component of IL-6R is enhanced by IL-6. The expres­
sion of both components, however, may be regulated by dif­

ferent mechanisms.

Preliminary results of ex vivo whole blood assays suggest that circulating cells are probably not involved in the produc­
tion or use of sIL-6R. When LPS is added to whole blood, IL-6 is produced in large amounts (≤100,000 pg/mL). How­
ever, compared with concentrations in control blood sam­

ples, without the addition of LPS the concentration of sIL-6R does not change (unpublished data).

Other researchers have also found elevated levels of circulat­
ing TNF receptors in patients with severe infections (e.g.,

sepsis, malaria, and meningococcemia). In our study group,

both TNF and IL-6 were increased during sepsis, but the pattern of their soluble receptors is different and the concen­

trations were not correlated. These findings suggest a differ­
et biologic role for both cytokine receptors: sTNFR may inhibit and sIL-6R enhances the biologic effects of TNFα and IL-6, respectively.

It is well known that concentrations of sTNFRs in sepsis are influenced by renal function [16]. In this study, we con­
firm that septic patients with renal impairment have higher

sTNFR concentrations. For sIL-6R, no relation with the cre­
tinine levels was found. This suggests that the renal clear­
ance of sIL-6R is not an important way to regulate levels.

This idea is supported by the fact that only small amounts of

sIL-6R are found in the urine of healthy humans compared

with circulating concentrations [6]. However, because pa­
tients with a renal clearance of <10 mL/min were excluded

from this study, no definitive conclusions can be drawn. Pre­
liminary results in patients with chronic hemodialysis indi­
cate an increase in basal levels of sIL-6R. This means that

moderate renal impairment influences sTNFR concentra­
tions but not sIL-6R concentrations.

In previous in vitro studies, the biologic effects of sIL-6R

[4, 5] were different from the biologic effects of sTNFR [2].

Here we demonstrate that the in vivo pattern of sIL-6R in

septic patients is also different from that of sTNFR. We can

only speculate on the biologic significance of the reduction

of sIL-6R during sepsis. It might be a way to decrease the

biologic activity of IL-6 or just a result of internalization of

IL-6 together with its receptor.

Discussion

The principal finding in this study was that patients with

sepsis syndrome have decreased circulatory concentrations of

sIL-6R with concomitantly high concentrations of IL-6.

The concentrations of sIL-6R in normal volunteers were

comparable to those previously described [1, 5]. Compared

with the control group, septic patients in the current study

had significantly lower concentrations of sIL-6R. These data

contrast with the findings reported in HIV-seropositive pa­

patients and patients with monoclonal gammopathy, in whom

levels of sIL-6R were elevated compared with controls [1, 5]. These diseases are obviously fundamentally different from sepsis, but increased IL-6 concentrations have been de­

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its detection and enhanced release by HIV infection. J Immunol
Interleukin-10 and the Monocyte/Macrophage-Induced Inflammatory Response in Septic Shock


Interleukin (IL)-10 is a potent immunosuppressant of monocyte/macrophage function and may help control the inflammatory response induced by bacterial infection. To analyze whether IL-10 is detectable in plasma of patients with septic shock and to evaluate its relationship with endotoxin (lipopolysaccharide [LPS])-induced and monocyte/macrophage-induced inflammatory response, plasma IL-10, tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8, LPS, and neopterin were studied in 24 patients with septic shock and in 12 critically ill patients. Eighty-three percent of patients with septic shock and 25% of critically ill patients had detectable levels of IL-10 (P < .001). There was a significant correlation between plasma IL-10, neopterin (r = .72), TNF-α (r = .76), IL-6 (r = .68), and IL-8 (r = .61) levels in patients with septic shock. Monocyte/macrophage activation leads to massive secretion of IL-10, which, however, seems to be unable to control the increased production of proinflammatory mediators during septic shock.

Interleukin (IL)-10 is a recently characterized cytokine [1, 2], which has been implicated in the regulation of lymphoid and myeloid cell functions [3] because of its ability to suppress the synthesis of proinflammatory cytokines from T cells [2], polymorphonuclear leukocytes [4], and monocytes/macrophages [5-7]. In the latter case, IL-10 profoundly suppresses the induced production of tumor necrosis factor (TNF)-α, IL-1β, IL-6, and IL-8 by human monocytes [5]. Recent studies suggest that IL-10 production in human monocytes is controlled by bacterial lipopolysaccharide (LPS)-induced TNF-α [8].

To analyze whether IL-10 is detectable in plasma of patients with septic shock, we compared the levels of IL-10, neopterin, endotoxin, TNF-α, and IL-1β, IL-6, and -8 in septic shock patients with those in critically ill patients. We also used these levels to analyze the relationship between plasma

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Informed consent was obtained from all subjects or their parents or guardians; all guidelines for human experimentation from the ethical committee of the Vall d’Hebron General Hospital were followed in the conduct of this research.
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