Increased Plasma Levels of Interleukin-6 in Sepsis


Interleukin-6 (IL-6) is likely to be an important mediator of the inflammatory response. We measured levels of this cytokine in plasma samples from 37 patients with sepsis or septic shock obtained at the time of admission to the intensive care unit and related these levels to hemodynamic and biochemical parameters as well as to clinical outcome. In 32 of the 37 patients, increased levels of IL-6 were found, occasionally up to 7,500 times the normal level. The highest IL-6 levels were encountered in patients who suffered from septic shock (P value of the difference between patients with and without shock <.0001). In addition, IL-6 significantly correlated with plasma lactate (P < .0001), heart rate (P = .05), and, inversely, with mean arterial pressure (P = .01) and platelet counts (P = .0002). Significant correlations of IL-6 with the anaphylatoxins C3a (P = .0001) and C4a (P = .0002) and with the main inhibitor of the classical pathway of complement, C1-inhibitor (inverse correlation, P = .05), were also observed. IL-6 on admission appeared to be of prognostic significance: levels were higher in septic patients who subsequently died than in those who survived (P = .0003), in particular when only patients with septic shock were considered (P < .0001). All nine septic patients with levels of less than 40 U/mL on admission survived, whereas 89% of the nine patients with levels exceeding 7,500 U/mL died. These data provide evidence for a role of IL-6 in the pathophysiology of septic shock. Further studies are needed to reveal whether IL-6 in sepsis is directly involved in mediating lethal complications or whether it is to be considered as an "alarm hormone" that reflects endothelial cell injury probably mediated by the anaphylatoxines.

In sepsis, mediator systems such as the complement system and the contact system of coagulation are thought to play a key role via generation of small peptides that can cause an increased vasopermeability and vasodilatation.1-3 Recently, attention has been directed to the role of cytokines, since these polypeptides that are released by activated monocytes and macrophages4-9 can mediate a wide variety of biological effects. Evidence that cytokines are involved in sepsis is derived from the following observations: administration of cytokines such as recombinant interleukin-1 (IL-1) and tumor necrosis factor (TNF), alone or in combination with each other, induces a shock state in animals with hemodynamic and hematologic alterations that are characteristic for septic shock in man.10-11 Furthermore, plasma levels of TNF-alpha, IL-1, and of interferon-gamma correlate with the severity of meningococcemia.12-14 TNF has been shown to increase upon infusion of small amounts of endotoxin into human volunteers.15 Finally, passive immunization with antibodies against TNF protects animals against the lethal effects of endotoxins and bacteria.16-22

More recently it has become clear that another cytokine, interleukin-6 (IL-6), previously called hydroloma-dissociation factor, interferon β2, B-cell stimulating factor 2, 26-Kd protein, and hepatocyte stimulating factor, also is involved in the systemic changes that are associated with infection and tissue injury. The biological activities of IL-6 partially overlap those of IL-1. Some of the pleiotropic effects of IL-1 are actually caused by IL-6 or by synergistic actions of IL-1 and IL-6.23-26 IL-6 stimulates hepatic protein synthesis during acute-phase responses26-29 and acts as an endogenous pyrogen.30 Many cells, in particular fibroblasts, cells of the monocyte-macrophage lineage, and endothelial cells can secrete IL-6.30-31 Because of these functions IL-6 has been described as an alarm hormone: it is released by damaged tissues and induces the synthesis of proteins in the liver (acute-phase proteins) that protect the host against inflammatory reactions.32 The availability of a very sensitive bioassay for IL-6 allows the determination of this cytokine in clinical samples.32-34 In the present study we have measured plasma levels of IL-6 in patients with sepsis and have delineated the relation of this cytokine with clinical and biochemical parameters. Our results indicate that plasma IL-6 levels in patients with sepsis are markedly increased, particularly in patients who develop a fatal septic shock.

PATIENTS AND METHODS

Patients. The patients who were studied were all admitted to the Medical Intensive Care Unit of the Free University Hospital in Amsterdam with a clinical diagnosis of sepsis. This diagnosis was based on the presence of at least four of the following criteria: a suspected infectious focus; shaking chills, fever (greater than 38.5°C) or hypothermia (less than 35.5°C); tachypnea (greater than 20 breaths per minute); tachycardia (greater than 100 beats per minute); leukocytosis (greater than 15,000 cells per μL); thrombocytopenia (less than 100,000 platelets per μL). The clinical diagnosis of sepsis was made before the results of the bacteriologic studies were known. The diagnosis of septic shock was made when the patients met the criteria for sepsis as well as those for shock (ie, a fall in systolic BP of 50 mm Hg or more, or a systolic BP of 90 mm Hg or less, in combination with either an elevated lactate level [greater than 1.6 mmol/L], oliguria [less than 20 mL per hour], or altered mentation).

Forty-eight consecutive patients fulfilled the above-mentioned criteria. In 11 of these patients neither local nor blood cultures yielded pathogenic bacteria. These patients, who in most cases were already treated with antibiotics prior to admission, were excluded.

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The ages were 15 to 88 years. In 24 patients an underlying disease was present (malignancy in seven, cirrhosis in five, diabetes mellitus in three, vasculitis in one, postresuscitation state in one, paralytic syndrome in two, renal insufficiency in one, heroin addiction in one, herpes encephalitis in one, viral hepatitis in one, and recent abdominal surgery in one). Gram-positive bacteria were found in 11 patients; gram-negative in 23; three patients suffered from infections by both gram-positive and gram-negative bacteria.

Hemodynamic studies according to established procedures \(^\text{35,36}\) were performed in all patients at the same time that blood was sampled for this study. All patients were treated with appropriate antibiotics, and with standard intensive-care support. A detailed description of these patients as well as studies on their complement and contact system are published elsewhere. \(^\text{37,38}\)

Blood sampling. Blood was obtained from each patient upon admission and every six hours thereafter. The blood was collected in tubes that contained Polybrene (0.05%, wt/vol, final concentration) and EDTA (10 mmol/L) to prevent activation of the complement and of the contact system of coagulation. The tubes were centrifuged for ten minutes at 1,300 g, and the plasma was stored in aliquots at −70°C immediately.

Assay for IL-6 and other assays. Plasma IL-6 was measured with a biosassay, the B9 assay, as previously described. \(^\text{39}\) In this assay, B9 cells derived from a murine hybridoma cell line that only proliferates in the presence of IL-6 are cultured for 68 to 72 hours in the presence of heat-inactivated (30 minutes 56°C) plasma samples. Next, proliferation of the cells is measured by a \(^{3}H\) thymidine pulse for four hours. Plasma samples were titrated in duplicate in fourfold dilutions. Results were related to a dose-response curve obtained with monocytederived IL-6 and expressed as U/mL. One unit per milliliter is the amount of IL-6 that causes half-maximal \(^{3}H\) thymidine incorporation in the assay \(^\text{39}\) and is equal to approximately 1 pg/mL as appeared from experiments with purified recombinant IL-6. \(^\text{39}\) Titrations of plasma samples were started at one to 100 final dilutions, since at higher concentrations of plasma the EDTA and Polybrene present in it interfered in the assay. Under these conditions IL-6 in plasma at concentrations exceeding 20 U/mL yields a significant response in the assay. The specificity of the B9 assay for IL-6 has been reported before. \(^\text{39}\) Mouse IL-4 shows some activity in the assay, but in line with the well-known species specificity of IL-4, human IL-4 is inactive in this assay, as are other human cytokines like IL-1 alpha and beta, IL-2, IL-3, IL-4, GM-CSF, TNF alpha, or interferon alpha and gamma.

The anaphylatoxins C3a-desarg and C4a-desarg (further designated as C3a and C4a, respectively) were determined by specific radioimmunoassays (RIAs), as previously described. \(^\text{40}\) Results obtained with plasma samples were expressed in nmol/L.

Data analysis. Relationships of IL-6 to clinical and biochemical parameters were analyzed by weighted regression analysis. Differences in IL-6 levels between patient groups were analyzed by the Wilcoxon-Mann-Whitney (WMW) test. A P value of less than 0.05 was considered to represent a significant difference or relationship.

RESULTS

IL-6 levels in patients with sepsis. In plasma samples obtained from 23 normal donors, no IL-6 was detected (ie, levels were less than 20 U/mL). In contrast, significant amounts of IL-6 were detected in 32 of the 37 samples obtained from the patients on admission, in some cases up to 150,000 U/mL (Fig 1). Polyclonal rabbit antibodies raised against Escherichia coli-derived recombinant human IL-6, \(^\text{39}\) when added, completely abrogated the activity of the plasma samples in the IL-6 assay (Table 1). Patients with positive blood cultures did not have higher levels of IL-6 than patients in whom only local cultures were positive (data not shown). IL-6 levels in patients with gram-positive infections were comparable to those in patients with gram-negative infections (Fig 1).

Relation of IL-6 to hemodynamic and biochemical parameters. IL-6 levels in patients with septic shock appeared to be significantly \((P < .0001, \text{WMW test; Table 2})\) higher than in normotensive patients. We therefore analyzed the relation between IL-6 and the various hemodynamic parameters (Table 3). IL-6 significantly correlated with the heart rate \((P = .046)\) and with plasma lactate levels.

| Table 1. Neutralization of IL-6 Activity in Plasma From Septic Patients by Rabbit Anti–IL-6 |
|-----------------------------------------------|-----------------------------------------------|
| Patient                                      | \(^{3}H\)Thymidine incorporation               |
|                                               | No Anti–IL-6 | Plus Anti–IL-6 |
| 1                                            | 17,200       | 700           |
| 2                                            | 18,700       | 700           |
| 3                                            | 17,500       | 600           |
| 4                                            | 18,700       | 500           |
| Human IL-6 (8,000 U/mL)                      | 17,400       | 600           |
| Mouse IL-6 (8,000 U/mL)                      | 18,100       | 18,400        |

Plasma from four patients with the highest IL-6 levels and rabbit antiserum were diluted in culture medium (final dilutions: 1 to 10,000 and 1 to 100, respectively), and 200 μL of the mixtures were tested in the B9 assay as described under Methods. Results (mean of triplicate) are given as \(^{3}H\) thymidine incorporation (cpm). For comparison, results with monocyte-derived human IL-6 and mouse IL-6 (at final dilutions of 1 to 1,000) are also given.

Fig 1. IL-6 levels in 37 sepsis patients at the time of admission to the intensive care unit. (Left) Patients with gram-positive infections. (Middle) Patients with gram-negative infections. (Right) Patients with double infections. The number of patients within each group is indicated. Upper limit of normal values is indicated by the dotted line.
correlations with activation products of the contact system. An inverse relation was found between IL-6 and CI-inhibitor, the main inhibitor of the classical pathway of the complement system (Table 3).

Relation of IL-6 to mortality. IL-6 levels at the time of admission appeared to be significantly higher in the patients who died in the intensive care unit (P < .0003, Table 4) than in the patients who recovered from their septic episode. This was most evident when patients with septic shock were considered (P < .0001). However, also the normotensive septic patients who died had higher IL-6 levels than those who survived (P = 0.009, Table 4). There was no relation in the nonsurvivors between IL-6 levels on admission and the survival time (range: two to 168 hours).

The relation between IL-6 levels on admission and mortality is depicted in Fig 5. It appeared that all nine patients with IL-6 levels of 40 U/mL or less survived, whereas 89% of the nine patients with levels exceeding 7,500 U/mL died.

Course of IL-6 during the observation period. In the analysis mentioned above, IL-6 levels at the time of admission were used. We have tested IL-6 in serial samples from nine patients. IL-6 levels decreased during the observation period in six of the seven septic shock patients tested. This was found for all three patients who recovered but also for three of the four septic shock patients who died. In one of these patients, IL-6 levels were even undetectable at the last day before death (Fig 6). In one patient with a fatal septic shock, levels of IL-6 remained more or less constant (fluctuating between 164 and 234 U/mL). Examples of the longitudinal course of IL-6 in two patients who died from septic shock and in two who recovered are shown in Fig 6. In one patient with normotensive sepsis who died, levels ranged from less than 20 to 65 U/mL, whereas in another patient with normotensive sepsis who recovered, small amounts of IL-6 (ie, up to 70 U/mL) were only detected on the second and third day of the observation period. Thus IL-6 levels were at the highest on admission and in most cases decreased during the observation period, also in patients who died.

### DISCUSSION

In this study we showed that most patients with sepsis have markedly increased plasma levels of the cytokine IL-6 at the time of admission to the intensive care unit. Levels of IL-6 correlated with symptoms of shock and with clinical outcome.

IL-6 is regarded to be a multifunctional hormone: it is a differentiation factor for B lymphocytes, cytotoxic T lymphocytes, and for hematopoiesis in the bone marrow (BM). Furthermore, it can act as a growth factor for plasmacytomas and hybridomas, the property of which

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient of Correlation</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Heart rate</td>
<td>0.33</td>
<td>.0464</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>-0.42</td>
<td>.0106</td>
</tr>
<tr>
<td>Lactate</td>
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<td>&lt;.0001</td>
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<td>Thrombocytes</td>
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<td>.0002</td>
</tr>
<tr>
<td>C3a</td>
<td>0.59</td>
<td>.0001</td>
</tr>
<tr>
<td>C4a</td>
<td>0.58</td>
<td>.0002</td>
</tr>
<tr>
<td>C1-inhibitor</td>
<td>-0.33</td>
<td>.0497</td>
</tr>
</tbody>
</table>

*The logarithm of the concentration of IL-6 was used for weighted regression analysis.*

![Graph of IL-6 levels on admission to thrombocyte number](image-url)
is used to determine its concentration in biological fluids. In addition, IL-6 is an endogenous pyrogen. However, its main physiologic role is presumably to act as a messenger between damaged tissues and the liver, since IL-6 can be released by many cells and is the main inducer of acute-phase responses in the liver. IL-6 obviously resembles IL-1 in its biologic activities, which is not surprising, since IL-1 induces IL-6 secretion in a variety of cells. This important role of IL-6 in inflammatory reactions prompted us to study IL-6 in sepsis.

IL-6 levels have been assessed in several diseases. In patients with severe burns, plasma IL-6 correlates significantly with body temperature, which supports the concept that IL-6 is an endogenous pyrogen. Increased levels of IL-6 have also been reported in urine and in blood samples from patients following kidney transplantation, in particular during acute graft rejections. In these diseases levels of IL-6 do not exceed 1,000 U/mL (ie, less than 1 ng/mL). In the sepsis patients reported here, IL-6 levels were much higher: levels exceeding 1,000 U/mL were found in 15 patients and levels exceeding 10,000 U/mL in eight of the 37 patients (Fig 1). Such high serum levels have only been encountered in patients with meningococcal septic shock and in five patients with infections by other bacteria. In addition, high levels of IL-6 may occur in specimens obtained from local inflammations, such as the cerebrospinal fluid from patients with infections of the CNS and in the synovial fluid of patients with arthritis. In these latter diseases, IL-6 levels in plasma are within the normal range or only slightly elevated (ie, up to 100 U/mL). Recently we also observed very high IL-6 levels in patients who were treated with high-dose recombinant IL-2 and subsequently developed a clinical syndrome (the vascular leakage syndrome), which resembles septic shock (G.J. Wolbink et al, manuscript in preparation). Although data on IL-6 levels in other shock syndromes are lacking, it is tempting to speculate that excessive amounts of IL-6 in plasma are involved in the pathophysiology of septic shock, in particular since administration of low doses of endotoxin to animals or human volunteers not only elicits an increase in plasma TNF but
also of IL-6\textsuperscript{44} (S.J. van Deventer et al, manuscript submitted).

Helfgott et al reported high serum of IL-6 in five patients with bacterial infections.\textsuperscript{40} However, no clinical data on these patients were given. Waage et al\textsuperscript{49} reported on the serum levels of IL-6 (and also of IL-1 and TNF) in patients with meningococcal disease. IL-6 was higher in patients who developed signs of shock, compared with patients who had only meningitis or bacteremia. Interestingly, IL-6 levels in patients with both shock and meningitis were lower than levels in patients who had shock without meningitis.\textsuperscript{49} Only one of the patients described here suffered from meningococcal sepsis. We found no differences in IL-6 levels between patients with gram-negative or gram-positive infections (Fig 1). Thus increased IL-6 levels are not a particular feature of meningococcal disease but seem to be a general phenomenon of severe bacterial infections, and are higher in patients who develop signs of shock (Tables 2 and 3) and who die (Table 4, Fig 5). These findings strongly suggest a role for IL-6 in the pathophysiology of sepsis.

Many cell types have been demonstrated to secrete IL-6 in vitro upon appropriate stimulation.\textsuperscript{40,41} In vivo fibroblasts, cells of the monocyte-macrophage lineage, and endothelial cells probably are the main sites for IL-6 production. It is not clear which cell type was responsible for the increased plasma IL-6 in the patients with sepsis reported here. Cells of the monocyte-macrophage lineage obviously are candidates, since in vitro these cells produce large amounts of IL-6.\textsuperscript{43} Thus IL-6 may be released in vivo by activated macrophages just as TNF and IL-1.

Another possibility is that the increased IL-6 levels in the patients with sepsis mainly were the result of an increased secretion by endothelial cells. Hemodynamic studies indicate that the basic pathogenic mechanism in sepsis is an increased vasopermeability presumably caused by endothelial damage in combination with vasodilatation.\textsuperscript{35,36} IL-6 in our patients correlated significantly with parameters that reflect a disturbed microcirculation and microcirculation (ie, lactate levels and mean arterial pressure [Table 3 and Fig 2]).

In addition, IL-6 significantly correlated with levels of the anaphylatoxins C3a and C4a, peptides that are generated during activation of the complement system and that may enhance vasopermeability and mediate vasodilatation.\textsuperscript{35,36} Recently we demonstrated that levels of these anaphylatoxins correlate with mortality in the patients also reported here.\textsuperscript{34} An attractive explanation for our findings would be that in patients with sepsis activation of the complement system results in damage of endothelial cells, which contributes to the disturbances in the microcirculation. The injured endothelium in turn secretes IL-6 as an alarm hormone to increase body defenses against inflammatory reactions.\textsuperscript{31} It is tempting to speculate that the inverse relationship between platelets and IL-6 (Fig 3) is also explained by this mechanism: upon interaction with stimuli like the anaphylatoxins and in particular IL-1 and TNF, the endothelial cell is known to down regulate the normal anticoagulant properties of its surface and expresses procoagulant activity that may induce aggregation of platelets.\textsuperscript{37,40} Consistent with this model is the recent observation by Sironi et al,\textsuperscript{44} who reported that IL-1 is able to stimulate IL-6 production in cultured endothelial cells.

IL-6 decreased during the observation period in most patients tested (Fig 6). In particular, the decrease in patients who died was remarkable. At this moment it is difficult to explain this phenomenon, as data on the systemic and toxic effects of IL-6 are hardly available. Apparently more studies are needed to establish whether IL-6, either alone or in concert with TNF and IL-1, can induce a septic shock syndrome or whether it is released by injured cells as an alarm hormone to induce protective acute-phase responses in the liver.\textsuperscript{31}

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