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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 (sTNFR) 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 \pm SD, 20.0 \pm 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

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Local ethical committees approved this study. Informed consent was obtained from patients or their relatives.

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(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 μ L of 15% EDTA(K₃) and 250 μ L 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 \pm 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-

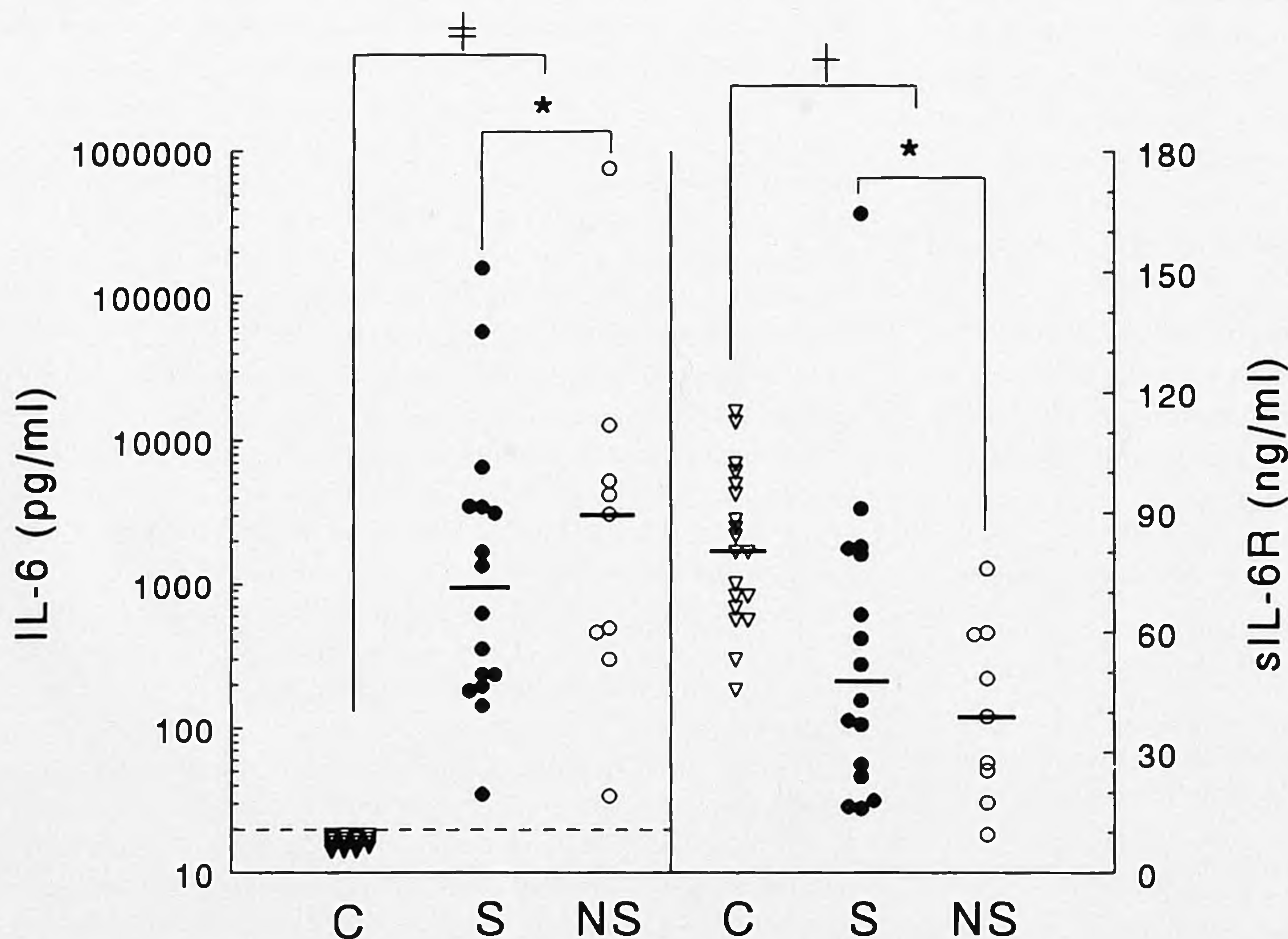


Figure 1. Median IL-6 and soluble IL-6 receptor (sIL-6R) concentrations of patients and healthy control group (C). S, survivors; NS, nonsurvivors. *, $P > .05$; †, $P = .0001$; ‡, $P < .0001$ (Wilcoxon rank sum test).

ing a stable concentration during the study period. sTNFR concentrations, determined in 23 patients, ranged from 2.4 to 30.9 ng/mL for p55 and from 5.4 to 75.8 ng/mL for p75. Neither receptor level differed significantly between survivors and nonsurvivors during the study. There was no significant correlation between sTNFRs and sIL-6R.

To investigate the role of renal function in maintaining soluble receptor levels, patients were divided into groups with normal ($n = 18$) or impaired ($n = 7$) renal function (creatinine concentration range, 0.6–1.9 and 2.0–3.4 mg/100 mL, respectively). Median concentrations of sIL-6R in patients with normal renal function (43.3 ng/mL) did not significantly differ from patients with impaired renal function (43.0 ng/mL). However, sTNFR levels were significantly higher in patients with impaired renal function (p55, 17.6 ng/mL; p75, 31.6 ng/mL) than in patients with normal renal function (p55, 6.4 ng/mL [$P < .001$]; p75, 11.7 ng/mL [$P < .005$]).

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 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 described in patients with all three diseases [10, 11]. Apparently, in HIV-seropositive patients and patients with monoclonal 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. Possibly 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 membrane-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 determined. Protein kinase C (PKC) seems to play an important role in shedding. Inhibitors of PKC might have a down-regulating 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 expression of both components, however, may be regulated by different mechanisms.

Preliminary results of ex vivo whole blood assays suggest that circulating cells are probably not involved in the production or use of sIL-6R. When LPS is added to whole blood, IL-6 is produced in large amounts ($\leq 100,000$ pg/mL). However, compared with concentrations in control blood samples, without the addition of LPS the concentration of sIL-6R does not change (unpublished data).

Other researchers have also found elevated levels of circulating TNF receptors in patients with severe infections (e.g., sepsis, malaria, and meningococemia). In our study group, both TNF and IL-6 were increased during sepsis, but the pattern of their soluble receptors is different and the concentrations were not correlated. These findings suggest a different biologic role for both cytokine receptors: sTNFR may inhibit and sIL-6R enhances the biologic effects of TNF α and IL-6, respectively.

It is well known that concentrations of sTNFRs in sepsis are influenced by renal function [16]. In this study, we confirm that septic patients with renal impairment have higher sTNFR concentrations. For sIL-6R, no relation with the creatinine levels was found. This suggests that the renal clearance 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 patients with a renal clearance of < 10 mL/min were excluded from this study, no definitive conclusions can be drawn. Preliminary results in patients with chronic hemodialysis indicate an increase in basal levels of sIL-6R. This means that moderate renal impairment influences sTNFR concentrations 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.

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Interleukin-10 and the Monocyte/Macrophage-Induced Inflammatory Response in Septic Shock

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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-1 β , 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 sup-

press 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-1 β , 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-1 β , -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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