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High Levels of Interleukin 10 in Serum Are Associated with Fatality in Meningococcal Disease

ANNE K. LEHMANN,[1]* ALFRED HALSTENSEN[1], STEINAR SØRNES,[1]
OLA RØKKE[2], AND ANDERS WAAGE[3]

Medical Department B[1] and the Department of Surgery,[2] University of Bergen, Bergen, and the Institute of Cancer Research, University of Trondheim, Trondheim,[3] Norway

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Interleukin 10 (IL-10) suppresses the production of proinflammatory cytokines in vitro and in murine models of endotoxemia and has been suggested as a candidate for treatment of bacterial septicemia. To investigate the role of IL-10 in meningococcal disease, a sandwich IL-10 enzyme-amplified sensitivity immunoassay was used to quantitate IL-10 in serum and cerebrospinal fluid samples from 41 patients with meningococcal bacteremia or meningitis with or without septic shock. High levels of IL-10 were demonstrated in sera from patients with meningococcal septic shock (mean, 21,221 pg/ml; range, 25 to 64,500 pg/ml). All cases involving fatalities had IL-10 levels in serum of ≥1,000 pg/ml (mean, 23,058 pg/ml; range, 1,000 to 64,500 pg/ml). Patients with meningococcal meningitis without septic shock had comparably low concentrations of IL-10 in serum (mean, 119 pg/ml; range, 0 to 1,050 pg/ml) but exhibited compartmentalized release of IL-10 in cerebrospinal fluid. Concentrations of IL-10 in serum were positively correlated with the previously reported concentrations of tumor necrosis factor alpha, IL-6, and IL-8 in serum in the same patients. We conclude that IL-10 is extensively activated along with the proinflammatory cytokines during the initial phase of meningococcal septic shock and that IL-10 is associated with fatality in meningococcal disease.

Cytokines appear to be essential mediators in the host immune response (17). In gram-negative septicemia, an excessive release of lipopolysaccharide-induced proinflammatory cytokines is believed to elicit the systemic cascade reactions associated with clinical deterioration, multiorgan failure, and death (4, 17, 19).

Human interleukin 10 (IL-10) is a polypeptide produced by the Th2 subset of T helper lymphocytes, B lymphocytes, macrophages, and monocytes in response to an immunological challenge (5, 7, 11, 15). Like most other cytokines, IL-10 exhibits numerous effects on the regulation of immune responses; which activity is the most important probably depends on the local context in which the cytokine is produced (13). Recent studies concerning severe bacterial infections have focused on the suppressive effects of IL-10 on the synthesis of proinflammatory cytokines. IL-10 has been shown to inhibit the in vitro production of tumor necrosis factor alpha (TNF-α), IL-1α, IL-1β, IL-2, IL-6, IL-8, interferon-γ, and granulocyte-macrophage colony-stimulating factor by human monocytes and mouse peritoneal macrophages (2, 7–9, 11, 16, 18) and to protect from lethality in murine models of endotoxemia (1, 9, 10).

Hypersecretion of IL-10 in plasma from patients with septicemia and septic shock has been proposed to be involved in the control of the inflammatory response induced by bacterial products (12). Furthermore, IL-10 has been suggested as a candidate for treatment of bacterial septicemia (1, 9, 10).

Fulminant cases of meningococcal disease exhibit one of the most rapidly developing forms of septic shock, involving an extensive release of proinflammatory cytokines in previously healthy individuals. We have previously demonstrated high levels of TNF-α, IL-1, IL-6, and IL-8 in sera and cerebrospinal fluid (CSF) from patients with systemic meningococcal disease (19). The aim of the present study was to evaluate the role of IL-10 in meningococcal disease. A sandwich IL-10 enzyme-amplified sensitivity immunoassay (EASIA) was used to quantitate IL-10 in sera and CSF from patients with meningococcal bacteremia or meningitis with or without septic shock. We also describe the compartmentalization of IL-10 in serum and CSF, the kinetics of IL-10 in serum during the initial phase of meningococcal septic shock, and the association between IL-10 and the previously reported levels of TNF-α, IL-1, IL-6, and IL-8 in meningococcal disease (19).

MATERIALS AND METHODS

Patients and materials. Serum samples from 20 male and 21 female previously healthy patients with bacteriologically verified meningococcal disease were collected on admission to the University Hospital of Bergen, Bergen, Norway, before the initiation of antibiotic therapy. A consecutive series of serum samples (0 h to 17 days after admission) were obtained from three of the meningococcal septicemic patients. Serum samples from three healthy individuals and seven surgical patients with serious, nonmeningococcal bacteremias (including those with *Escherichia coli* [$n = 2$], *Staphylococcus aureus* [$n = 2$], *Bacteroides fragilis* [$n = 1$], *Enterococcus faecalis* [$n = 1$], and *Klebsiella oxyfila* [$n = 1$]) with or without underlying or chronic illnesses ($n = 6$), recent surgery ($n = 4$), and septic shock and death ($n = 1$) were also included. All sera were separated rapidly after coagulation of nonheparinized whole blood in sterile tubes (6). Avoiding lumbar puncture on critically ill patients, we obtained CSF from 27 of the patients with meningococcal disease in the acute stages of the disease. CSF from six patients with nonbacterial neurologic diseases including cerebrovascular diseases (two), hemiplegia (two), hydrocephalus (one), and astrocytoma (one) were included as control specimens. Serum and CSF samples were stored in pyrogen-free aliquots at −20°C or −70°C until used.

The selection of patients included in this study was based on the availability of appropriate serum and CSF specimens, age (10 to 93 years old), and clinical diagnosis. The patients were allocated into four clinical disease categories: patients with meningitis ($n = 14$), septic shock ($n = 15$), septic shock and meningitis ($n = 8$), and bacteremia ($n = 4$). The patients were considered to have bacteremia if blood cultures were positive, meningitis if the CSF contained >100 cells per μl, and septic shock if the systolic blood pressure was ≤70 mm Hg (in patients ≤12 years of age) or ≤100 mm Hg (in patients >12 years of age) and associated with clinical signs of altered organ perfusion within 3 h of admission. The patients with meningococcal disease were also classified into four groups

* Corresponding author. Mailing address: Medical Department B, University of Bergen, Haukeland Hospital, N-5021 Bergen, Norway. Phone: 47 55973076 and 47 55973067. Fax: 47 55972950. Electronic mail address: Anne.Lehmann@ffhs.uib.no.

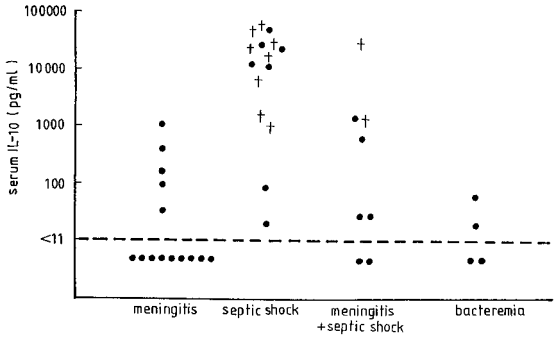

FIG. 1. IL-10 levels in sera (in picograms per milliliter) of 41 patients with different manifestations of meningococcal disease. IL-10 levels were measured by an EASIA. The broken horizontal line denotes the detection limit (11 pg/ml). ● = case without fatality; † = case with fatality.

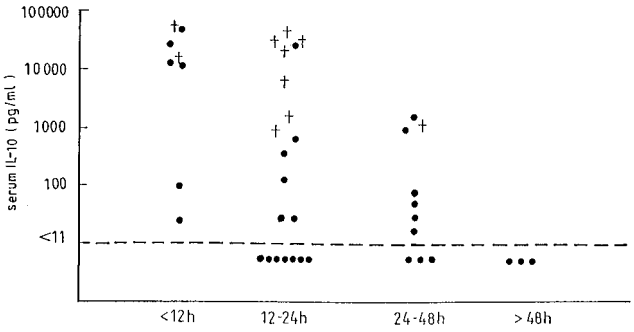

FIG. 2. IL-10 levels in sera (in picograms per milliliter) of patients with preadmission symptom histories of <12, 12 to 24, 24 to 48, and >48 h. IL-10 levels were measured by an EASIA. The broken horizontal line denotes the detection limit (11 pg/ml). ● = case without fatality; † = case with fatality.

according to the duration of symptoms before admission: <12 (n = 8), 12 to 24 (n = 20), 24 to 48 (n = 10), and >48 h (n = 3).

Cytokine assay. The IL-10 EASIA from Medgenix (Brussels, Belgium) was used to measure total IL-10 levels in sera and CSF. Briefly, standards, samples, and controls containing IL-10 react with capture monoclonal antibody 1 (MAb1) coated on plastic wells. After a washing to remove the excess of antigen, the addition of horseradish peroxidase-labelled MAb2 allows the formation of a sandwich: coated MAb1–IL-10–MAb2–HRP. Tetramethylbenzidine-H_2O_2 was used as revelation solution, and H_2SO_4 was used as a stopping reagent. The MAbs are primarily directed against epitopes not shared (<0.2% cross-reaction) with the Epstein-Barr virus BCRF1 protein (14). Optical density readings were done at 450 to 492 nm (reference filter, 620 nm). The lower limit of detection was 11 pg/ml (lowest positive standard value). Samples with high levels of IL-10 (offscale values at 450-nm readings) were read at 492 nm or diluted 1/25 or 1/50 and reassayed.

TNF-α, IL-1, and IL-6 have previously been measured by bioassays and IL-8 has been measured by an enzyme-linked immunosorbent assay in corresponding serum and CSF specimens from the majority of the patients with meningococcal disease (19). Because of the lack of remaining frozen specimens, some of the previously analyzed patients could not be included in the present study. Seven additional patients (four of whom died) with meningococcemia are included in the IL-10 analysis.

Statistics. The significance of differences in IL-10 concentrations was determined by paired and unpaired Student's t tests and chi-square tests, and correlations were tested by Pearson bivariant correlation analysis (SPSS for Microsoft Windows 6.0). A probability of <0.05 was considered statistically significant.

RESULTS

IL-10 in serum. Of the 41 patients with meningococcal disease, 27 (66%) had detectable levels of IL-10 in serum (Fig. 1). IL-10 was detected in all meningococcal septic shock patients, and they had significantly higher levels of IL-10 (mean, 21,221 pg/ml; range, 25 to 64,500 pg/ml) than patients in each of the other three disease categories (mean, 119 pg/ml, and range, 0 to 1,050 pg/ml [meningitis]; mean, 4,711 pg/ml, and range, 0 to 33,750 pg/ml [meningitis and septic shock]; and mean, 20 pg/ml, and range, 0 to 60 pg/ml [bacteremia categories]). The probability values for the latter three disease categories were <0.001, 0.043, and 0.050, respectively.

All fatalities involved meningococcal septic shock, and two of them involved septic shock and meningococcal meningitis (Fig. 1). A highly significant difference was observed between the IL-10 levels in cases involving fatalities (mean, 23,058 pg/ml; range, 1,000 to 64,500 pg/ml; n = 10) and those in cases without fatalities (mean, 4,102 pg/ml; range, 0 to 46,750 pg/ml; n = 31) (P = 0.001 [t test]). All cases involving fatalities had IL-10 levels of ≥1,000 pg/ml, compared with 7 of 31 cases without fatalities (P = 0.001 [chi-square test]). All but one of the cases involving fatalities had preadmission symptom histories of less than 24 h (Fig. 2).

Nine of the 15 patients in the meningococcal meningitis group and 2 of the 4 patients with bacteremia did not have IL-10 levels above the threshold (11 pg/ml). The IL-10 contents of serum controls from healthy individuals were also below the threshold. The nonmeningococcal bacteremic patients had low (≤270 pg/ml) or nondetectable (n = 1) levels of IL-10 in serum, and the surgical septic shock patient who died (*S. aureus* septicemia) had an IL-10 concentration in serum of 100 pg/ml (data not shown).

IL-10 in CSF. IL-10 was detected in 24 of the 27 CSF samples from patients with meningococcal disease (Fig. 3). CSF from patients with meningitis (with or without septic shock [n = 19]) had significantly higher contents of IL-10 (mean, 637 pg/ml; range, 0 to 2,000 pg/ml) than the CSF of patients without meningitis (mean, 41 pg/ml; range, 0 to 230 pg/ml; n = 8) (P = 0.001). CSF from all control patients had IL-10 contents of <11 pg/ml (data not shown).

IL-10 in serum-CSF pairs. IL-10 levels were compared in paired samples of serum and CSF obtained from 12 patients with meningococcal meningitis (without septic shock) and from 8 patients with meningococcal bacteremia with or without septic shock (Fig. 4). The patients with meningitis had higher levels of IL-10 in CSF (mean, 651 pg/ml; range, 0 to 1,825 pg/ml) than in serum (mean, 51 pg/ml; range, 0 to 340 pg/ml) (P = 0.001). Only 2 of the 12 meningitis patients had higher levels of IL-10 in serum than in CSF. The CSF from the bacteremic patients had low contents of detectable IL-10 (mean, 41 pg/ml; range, 0 to 230 pg/ml), whereas the serum had higher IL-10 levels (mean, 10,185 pg/ml; range, 0 to 46,750 pg/ml) (P = 0.136).

Correlations between IL-10 and TNF-α, IL-1, IL-6, and

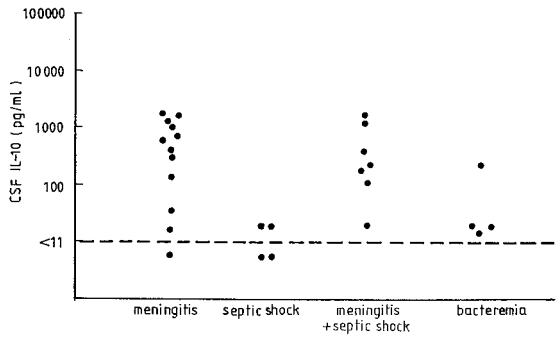

FIG. 3. IL-10 levels in CSF (in picograms per milliliter) of 27 patients with different manifestations of meningococcal disease. IL-10 levels were measured by an EASIA. The broken horizontal line denotes the detection limit (11 pg/ml). ● = case without fatality; † = case with fatality.

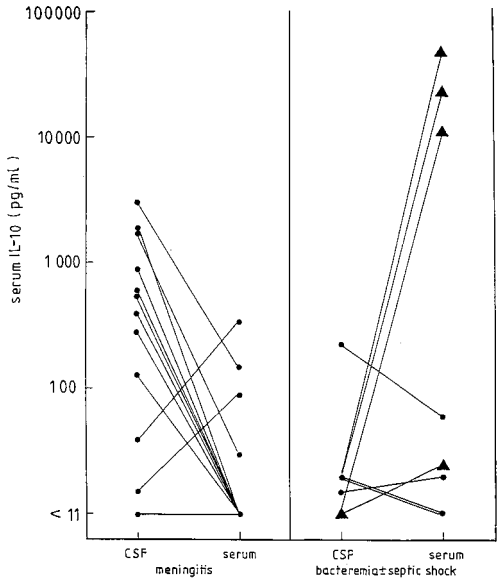

FIG. 4. IL-10 levels in paired samples of serum and CSF (in picograms per milliliter) of 12 patients with meningococcal meningitis and 8 patients with meningococcal bacteremia (with or without septic shock). IL-10 levels were measured by an EASIA. ● = case without septic shock; ▲ = case with septic shock.

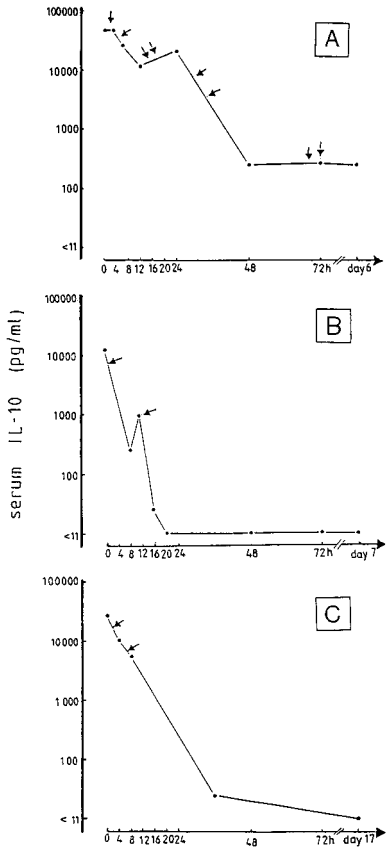

FIG. 5. Pattern of kinetics of IL-10 (in picograms per milliliter) as measured by an EASIA in consecutively drawn serum samples from 3 surviving patients (patients A, B, and C) with meningococcal septic shock. The times after admission are denoted in hours and days. The arrows denote the times of plasma infusions (600 to 800 ml).

IL-8. A positive correlation between levels of IL-10 in serum and those of TNF-α, IL-6, and IL-8 was observed ($r = 0.57$ and $P < 0.001$, $r = 0.87$ and $P < 0.001$, and $r = 0.70$ and $P < 0.001$, respectively). Levels of IL-10 in CSF correlated highly significantly with those of IL-1 ($r = 0.84$ and $P < 0.001$) and IL-6 ($r = 0.88$ and $P < 0.001$), while the correlations between corresponding levels of IL-10 in CSF versus those of IL-8 and TNF-α were less pronounced ($r = 0.30$ and $P = 0.32$ and $r = 0.49$ and $P = 0.014$, respectively).

Kinetics of IL-10. In consecutively drawn serum samples from three surviving patients with septic shock, the highest levels of IL-10 were detected in sera drawn on admission (Fig. 5), suggesting that the IL-10 levels in the circulation had peaked before or on admission and initiation of therapy, including antibiotics and plasma infusions. The half-life appeared to be only a few hours, but in the most severely ill patient (Fig. 5A) with a prolonged period of septic shock and extensive multiorgan failure, elevated levels of IL-10 (250 pg/ml) remained in the systemic circulation 6 days after admission.

DISCUSSION

In the present study, high levels of IL-10 are demonstrated in sera from meningococcal septic shock patients and are associated with fatal outcomes in meningococcal disease. The most severely ill patients exhibited IL-10 levels 10-fold higher than those measured in the nonmeningococcal, septicemic patients included in this study and also those in corresponding patients described elsewhere (12). Sera from patients with meningococcal meningitis or bacteremia without septic shock had low or nondetectable levels of circulating IL-10.

Considerable differences may exist among various categories of septic shock patients. Meningococcal septic shock is often marked by a sudden onset, and characteristically, the clinical condition of previously healthy individuals rapidly deteriorates. The vigorous activation of the cytokine network in these patients is probably due to the high number of bacteria and consequently high levels of endotoxin in the systemic circulation (3).

The high levels of IL-10 in serum on admission in severely ill patients with short preadmission symptom histories (<24 h) (Fig. 2) and the rapid diminution of IL-10 in the systemic circulation of septic shock patients (Fig. 5) suggest that IL-10 is activated early in the cascade of cytokines. The design of this study does not allow any evaluation of the effects of therapy on the observed patterns of kinetics of circulating IL-10 (Fig. 5).

Elevated levels of IL-10 in serum in septic shock patients are present concomitantly with a high content of circulating proinflammatory cytokines like TNF-α, IL-8, and IL-6. The pattern of kinetics of IL-10 also resembles the pattern of kinetics observed for TNF-α in corresponding cases (19). Thus, IL-10, TNF-α, IL-6, and IL-8 are profoundly activated in the initial phase of fatal meningococcal disease, and endogenous IL-10 does not appear to effectively dampen the extensive release of TNF-α, IL-6, and IL-8 in the most severely ill patients.

IL-10 has been reported to protect against lethality in murine models of induced endotoxemia (1, 9, 10). Correlative clinical studies of circulating cytokine levels and mortality do not establish causality, but the extensive activation of IL-10 in the most severe cases and cases involving fatalities implies that endogenous IL-10 does not protect these patients from fatal meningococcal septicemia.

While IL-10 has been shown to have in vitro cytokine-sup-

pressing, antiinflammatory effects, IL-10 has also been shown to act as a mediator of inflammation by recruiting inflammatory cells in vivo (21). Moreover, mice treated with anti-IL-10 MAbs initially demonstrated resistance to *Listeria monocytogenes* infection, indicating that IL-10 actually suppresses resistance during the early stage of this infection (20). Taken together, the present knowledge suggests that the role of IL-10 in serious systemic infections may be more complex than previously expected. This warrants caution on the part of researchers evaluating IL-10 as a candidate for the treatment of septic shock.

IL-10 was found in most CSF samples from patients with meningococcal meningitis. We have previously reported that patients with meningococcal disease localized to the meninges tend to have higher levels of TNF-α, IL-1, and IL-6 in CSF and not significantly higher levels of IL-8 in CSF than patients with meningococcal disease without meningitis (19). A higher level of IL-10 in CSF was also observed in the meningitis patients in the present study. In spite of an initially high IL-10 concentration gradient across the blood-brain barrier, comparably low amounts of IL-10 were detected in serum in the acute phase of meningitis, suggesting an initial compartmentalized release of IL-10 in the subarachnoid space in patients presenting with meningococcal meningitis without persistent circulatory impairment. Furthermore, adherence to tissue and cells in the systemic circulation and inactivation by inhibitors and soluble receptors may minimize the effects of leakage of cytokines during meningitis (19).

This study adds IL-10 to the complex mix of cytokines activated during meningococcal disease and confirms that the cytokine system is vigorously activated in septic shock patients. In fulminant meningococcal septic shock, the IL-10 concentration peaks early and reaches levels 10-fold higher than those observed in septicemia and septic shock induced by other bacterial species. The degree of systemic IL-10 release correlates with the severity of meningococcal disease, and IL-10 does not appear to effectively suppress the extensive release of proinflammatory cytokines in the most severe cases and cases involving fatalities.

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