CLINICAL ROLE OF CIRCULATING INTERLEUKIN-8 IN PATIENTS WITH BACTERIAL INFECTION

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Abstract: The cytokine network is activated in the pathogenetic mechanisms of septic diseases. We measured serum concentrations of interleukin (IL)-8, a leukocyte-mediated cytokine, in 152 patients with culture-proven or suspected septicemia and 25 control subjects. The IL-8 concentrations in patients who were diagnosed as septicemia were significantly higher than those in controls. IL-8 concentrations in septic patients with negative blood cultures were also significantly higher than in controls. In addition, serum IL-8 concentrations were assayed prospectively in 3 patients undergoing chemotherapy for acute myelogenous leukemia. Increased IL-8 concentrations preceded onset of fever and elevation of C-reactive protein by 1 day. Finally, to investigate modulation of cytokine release in vitro, U937 human monocytic leukemia cells were stimulated with phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS), or lipoteichoic acid (LTA). IL-8 mRNA expression was detected by reverse transcription-polymerase chain reaction 30 min after PMA or LPS exposure, and IL-8 was secreted into culture media 3 hr after PMA, LPS, and LTA stimulation. These data suggest that IL-8 is a useful early indicator of bacterial infection.

Key words: Interleukin-8, C-reactive protein, Interleukin-6, Leukemia, Infection

INTRODUCTION

Sepsis and its sequelae remain among the most common and devastating problems encountered in critically ill patients. Despite advances in antimicrobial agents and improved supportive management, an estimated 40 to 50% of patients with septic shock will die, mostly from refractory shock or multiple organ failure.1-3) Because of morbidity and the cost of critical care, early diagnosis and prevention of infectious episodes are one of major economic imperatives.4)

Interleukin (IL)-8, a recently described 6- to 10-kDa protein with leukocyte chemoattractant and activation properties in vitro, is an important mediator of host response to injury and infection.5) IL-8 is produced by a variety of cells including blood monocytes, macrophages, and endothelial cells5,6) when they are stimulated with cytokine inducers such as phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharide (LPS).7) In clinical settings, IL-8 has been studied in association with various acute and chronic infectious conditions including sepsis,8) empyema,9) and pneumocystis carinii pneumonia.10) However, the clinical utility of IL-8 as an early marker...
of bacterial infection or sepsis has not been determined as it has for other cytokines such as IL-6. Moreover, the *in vitro* action of IL-8 remains undetermined (Fig. 1).

We extensively studied a group of septic patients regarding activation of a variety of cells and mediators including neutrophils, C-reactive protein (CRP), and IL-6. To further delineate the role of IL-8 in sepsis generally and a leukemic patients undergoing chemotherapy, we correlated circulating IL-8 concentrations with clinical events. Finally, we determined whether bacterial antigens including PMA, LPS, and lipoteichoic acid (LTA) could induce IL-8 production by a human leukemia cell line *in vitro*.

**MATERIALS AND METHODS**

**Patients**

Patients studied included 152 individuals with a septic syndrome as defined using the criteria described by Bone. 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 of the criteria included treatment with glucocorticosteroids exceeding 20mg/day and a creatinine clearance < 10mL/min. Additionally, blood samples were collected from 3 patients under treatment for acute myelogenous leukemia (AML) at the Third Department of Internal Medicine at our University Medical Center. Healthy controls (n=25) were selected randomly from volunteers among our departmental staff.

**Serum sampling**

Serum was collected in sterile tubes at study entry and on days 1 and 10 following initial sampling in surviving patients. Aliquots of serum samples were prepared, coded by number, and stored in plastic tubes at -70°C until they were assayed blindly. No serum sample underwent more than two freeze/thaw cycles before being assayed.

**Reagents**

Phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS, from Escherichia coli O26:B6), and lipoteichoic acid (LTA, from Staphylococcus aureus and Streptococcus pyogenes), were purchased from Sigma Chemical Co (St. Louis, MO).

**IL-6 and IL-8 assays**

Cytokine concentrations were measured by enzyme-linked immunosorbent assay (ELISA; Fujirebio, Tokyo, Japan) according to a two-step sandwich method. The 96 wells of a microtiter plate were coated with affinity-purified polyclonal antibody to human IL-8. Serum samples were added to the wells. Then a horse radish peroxidase (HRP)-labeled anti-human IL-8 monoclonal antibody was allowed to form an antibody-antigen complex with IL-8 being sandwiched between the primary antibody immobilized on the well and the enzyme-labeled secondary antibody. Finally the complex-bound HRP was allowed to react with a color development system, permitting spectrophotometric measurement of bound IL-8. The limit of detection of the assay was less than 1 pg of human recombinant IL-8/mL. Coefficient of variation (C.V.) in intra assay was 7.1%.

ELISA also was carried out using antibodies against IL-6 as described for IL-8. The assay was specific for IL-6 and was not affected by the presence of other interleukins, tumor necrosis factor-α (TNF-α), interferons, or colony-stimulating factors in serum. The limit of detection of the assay was less than 1 pg of recombinant IL-6/mL.

**Stimulation of human leukemia cells in culture**

The human monocytic cell line U937 was obtained from the American Type Culture Collection (Rockville, MD) and cultured by routine methods in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Gibco). Cultures at a density of about 2×10⁵ cells/mL were treated with 10nM PMA, 1µM LPS, or 1µM LTA. After 10, 30, or 60min of incubation the cells were processed as described below.

**Transcript amplification and detection**

Total RNA was reverse-transcribed in a 25µL reaction volume containing 50 mM Tris (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10mM DTT, 2.5µM oligo (dT) 20 and 200 units of Superscript II reverse transcriptase at 37°C for 1.5hr. One microliter of the first-strand cDNA was synthesized and mixed with 200µM each of dATP, dGTP, dCTP, and dTTP and 0.5µM each of sense and antisense primer, 1.5mM MgCl₂, 1x polymerase chain reaction (PCR) buffer, and 2.5 U of Taq polymerase (Perkin Elmer Fostercity, CA) in a final volume of 10µL. PCR was then carried out in a DNA thermal cycler (UNO II, Biometra, Gottingen, Germany) with 80 cycles of denaturation (94°C for 1 min), annealing (60°C for 5 min), and extension (72°C for 1 min). After PCR, PCR products were analyzed by 1.0% agarose gel electrophoresis followed by staining with ethidium bromide.

**Probe sequence design and synthesis**

Probe sequences were determined using a computer program (HYBsimulator, Advanced Gene Computing Technologies, Irvine, CA). In brief, oligonucleotide sequences were extracted at a temperature of 64°C from
each position of interest in the target gene. Then each oligonucleotide sequence was screened for possible cross-hybridizable genes and their binding strength was estimated using the Genbank sequence database. Oligonucleotide sequences were selected mainly for maximum specificity and minimum likelihood of cross-hybridization against other gene sequences in the database, taking into account their secondary structures. As a result, oligonucleotide probes were designed [IL-8, amplicon size 719; sense, 5'-ACTGAGAGTGATTGAGAGTGGACCA-3' (#1); antisense; 5'-TGGTCCACTCTCAATCACTCTCAGT-3' (#2)] and produced using a synthesizer (Applied Biosystems, Foster City, CA).

Statistical analyses
Statistical analyses were done using chi-squared or Fisher’s exact tests for comparisons of proportions and analysis of variance for comparisons of intergroup differences. All significance levels were two-sided.

RESULTS
IL-8 in patients with sepsis and in healthy controls
Concentrations of IL-8 in serum samples obtained from 25 healthy donors ranged from 1 to 10 pg/mL, with a mean of 4 pg/mL and a standard deviation of 4 pg/mL. On the basis of these results, IL-8 concentra-

![Graph showing circulating interleukin(IL)-8 concentrations in healthy controls (n=25) and in septic patients with bacteremia (Bacteremia +; n=29) or without culture-proven bacteremia (Bacteremia -; n=123). Bacteremia (+): Blood sample was positive with bacterial infection. Bacteremia (-): Blood sample was negative with bacterial infection. * p<.05 vs. healthy controls.](image-url)

![Graph showing correlation between interleukin (IL)-8 and other inflammatory markers including C-reactive protein (CRP), leukocyte count, and IL-6. A significant correlation was found between IL-8 and CRP as well as IL-8 and IL-6.](image-url)
Fig. 4. Changes in inflammatory markers over time in three patients with acute myelogenous leukemia undergoing chemotherapy. Arrows indicate the increase of interleukin (IL)-8 prior to other markers, such as increases of leukocytes and C-reactive protein.
IL-8 in bacterial infection

In serum samples from septic patients, considerably higher IL-8 concentrations were detected, ranging from 8 to 179 pg/mL (Fig. 2). No significant differences in IL-8 concentrations were seen between patients with proven bacteremia and clinically septic patients with negative bacteriologic studies, nor were significant differences evident between patients with gram-positive infections and those with gram-negative or double infections. Moreover, IL-8 concentrations did not differ significantly according to a variety of bacterial pathogen (pathogen data were not shown). Kinetics of IL-8, IL-6, leukocyte counts, and CRP

No significant correlation was seen between IL-8 concentrations and numbers of leukocytes. However, IL-8 correlated positively with circulating CRP and IL-6 in patients with sepsis syndrome (Fig. 3).

IL-8 in AML patients undergoing chemotherapy

We analyzed serial serum samples from three patients with AML undergoing chemotherapy. The time course of IL-8 concentration in these patients is shown in Fig. 4. Case 1 showed a significant elevation in serum IL-8 concentration preceding onset of fever and elevation of CRP. Cases 2 and 3 showed the same sequence. The elevation of IL-8 concentration preceded the IL-6 and CRP in patients with leukemia during bacterial infection (Fig. 5).

Release of IL-8 in human leukemia cell cultures

We assessed whether bacterial antigens including PMA, LPS, and LTA could induce IL-8 production in vitro by a human leukemia cell line. Human leukemia cells (10^6 cells per mL) were challenged with either PMA, LPS, or streptococcal or staphylococcal LTA at time zero, and IL-8 protein levels were determined during incubation. In addition of PMA, LPS, and staph LTA, but not of strep LTA, progressive production of IL-8 was observed until 24 hr incubation (Fig. 6).

Expression of IL-8 mRNA in human leukemia cells

PMA induced expression of IL-8 mRNA in U937 cells at 30 min but no longer induced its expression at 60 min. LPS induced IL-8 mRNA at 10 and 30 min. However, LTA did not induce detectable expression during the experimental period (Fig. 7).

DISCUSSION

We found that patients with the septic syndrome had elevated serum concentrations of IL-8 independently of the presence of a culture-documented infection and changes in other inflammatory markers such as IL-6 concentration, leukocyte counts, or CRP. Early production of IL-8 protein preceded by expression of IL-8 mRNA was confirmed in vitro using a human leukemia cell line. These data suggest that IL-8 is an early initiator in the pathogenesis of sepsis, and that elevation of IL-8 may be as an early marker for bacterial infection or the septic syndrome both generally and in patients undergoing chemotherapy for leukemia.

Enhanced endogenous release of IL-8 has been associated with infection in AML patients with chemotherapy-induced neutropenia. Serum concentrations of this cytokine are higher in patients who have experienced at least one febrile episode, but production of IL-8 preceded with the onset of febrile episodes or the production of IL-6. In clinical settings, measurement of IL-8 shows one promise as an early indicator of bacterial infection, particularly in patients with leukopenia or other immunocompromised hosts. The measurement of IL-8 is useful for an early...
indicator of severe infection. Especially in neutropenic patients, no clinical or laboratory parameters have so far been prospectively confirmed to predict the risk for development of severe infection or death. Therefore, parameters which reliably identify high risk patients for severe infection would be of major importance for a safe supportive treatment of neutropenic cancer patients after chemotherapy. In addition, the measurement of IL-8 should be needed in patients with leukemia, immuno-compromised hosts, graft versus host disease, sepsis, and adult respiratory distress syndrome (ARDS).

Despite differences in the cytokine release between patients with signs of infection and those remaining afebrile, no direct correlation was observed between serum cytokine concentrations and body temperature. Significant concentrations of the potentially pyrogenic cytokine IL-8 were detected in serum samples from a proportion of afebrile subjects as well as from some patients before the onset of fever. The presence of pyrogenic cytokines in serum is not invariably associated with fever.

U937 cells, a leukemic human mononuclear cell line, have been used to investigate a number of human monocyte signaling pathways such as increase of intracellular calcium, and tyrosine phosphorylation. A previous study has demonstrated that PMA and LPS induced expression of IL-8 mRNA and protein secretion in combination with an increase of intracellular calcium. Therefore, we used U937 cells to determine IL-8 mRNA and protein expression in response to PMA, LPS, and LTA. We observed significant expres-

![Fig. 6. Time course of interleukin (IL)-8 secretion from U937 cells stimulated by PMA, LPS, and streptococcal (strep) LTA or staphylococcal (staph) LTA. After 3 hr, all pyogens significantly stimulated IL-8 secretion (P < .05). Titers of IL-8 stimulated by PMA were significantly higher than those stimulated by LTA. Bars show the mean value for three different experiments.](image)

![Fig. 7. Expression of interleukin (IL)-8 mRNA in U937 cells stimulated by PMA, LPS, and staphylococcal (staph) LTA. After 30 min of stimulation, both PMA and LPS induced expression of IL-8 mRNA.](image)
sion of IL-8 mRNA and elevation of IL-8 protein within 3 hr of PMA and LPS treatment.

LPS is believed to be the major trigger of immune cell activation in this setting, leading to release of host-derived proinflammatory cytokines and further amplification of the inflammatory response.17,18) Gram-negative bacteria are associated with LPS and vigorous clinical and experimental inflammatory responses.19,20) LTA is an immunologically active constituent of the gram-positive bacterial cell wall. LTA has been reported to induce IL-8 protein in human blood monocytes.7) Our investigation showed that only staphylococcal LTA induced IL-8 protein. These results suggest that not only gram-negative, but also gram-positive organisms can induce IL-8 protein secretion by human blood cells.

Standiford T. et al found that although LTA can induce the secretion of IL-8 from human peripheral blood monocytes, the concentration of LTA at which IL-8 production occurs is approximately 100-fold greater than that of LPS. Furthermore LPS at the maximal doses tested induces approximately twice as much IL-8 mRNA and protein than did comparable concentrations of staphylococcal and streptococcal LTA. At the same dose, streptococcal LTA weakly induced the IL-8 mRNA in comparison with staphylococcal LTA.7) The above-mentioned fact was compatible with our result. Bhakdi S. et al reported that certain LTAs could provoke maximal stimulation of IL-1, IL-6, and TNF release from human monocytes, whereas staphylococcal LTA was not a strong inducer of cytokine production.21) This might show the difference between IL-8 and other cytokines. The further study will be needed to clear this difference.

Circulating cytokines have been examined extensively in the septic syndrome.20) Circulating TNF-α, IL-1, and IL-6 concentrations were elevated independently of culture-documented infection in these patients. IL-6 concentrations have been linked to mortality in sepsis.422) and an IL-6 peak is prominent at the onset of sepsis in contrast to circulating TNF-α, which rapidly declines after reaching an irrespective peak of the course of the underlying infection.23) Circulating IL-8 concentrations rise rapidly after onset of bacterial infection, as early as those of TNF-α, which usually exceed a 10-fold excess over IL-8 concentrations in uninfected control subjects.4) Our in vitro studies demonstrated IL-8 mRNA in stimulated human cells only 30min after exposure. Therefore, IL-8 may be a particularly early marker for bacterial infection. During viral infection, a variety of cytokines are induced,24,25) but cytokine response patterns may differ from those in bacterial infection.

In recent study of volunteers experimentally infected with influenza A virus increased serum concentrations of IL-6 and TNF-α preceded an increase in serum IL-8 concentration.26) This study implicated that IL-6 was a marker in the early phase of host defense against influenza viral infection, and that IL-8 was a specific type representing a second wave of proinflammatory cytokine response in this specific type of infection. Our findings for bacterial infection showed the opposite sequence. In this regard, combined measurements of IL-8 and IL-6 may be clinically useful for early differentiation of bacterial from viral infection. Further study is needed to test this idea.

In summary, our findings indicate that measurement of serum IL-8 concentration may provide an early indication of human bacterial infection. The roles of IL-8 in septic shock and the host defense mechanism are important matters for further study.

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