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Interleukin-8 in Sepsis: Relation to Shock and Inflammatory Mediators

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Because of its neutrophil-activating properties, interleukin-8 (IL-8) may play an important role in the pathophysiology of sepsis. We measured circulating IL-8 levels in 47 patients with clinical sepsis. Levels on admission were elevated in 42 of the 47 patients (89%) and were comparable in patients with gram-positive or gram-negative infections. Patients with shock had significantly higher IL-8 levels than normotensive patients ($P = 0.0014$, Wilcoxon-Mann-Whitney test), whereas no differences in IL-8 levels were found between patients with or without adult respiratory distress syndrome. Patients who died had higher IL-8 levels on admission than the patients who survived. The largest differences in IL-8 levels between survivors and nonsurvivors was found when only patients with positive cultures were considered ($P = 0.0342$). IL-8 levels appeared to correlate significantly with lactate levels and inversely with leukocyte and platelet numbers and mean arterial pressure. In addition, the IL-8 level in the sepsis patients was found to correlate significantly with levels of IL-6, elastase-α1-antitrypsin, and C3a. Serial observations revealed that in most patients IL-8 levels decreased, irrespective of the outcome. Thus, our results demonstrate that IL-8 levels are increased in most patients with sepsis and correlate with some important clinical, biochemical, and inflammatory parameters. These findings suggest a role for IL-8 in the pathophysiology of sepsis.

Sepsis is a clinical syndrome induced by severe bacterial infections and has a high mortality (7, 38). The syndrome is caused by an excessive host response to the invading microorganisms and their products, which results in the activation of endogenous inflammatory mediators (4, 5, 7, 30, 38). Among the mediators involved are neutrophils; plasma cascade systems such as the coagulation, fibrinolytic, contact, and complement systems; and cytokines. In particular, the latter are considered to be key mediators in the pathogenesis of sepsis for various reasons. First, intravenous administration of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) induces septic shock-like syndromes in humans and animals (31, 37, 46, 48) and leads to activation of neutrophils and of plasma cascade systems in a pattern similar to that seen after endotoxin challenges (45, 51–53). Second, in vivo inhibition of cytokines by the administration of neutralizing antibodies or receptor antagonists attenuates lethal sepsis or endotoxemia in animals (2, 5, 22, 24, 36, 44, 47). Finally, increased levels of circulating cytokines are found during experimental endotoxemia and in sepsis (16, 17, 29, 53, 55).

The proinflammatory cytokines TNF-α, IL-1β, and IL-6 have been studied extensively with regard to their possible role in sepsis. Recently, a novel cytokine with potential proinflammatory effects has been described (3, 49, 56). This cytokine, presently known as IL-8, has chemoattractant activity (12, 13, 43, 50, 57) and is able to activate and degranulate neutrophils (40, 43). In addition, it is able to inhibit adhesion of neutrophils to endothelial cells (15), although enhancement of adhesion also has been described (10). Thus, in vivo IL-8 presumably is an important regulator of neutrophil activation and migration (20). In vitro IL-8 is produced by a variety of cells, including blood monocytes, macrophages, and endothelial cells (3, 49). Agonists that induce the release of IL-8 in these cells are TNF-α, IL-1β, and endotoxin (3, 49), all of which are involved in the pathogenesis of sepsis. A possible role for IL-8 in sepsis is further suggested by observations that circulating levels of this cytokine increase during experimental endotoxemia, in animal sepsis, and upon intravenous administration of IL-1α or TNF-α (28, 41, 53a, 54).

We have extensively studied a group of sepsis patients with regard to activation of a variety of mediators, including IL-6, neutrophils, complement and contact systems, and proteinase inhibitors (1, 17, 19, 32–34). To further delineate the role of IL-8 in sepsis, we measured circulating levels of this cytokine in these patients and related these levels to the clinical course and to levels of other inflammatory mediators.

MATERIALS AND METHODS

Patients. The patients included in this study were admitted to the intensive care unit 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 (>38.5°C) or hypothermia (<35.5°C); tachypnea (>20 breaths per min); tachycardia (>100 beats per min); leukocytosis (>15,000 leukocytes per mm^3); or thrombocytopenia (≤100,000 platelets per mm^3). A diagnosis of septic shock was made when patients fulfilled the criteria for sepsis as well as those for shock; the criteria for shock were: a fall in systolic blood pressure of ≥50 mm Hg or a systolic blood

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pressure of ≤90 mm Hg, in combination with at least one of the following signs: oliguria (≤20 ml/h); increased plasma lactate (≥1.6 mmol/liter); or an altered mentation. The diagnosis was made before the results of the bacteriologic studies were known. Adult respiratory distress syndrome (ARDS) was diagnosed when patients met the following criteria: diffuse pulmonary infiltration resembling pulmonary edema on chest X-ray photographs, pulmonary arterial occluded wedge pressure of <18 mm Hg, and a PaO_2 of <75 mm Hg at an FiO_2 of ≥0.5.

Blood sampling. Blood samples were obtained via arterial catheters from the patients upon admission. Blood was collected into siliconized Vacutainer tubes (Becton Dickinson, Plymouth, United Kingdom) to which EDTA and Polybrene (final concentrations, 10 mM and 0.05% [wt/vol], respectively) had been added to prevent in vitro activation of complement, coagulation, and contact systems (35). The tubes were centrifuged at 1,300 × *g*, and the plasma was stored in aliquots at −70°C. Eleven of the plasma samples tested had never been thawed, whereas the others had been thawed and frozen for other studies up to a maximum of three times. In experiments using samples from patients as well as those using normal plasma to which IL-8 had been added, we established that five cycles of thawing and freezing at −70°C did not influence the results in the assay, in agreement with the known stability of IL-8 with regard to pH and temperature variations (49). Serial samples from 11 patients (4 with lethal septic shock, 4 with nonlethal septic shock, 1 with fatal sepsis not accompanied by shock, and 2 with nonfatal normotensive sepsis) were also available for the present study (serial samples also had been collected from the other patients, but the longitudinal series of these patients were incomplete owing to the use of samples for other studies). The serial samples, collected and processed as described above, were obtained every 6 h for the first three days and thereafter every 12 h until patients died or recovered. The serial samples had never been thawed before.

Blood samples from healthy donors were obtained by venipuncture and processed as described above. The plasma samples obtained from healthy volunteers used in this study were collected in the same period as those from the patients and stored under identical conditions. Comparison of IL-8 levels in these plasma samples with those in samples freshly obtained from healthy donors revealed no significant difference.

IL-8 assay. IL-8 was measured with an enzyme-linked immunosorbent assay (ELISA) in which monoclonal antibodies (MAbs) and polyclonal antibodies to human recombinant IL-8 (rIL-8) were used. Briefly, rIL-8 was obtained by transfecting *Escherichia coli* DH5 (8, 9) with the plasmid pMBL11 that contained cDNA encoding the 72-amino-acid species of human IL-8 (British Biotechnology Ltd, Oxford, United Kingdom), i.e., the molecular species secreted most abundantly by human leukocytes (49). rIL-8 was purified from *E. coli* sonic extract supernatant by sequential gel filtration on AcA 54 (Pharmacia Fine Chemicals, Uppsala, Sweden) and Sephadex G50 (Pharmacia) columns employing the different migration behaviors of IL-8 on these gel materials. Purified rIL-8 migrated as a single protein band with an M_r of ~8,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis performed with the Phast Gel System (Pharmacia). MAbs against rIL-8 were prepared according to a procedure that we previously described for IL-6 (23). Five different MAbs were obtained from two fusion experiments. Polyclonal rabbit antibodies against rIL-8 were obtained by repeated immunization of rabbits with rIL-8 (10 μg each time). These polyclonal antibodies were affinity purified from antiserum according to the procedure described for the purification of anti-C1-esterase inhibitor antibodies (18). The ELISA system used was that described previously for IL-6 (23) and included an amplification step with catalyzed reporter deposition (6). Several combinations of purified (biotinylated) MAbs and polyclonal antibodies were attempted by using rIL-8 as a standard. The most sensitive assay appeared to be the ELISA in which plates were coated with MAb CLB-IL8/1 (1 μg/ml) and bound IL-8 detected by polyclonal biotinylated rabbit anti-IL-8 (1 μg/ml). However, in this assay an increased aspecific background was observed with plasma samples collected on Polybrene. Therefore, an ELISA was used in which the polyclonal antibodies were replaced by an MAb against human rIL-8, A5-12-14 (0.1 μg/ml) (kindly provided by K. J. Kim, Genentech Inc., San Francisco, Calif.). The lower limit of sensitivity of this ELISA was approximately 5 pg of IL-8 per ml of plasma; intra- and interassay variation coefficients were less than 15%. Dose-response curves of human rIL-8, of IL-8 in the supernatant of endotoxin (*E. coli* O55:B5; Sigma)-stimulated monocytes (23), and of IL-8 in patient samples yielded identical slopes (Fig. 1). Therefore, rIL-8 was used as a standard in the assay; results obtained with plasma samples were expressed in picograms per milliliter.

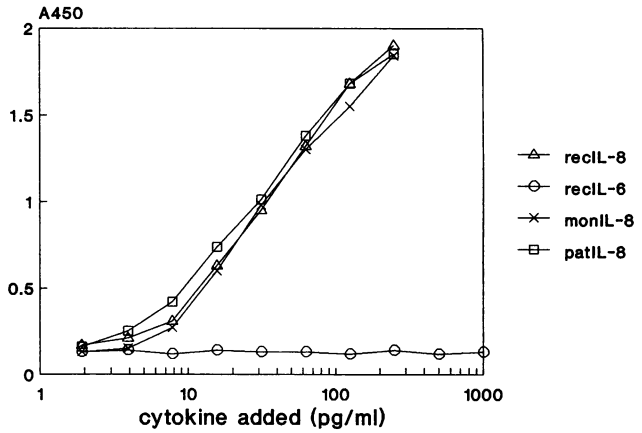

FIG. 1. Dose response curves of rIL-8 (recIL-8), monocyte-derived IL-8 (monIL-8), and IL-8 in plasma from a septic patient (patIL-8) in the ELISA. As a control, results obtained with recombinant IL-6 (recIL-6) are also shown.

Measurement of other inflammatory mediators. Results of studies on the complement activation product C3a, the contact system, the cytokine IL-6, and activation of neutrophils in the patients described here, as well as the assays used, have been described previously (17, 19, 32–34).

Analysis of data. Differences in the IL-8 levels between patients subgroups were evaluated with the Wilcoxon-Mann-Whitney (WMW) test because IL-8 levels were not normally distributed as assessed with the Kolmogorow-Smirnov test. The following subgroups of patients were analyzed (the number of patients belonging to each group is given in the appropriate table): patients with positive blood and/or local bacteriological cultures (hereafter referred to as patients with definite sepsis) versus patients with negative cultures (hereafter designated as critically ill patients); patients with shock versus normotensive patients; patients with definite sepsis with shock versus those without shock; patients with

ARDS versus patients without ARDS; patients with definite sepsis with ARDS versus those without ARDS; and nonsurvivors versus survivors within the various subgroups, i.e., all patients, patients with definite sepsis, critically ill patients, patients with definite sepsis and shock, and patients with normotensive definite sepsis. All subgroups were defined prospectively.

Correlations between IL-8 and other parameters were analyzed by linear regression. Prior to analysis, parameters not normally distributed were log transformed. All log-transformed parameters were distributed normally (Kolmogorow-Smirnov test). The parameters tested were serum creatinine, leukocyte numbers, platelet numbers, plasma lactate level, mean arterial pressure, systemic vascular resistance index, and levels in plasma of the cytokine IL-6, elastase-α1-antitrypsin complex, lactoferrin, the complement activation fragment C3a, and the intrinsic coagulation proteins factor XII and prekallikrein. The hemodynamic and laboratory measurements used for this correlation analysis were all obtained at the same time, i.e., at the time that the patients were admitted to the intensive care unit. The correlations were analyzed for the whole group as well as for the subgroups of definite sepsis and of septic shock. The coefficients of correlation observed in these latter subgroups were not different from those observed for the whole group. Therefore, only the data obtained for the whole group are presented. All calculations were done by using the SPSS statistical package.

RESULTS

Patients. Forty-eight consecutive patients (aged 15 to 88 years) who met the criteria for sepsis or septic shock entered the study. However, for the present study samples from one patient with fatal gram-negative septic shock were not available. Thirty-two of the 47 patients had an underlying disease (malignancy in 11 patients, liver cirrhosis in 5, diabetes mellitus in 4, vasculitis in 2, postresuscitation state in 2, paralytic syndromes in 2, chronic renal insufficiency in 1, alcohol abuse in 1, heroin addiction in 1, herpes encephalitis in 1, viral hepatitis in 1, and abdominal surgery in 1). Septic shock was diagnosed in 22 patients, and ARDS was diagnosed in 15.

Bacteriological cultures yielded no pathogenic microorganisms in 11 patients. These patients are referred to hereafter as critically ill patients. None of these patients had shock, and three of them had ARDS. In the other 36 patients, hereafter designated as definite sepsis patients, cultures yielded pathogenic microorganisms: gram-negative bacteria in 22, gram-positive bacteria in 11, and both gram-positive and gram-negative bacteria in 3. Blood cultures were positive in 17 of these 36 patients.

All patients were treated with appropriate antibiotics according to the sensitivity pattern of the infecting microorganism and with fluid and vasopressor therapy when indicated. Further details on the patients can be found in the published results of previous studies (1, 17, 19, 32–34).

IL-8 in the patients with sepsis and in healthy volunteers. Levels of IL-8 in plasma samples obtained from 34 healthy donors ranged from 6 to 41 pg/ml, with a mean of 19 pg/ml and a standard deviation of 9 pg/ml (Table 1). On the basis of these results, IL-8 levels exceeding 40 pg/ml were considered to be elevated.

In the plasma samples from the septic patients, considerably higher IL-8 levels ($P < 0.0001$ for the difference between the levels in patients versus those in healthy controls, WMW test) were detected, ranging from 7 to 66,000 pg/ml (Table 1, Fig. 2). Only 5 of the 47 patients had normal levels on admission (4 patients with normotensive sepsis, who all recovered, and 1 critically ill patient, who died). There were no significant differences in IL-8 levels in patients with definite sepsis compared with those in the critically ill patients (i.e., patients with the sepsis syndrome but with negative results of bacteriological studies), nor were there significant differences between patients with gram-positive infections and those with gram-negative or with double infections (Table 1). Moreover, IL-8 levels were not different in definite sepsis patients with or without positive blood cultures (Table 1).

TABLE 1. IL-8 levels on admission in the various groups of patients

Group	Median IL-8 level (pg/ml)	Range (pg/ml)	% of patients with elevated IL-8 level
All patients (n = 47)	280	7–66,000	89
Definite sepsis (n = 36)	282	27–66,000	89
Critically ill (n = 11)	220	7–2,768	91
Patients with:			
Double infection (n = 3)	7,955	168–30,000	100
Gram-negative infection (n = 22)	280	27–8,164	91
Gram-positive infection (n = 11)	379	32–66,000	82
Positive blood culture (n = 17)	284	40–66,000	94
Healthy controls[a] (n = 34)	16	6–41	3

[a] Mean, 19; standard deviation, 9 pg/ml.

Relationship of IL-8 levels on admission to the presence of shock. Twenty-two of the 47 patients with clinical sepsis fulfilled the criteria for shock. IL-8 levels on admission in these septic shock patients were significantly higher than in the normotensive patients, regardless of whether critically ill

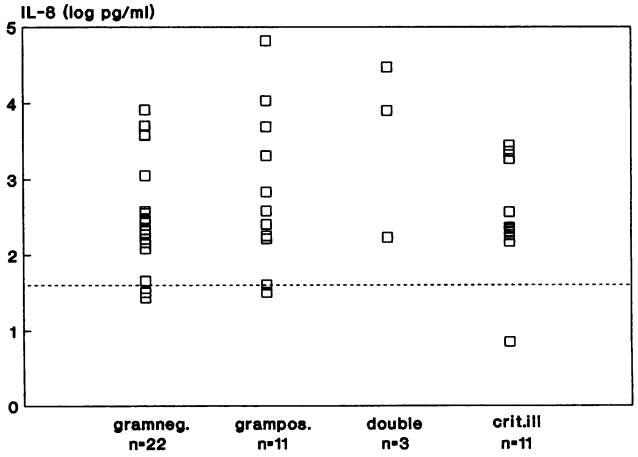

FIG. 2. IL-8 levels on admission in the septic patients with gram-negative (gramneg), gram-positive (grampos), or double infections (double) and in the patients with negative bacteriological cultures (crit.ill). The dotted line indicates the upper limit of normal values as assessed in 34 healthy individuals.

TABLE 2. IL-8 levels on admission in patients with and without shock

Group	Median IL-8 level (pg/ml)	Range (pg/ml)	% of patients with elevated IL-8 level
All patients			
With shock (n = 22)	900[a]	145–66,000	100
Normotensive (n = 25)	203[a]	7–2,768	80
Definite sepsis			
With shock (n = 22)	900[b]	145–66,000	100
Normotensive (n = 14)	177[b]	32–376	79

[a] P = 0.0014 (WMW test).
[b] P = 0.0009.

TABLE 3. IL-8 levels on admission in patients with and without ARDS

Group	Median IL-8 level (pg/ml)	Range (pg/ml)	% of patients with increased IL-8 level
All patients			
With ARDS (n = 15)	280	27–66,000	87
Without ARDS (n = 32)	255	7–10,788	91
Definite sepsis			
With ARDS (n = 12)	282	27–66,000	83
Without ARDS (n = 24)	290	32–10,788	92

patients were excluded from the analysis (P = 0.0009, WMW test) or not (P = 0.0014, Table 2). When the patients with definite sepsis were divided into four groups (each consisting of nine patients) on the basis of their IL-8 levels on admission, it appeared that the group with the highest levels also had the highest incidence of shock (Fig. 3). Thus, high IL-8 levels on admission were significantly associated with the presence of shock.

Relationship of IL-8 levels on admission to the presence of ARDS. Fifteen of the 47 patients who entered the study suffered from ARDS. Levels of IL-8 in plasma on admission in these 15 patients were not different from those observed in the patients who did not develop ARDS (Table 3). Moreover, the incidence of ARDS in the patients with definite sepsis was comparable in patients with the highest and the lowest IL-8 levels on admission (Fig. 3). Thus, we found no evidence for an association of levels of IL-8 in plasma on admission with the development of ARDS.

Relationship of IL-8 levels on admission to the outcome. Twenty-six of the 47 patients (55%) with clinical sepsis died in the intensive care unit. Although IL-8 levels on admission in these nonsurvivors were higher than those found in the patients who recovered (Table 4) and although all 8 patients with IL-8 levels above 4,000 pg/ml died, the difference in IL-8 levels between survivors and nonsurvivors did not reach statistical significance (P = 0.0723). The largest difference was found when only the patients with definite sepsis were considered (Table 4). However, considering that multiple comparisons were made, this difference (P = 0.0324, WMW test) probably was not significant. The lack of a significant difference in IL-8 levels on admission between

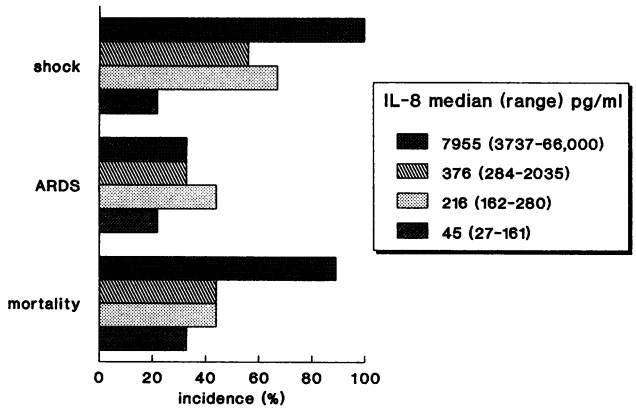

FIG. 3. Relationship of IL-8 levels on admission to the presence of shock, ARDS, and mortality. Patients with definite sepsis were divided into four groups, each consisting of nine patients, according to their IL-8 levels on admission. The incidence of shock, ARDS, and mortality in each group is expressed as a percentage of the number of patients. The uppermost bar of each group represents the group of patients with the highest IL-8 levels, and the lowest bar represents that with the lowest IL-8 levels. IL-8 levels in the various groups are given in the inset.

TABLE 4. IL-8 levels on admission in surviving and nonsurviving patients

Group	Median IL-8 level (pg/ml)	Range (pg/ml)	% of patients with elevated IL-8 level
All patients			
Survivors (n = 21)	251	27–3,856	81
Nonsurvivors (n = 26)	335	7–66,000	96
Definite sepsis			
Survivors (n = 17)	271[a]	27–3,856	76
Nonsurvivors (n = 19)	379[a]	145–66,000	100
Septic shock			
Survivors (n = 7)	301	251–3,856	100
Nonsurvivors (n = 15)	3,737	145–66,000	100
Normotensive sepsis			
Survivors (n = 10)	83	27–376	60
Nonsurvivors (n = 4)	231	161–303	100
Critically ill			
Survivors (n = 4)	208	149–2,313	100
Nonsurvivors (n = 7)	229	7–2,768	91

[a] P = 0.0324.

TABLE 5. Relationship of IL-8 levels on admission to biochemical and hemodynamic parameters in patients with clinical sepsis[a]

Parameter	Correlation coefficient[b]	P
Creatinine	−0.03	Not significant
Leukocytes	−0.46	0.001
Platelets	−0.42	0.004
Lactate	0.45	0.002
MAP[c]	−0.31	0.04
SVRI[d]	−0.13	Not significant

[a] The values for lactate and systemic vascular resistance index (SVRI) were log transformed.
[b] Pearson's product moment correlation.
[c] MAP, mean arterial pressure.
[d] SVRI, systemic vascular resistance index.

survivors and nonsurvivors within the subgroup of patients with septic shock was due to the fact that not only the 7 patients with the highest IL-8 levels within this group died but also the 5 with the lowest IL-8 levels died. The mortality rate of the 9 patients with definite sepsis and with the highest IL-8 levels on admission was 89%, versus 22% in the 9 patients with the lowest levels (Fig. 3). Thus, IL-8 level on admission showed a weak association with clinical outcome.

Relationship of IL-8 level on admission to biochemical and hemodynamic parameters. All patients were extensively monitored for hemodynamic changes. In addition, routine biochemical and hematological parameters were assessed. Of the hemodynamic parameters, only the mean arterial pressure showed a significant inverse correlation to IL-8 levels on admission (Table 5). Of the routine biochemical and hematological parameters, plasma lactate, presumably reflecting tissue hypoxygenation, as well as platelet and leukocyte numbers, showed significant correlations with IL-8 levels (Table 5). On the other hand, no significant correlation with serum creatinine was observed, which virtually excluded the possibility that the increased levels of IL-8 were due to a decreased clearance by an impaired kidney function (Table 5).

Relationship of IL-8 level on admission to levels of other inflammatory parameters in plasma. Previously, we analyzed changes in levels of several other inflammatory mediators in plasma in the patients studied here (17, 19, 32–34). This enabled us to assess the relationship of IL-8 level to levels of other inflammatory mediators in these patients. IL-8 level correlated very significantly with levels of another cytokine, IL-6 (Table 6, Fig. 4). Also, a significant correlation with C3a was observed, whereas that with elastase-α1-antitrypsin

TABLE 6. Relationship of IL-8 levels on admission to levels of other inflammatory parameters in patients with clinical sepsis

Parameter[a]	Correlation coefficient[b]	P
IL-6	0.71	<0.001
Elastase-α1AT	0.35	0.018
Lactoferrin	−0.02	Not significant
C3a	0.53	<0.001
Prekallikrein	−0.29	Not significant
Factor XII	−0.26	Not significant

[a] The values for IL-6, elastase-α1-antitrypsin (elastase-α1AT), and C3a were log transformed.
[b] Pearson's product moment correlation.

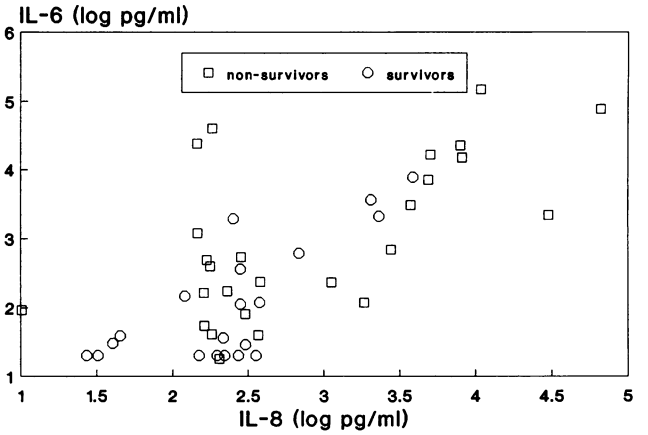

FIG. 4. Relationship of IL-8 levels to IL-6 levels in the patients on admission.

complexes was only weak (Table 6). Moreover, neither the correlation of IL-8 with lactoferrin levels nor that with the contact system proteins prekallikrein and factor XII was significant (Table 6).

Course of IL-8 in clinical sepsis. From 11 patients (5 nonsurvivors and 6 survivors), we analyzed serial samples. The course of IL-8 in 4 of these patients is shown in Fig. 5.

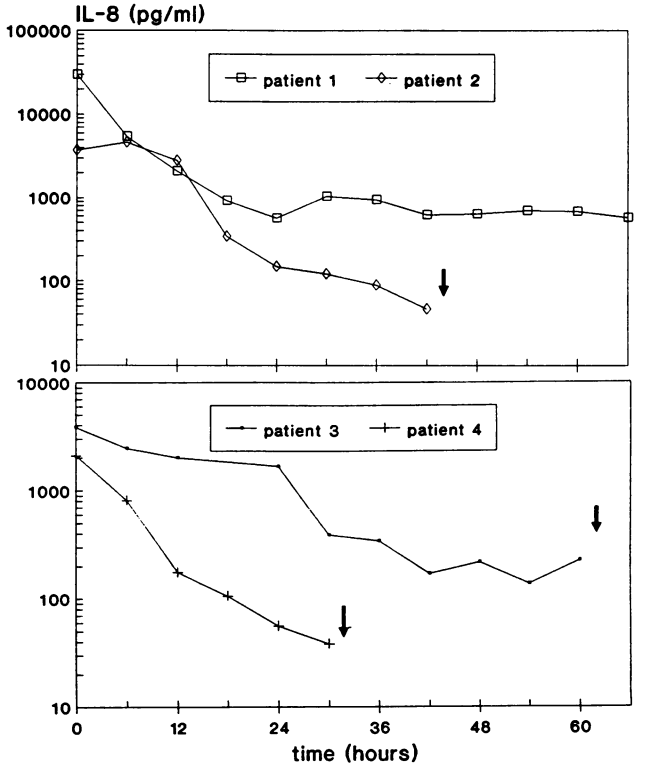

FIG. 5. Course of IL-8 in two patients with fatal septic shock (patients 1 and 2, upper panel) and in two patients with nonfatal septic shock (patients 3 and 4, lower panel). Arrows indicate time of death (upper panel) or time of discharge from the intensive care unit (lower panel). Patient 1 died at 221 hours after admission to the intensive care unit. IL-8 levels during the period not shown ranged from 300 to 1,400 pg/ml.

Eight of the 11 patients had septic shock. In 5 of these septic shock patients (including 2 fatal cases), with IL-8 levels on admission ranging from 680 to 30,000 pg/ml, IL-8 levels decreased at least 10-fold during the observation period. In 2 other septic shock patients (including 1 fatal case) with initial levels of 2,035 and 28,000 pg/ml, levels decreased two- to threefold. In 1 patient with fatal septic shock, levels were 200 pg/ml during the whole observation period. In 2 patients with nonfatal normotensive sepsis, levels remained constant during the course, being 40 to 100 pg/ml in one and 1,000 to 1,300 pg/ml in the other. In one patient with fatal sepsis not accompanied by shock, IL-8 decreased from 161 pg/ml on admission to 47 pg/ml shortly before death. Thus, there was no clear relationship between the course of plasma IL-8 and the clinical outcome in these 11 patients, similar to what we found previously for IL-6 (17). In addition, it may be noted that despite the decrease in IL-8 levels observed in 8 of the 11 patients, in all 11 patients IL-8 was increased in at least five consecutive plasma samples, indicating a sustained release of this cytokine in all of these patients.

DISCUSSION

IL-8 has been suspected to play a role in the pathophysiology of sepsis because this cytokine is able to activate and degranulate neutrophils (40, 43). In support of this notion are recent observations that IL-8 is released into the circulation in an animal model for septic shock (41, 54) and upon intravenous administration of endotoxin (28, 54). The aim of this work was to establish the extent to which circulating IL-8 is increased in sepsis as well as to delineate the relationship of this cytokine to the clinical course and to inflammatory mediators, most notably to activation of neutrophils.

Levels of IL-8 in plasma were increased in 89% of the patients, with no significant differences between patients with gram-positive or gram-negative infections (Table 1, Fig. 2). Occasionally, IL-8 was increased in a patient by more than 1,000-fold relative to values in healthy controls. Similar increases were previously reported for IL-6 in sepsis (17), and levels of both cytokines strongly correlated (Fig. 4). We do not know exactly which forms of IL-8 are detected in our ELISA. Because the antibodies used in the assay were raised against the 72-amino-acid form, they very likely also will bind to the 77-amino-acid form of IL-8, and these forms account for more than 80% of the IL-8 produced (49). In vitro, the conditions to produce IL-6 and IL-8 in monocytes and fibroblasts show a striking parallelism (49). Thus, it is conceivable that the stimuli that cause the release of IL-6 in sepsis also induce the production of IL-8. Our data do not allow definite conclusions with regard to the source(s) of IL-8 in sepsis or to the trigger(s) for synthesis and release of this cytokine. In vitro studies have indicated that many cell types, including monocytes, macrophages, and endothelial cells, can produce IL-8 in response to stimulation with endotoxin, IL-1, or TNF-α (3, 49, 56). These latter agents also have been shown to induce IL-8 release in vivo (53a, 54). Thus, endothelial and mononuclear cells stimulated by endotoxin and/or IL-1 and TNF-α might well be responsible for the IL-8 release in sepsis.

To our knowledge, circulating IL-8 levels as high as those observed in our patients have been reported only in primates challenged with a lethal dose of *E. coli* (41, 54). Moreover, we are not aware of reports on circulating IL-8 levels in other critically ill but nonseptic patients. We did not measure levels of IL-8 in these latter patients, and therefore, it remains to be established whether high IL-8 levels in plasma are specific for sepsis or whether they may occur in other disease states as well.

Although IL-8 levels on admission were higher in the patients who died compared with levels in patients who survived, the largest difference was observed when the patients with definite sepsis were considered ($P = 0.0324$, Table 4). Thus, the differences in levels of this cytokine between survivors and nonsurvivors were less marked than those previously noted for IL-6 (17). Our results are in contrast with data reported in a preliminary communication by Danner et al. (14). These authors found that in patients with septic shock IL-8 levels on admission were lower in nonsurvivors than in survivors. Although the difference in IL-8 levels between survivors and nonsurvivors within the subgroup of patients with septic shock was not significant in our study, nonsurvivors in this subgroup had higher IL-8 levels than survivors (Table 4). Because detailed data from the study by Danner et al. (14) are not available yet, it is not clear whether this discrepancy is due to differences in selection of patients or in the stage of sepsis at which patients were analyzed or to other reasons.

The difference in IL-8 levels between patients with and without shock was highly significant (Table 2), which suggests a role for this cytokine in the pathophysiology of the hemodynamic disturbances of septic shock. This was further supported by the observation that IL-8 levels correlated with lactate levels, which presumably reflect tissue hypoxygenation, and inversely with mean arterial pressure (Table 5). Conversely, we found no significant differences in IL-8 levels in patients with and without ARDS (Table 3). Experimental evidence suggests that activated neutrophils are important mediators of the endothelial damage in ARDS (21, 26, 27, 39). We found only a weak positive correlation between elastase-α1-antitrypsin complexes and IL-8 (Table 6). Thus, the lack of association between circulating IL-8 levels and ARDS may be due to the fact that in sepsis IL-8 is less important as an agonist for neutrophils than, for example, C5a (19, 32). However, for other inflammatory mediators also, such as IL-6, C3a, and elastase-α1-antitrypsin complexes, we found no significant differences in IL-8 levels in plasma between patients with and without ARDS (19) (unpublished results). Although we cannot exclude the possibility that this lack of association between circulating inflammatory mediators and the development of ARDS was due to difficulties in the diagnosis of this condition, we favor the explanation that this lack of association is due to the fact that mediators locally produced also are involved in the inflammatory response in the lungs, thereby obscuring the correlation with circulating mediators. For example, Cohen et al. have demonstrated that the concentration of a peptide that causes the release of elastase from neutrophils and which very likely is IL-8 is increased in bronchoalveolar lavage fluid from patients with ARDS and correlates with the severity of ARDS (11).

Although our results indicate that circulating levels of IL-8 are increased in sepsis, the data do not allow definite conclusions concerning the precise role of IL-8 in the pathogenesis of this condition. In vitro, IL-8 is able to induce degranulation, to elicit a respiratory burst, and to activate arachidonate-5-lipoxygenase in neutrophils (42, 43, 49), processes that enhance inflammation. In addition, IL-8 may promote adherence of neutrophils to endothelium by increasing β2-integrin expression and may regulate transendothelial migration of these cells (10, 25). Moreover, when produced at local sites, IL-8 elicits edema formation due to

neutrophil-mediated endothelial damage and subsequent plasma leakage (12, 13). These proinflammatory effects of IL-8, observed under experimental conditions, may explain the association of IL-8 levels with shock (Table 2) and mortality (Table 4) and the correlation with lactate levels (Table 5) in our patients. However, some findings in the literature suggest that IL-8 may have antiinflammatory effects; it has been shown that in vitro at certain concentrations IL-8 can inhibit adherence of neutrophils to endothelium (15). Moreover, an intravenous bolus injection of IL-8 into rabbits induces a rapid and transient neutropenia due to sequestration in the lungs, followed by a sustained leukocytosis during which accumulation of neutrophils at extravascular sites is inhibited (20). However, it has also been demonstrated that when additional IL-8 is injected intravenously during the phase of inhibited tissue infiltration by neutrophils, neutropenia again develops (20). These experimental data indicate that a sustained release of IL-8 into the circulation may lead to a prolonged neutropenia due to sequestration of neutrophils in the lungs and presumably other organs. Although the course of IL-8 was studied in only 11 patients, in all of them IL-8 was increased in at least five consecutive plasma samples, which were taken over a period of at least 24 h (Fig. 5). Therefore, in sepsis presumably there is a sustained release of IL-8, and this, in keeping with the experimental data just mentioned, probably explains the inverse correlation between IL-8 levels and leukocyte numbers observed in the patients (Table 5). The mechanism behind the neutropenia-inducing effect of IL-8 is not clear but may be related to the proinflammatory effects of this cytokine (20). Apparently, studies on the effects of monoclonal antibodies that neutralize IL-8 in vivo are needed to establish the exact role of IL-8 in sepsis.

In conclusion, we showed that circulating levels of IL-8 are increased in most patients with sepsis and correlate with several important biochemical and clinical parameters. These data suggest a role for IL-8 in the pathophysiology of sepsis.

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