Increased Expression of the Interleukin 1 Receptor on Blood Neutrophils of Humans with the Sepsis Syndrome

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Abstract

Because of the potential importance of interleukin 1 (IL-1) in modulating inflammation and the observations that human blood neutrophils (PMN) express IL-1 receptors (IL-1R) and synthesize IL-1α and IL-1β, we studied the IL-1R on blood PMN from a group of patients with the sepsis syndrome. We report a marked enhancement in the sites per cell of IL-1R expressed on sepsis-PMN of 25 consecutively studied patients compared to 20 controls (patient mean = 9,329±2,212 SE; control mean = 716±42 SE, respectively). There was no demonstrable difference in the K_d of IL-1R on sepsis-PMN ($\sim$ 1 nM) as determined by saturation curves of ^{125}I-IL-1α binding and the IL-1R on sepsis-PMN had an apparent $M_r \sim 68,000$, a value like that of normal PMN. Cytofluorographic analysis indicated that the sepsis-PMN phenotype is a single homogeneous population with respect to IL-1R expression. In contrast, expression of the membrane complement receptor CR3 is not increased on sepsis-PMN. Similar increases in expression of IL-1R were not observed in various other inflammatory processes, including acute disseminated inflammation and organ failure not caused by infection, acute infection without organ failure, and immunopathologies such as active systemic lupus erythematosus and rheumatoid arthritis. Enhanced expression of IL-1R was not related simply to the state of myeloid stimulation. Increased expression of IL-1R on normal PMN was induced in vitro by incubating cells with recombinant human granulocyte-macrophage/colony-stimulating factor for 18 h and this response was inhibited by cycloheximide, suggesting the possibility that de novo synthesis of IL-1R might occur in PMN during the sepsis syndrome. (*J. Clin. Invest.* 1991. 88:1452–1459.) Key words: septic shock • inflammation • complement CR3

Introduction

Interleukin-1 (IL-1) occurs as α and β polypeptides that activate specific receptors for IL-1 (IL-1R)[1] (reviewed in reference

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1. *Abbreviations used in this paper:* GM-CSF, granulocyte-macrophage/colony-stimulating factor; IL-2R, IL-2 receptor(s); rhIL-1, recombinant human IL-1; TNFα, tumor necrosis factor-α.

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1). IL-1 exerts a wide range of biological effects on many cells. As a mediator of inflammation and host responses to infection, IL-1 has effects on thermoregulation, lymphocyte function, synthesis of acute phase proteins, production of arachidonic acid metabolites, granulocytosis, carbohydrate, and protein and lipid metabolism (2–4). The importance of IL-1 and tumor necrosis factor-α (TNFα) in the sepsis syndrome has recently been documented (5, 6), making the characterization of the IL-1R of prime importance. Recently, we (7) and others (8) reported that a single class of high-affinity IL-1R exists on the surface of circulating human neutrophils (PMN). Normal resting PMN express $\sim$ 700 receptor sites per cell with an apparent K_d of $\sim$ 1.0 nM as determined by saturation curves and Scatchard analysis of ^{125}I-IL-1α binding. Based on cross-linking analysis, the IL-1R of the PMN has an apparent M_r of $\sim$ 68,000, a value smaller than the IL-1R of T-cells and fibroblasts ($M_r \sim 80,000$) (9–12) but similar in size to the IL-1R of B-cells and monocytes (13–15).

Detectable levels of circulating TNFα and IL-1 occur in serum during sepsis in humans (5, 6) and both of these cytokines can mimic the sepsis syndrome in experimental animals (16–18). Children with severe meningococcemia and other forms of sepsis have elevated levels of TNFα and IL-1 in serum and the quantity of these circulating cytokines may correlate with the severity of the disease (5, 6).

We recently reported that normal human blood PMN synthesize IL-1β and IL-1α in response to endotoxin or IL-1 itself (19). Synthesis of IL-1β by human PMN predominates over that of IL-1α and IL-1β, but little IL-1α is secreted by these cells. Because of the potential importance of IL-1 in the sepsis syndrome, we studied the expression of IL-1R on human blood PMN obtained from patients with this highly lethal disseminated inflammatory process. We report that sepsis-PMN consistently express a marked increase in the number of IL-1R without a change in K_d (apparent $M_r \sim 68,000$). The enhanced expression of IL-1R on sepsis-PMN occurs as a unimodal and homogenous event that is reversible, and it is not accompanied by increased expression of the complement receptor CR3. Enhanced expression of IL-1R on sepsis-PMN is not related simply to a state of myeloid stimulation. Marked amplification of IL-1R expression is not observed in other inflammatory processes, whether induced by infection or immunopathologic events.

Methods

Patient selection and chart review. Patients admitted to the Intensive Care Unit of the Wake Forest University Medical Center were screened for signs and symptoms of the sepsis syndrome, and then selected for entry into the study on the basis of the criteria of Bone et al. (20) (Table I). Patients with known immunodeficiencies (primary or secondary) were excluded from the study, as were trauma or burn victims within 1 wk of presentation. Once selected, informed consent was obtained

Table I. Clinical Criteria for Enrollment of Subjects with the Sepsis Syndrome

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Inclusion (all of the following) essentially as defined by Bone et al. (20):

(a) Clinically apparent focus of infection

(b) Fever (> 38.3°C, rectal) or hypothermia (< 35.6°C, rectal)

(c) Tachycardia: heart rate > 90 beats/min

(d) Tachypnea: > 20 spontaneous breaths per minute or a P_aCO_2 < 32 mmHg

(e) Evidence of organ dysfunction—at least one of the following:
  Altered mentation (in relation to patient's baseline)
  Hypotension (< 90 mmHg systolic arterial pressure)
  Hypoxemia: P_aO_2 < 75 mmHg on room air, or an equivalent
  oliguria: < 30 ml or < 0.5 ml/kg·h for > 2 h
  Metabolic acidosis: elevated serum lactate (> 2.2 mg/dl) or decreased serum bicarbonate (≤ 15 meq/liter) with an anion gap of > 15 accepted as criteria in the absence of a serum lactate level, unless the patient had chronic renal failure

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from the patient or next of kin and 30–60 ml of heparinized venous or arterial blood were drawn and delivered to the lab within 30 min. A venous blood sample was obtained simultaneously on healthy adults to serve as a source of control cells, and the samples were handled and processed in similar fashions. A chart review was conducted on all patients and data analysis conducted.

Neutrophil isolation. Human PMN (98–99% pure) were isolated from heparinized venous or arterial blood using a modification of a previously described technique (21), with Isolymph (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY) sedimentation and gradient centrifugation, followed by hypotonic lysis of erythrocytes. The cells were suspended in sterile binding media (RPMI 1640, 5% fetal calf serum, 25 mM Hepes, pH 7.4) at a concentration of $1.2-6.0 \times 10^7$ cells/ml and kept on ice until assayed. Cell viability using the trypan blue exclusion method remained unchanged over 8 h. The percentage of early forms of PMN after isolation did not differ significantly from the whole blood differential.

Iodination of IL-1. Recombinant human IL-1α (rhIL-1α) (a gift from Dr. Peter Lomedico, Hoffmann-LaRoche, Inc., Nutley, NJ or Drs. Steve Gillis and Steve Dower, Immunex Corp., Seattle, WA) was radioiodinated with carrier-free Na-^{125}I (Amersham Corp., Arlington Heights, IL) by using Enzymobeads (Bio-Rad Laboratories, Richmond, CA) as previously described by Paganelli-Parker et al. (8). The ^{125}I-IL-1α among preparations exhibited a specific activity of 30–70 μCi/μg of protein and retained 90% of its biological activity as measured by the D10.G4.1 murine co-mitogen assay. The radiolabeled protein was stored at 4°C and used within 3 wk.

^{125}I-IL-1α radioreceptor binding assay. PMN from patients and controls and ^{125}I-IL-1α at varying concentrations were incubated in binding media in a total volume of 150 μl for 4 h at 4°C on a rocker platform (7). Nonspecific binding was measured by incubations in the presence of at least a 100-fold molar excess of unlabeled rhIL-1α. Samples were performed in triplicate with variation in triplicate binding being < 10% in most instances. Cell-bound and free ^{125}I-IL-1α were separated by centrifugation through 100 μl of a silicone oil mixture (1 part Thomas oil to 2 parts Gallard-Schlesinger oil). Tips containing pelleted cells and bound labeled protein were then counted on a gamma counter and Scatchard analysis of saturation curves was performed.

Internalization of human ^{125}I-IL-1α in human PMN. 2×10^7 PMN were incubated with 5×10^{-10} M ^{125}I-IL-1α for 1 h at 4°C in the presence and absence of a 100-fold excess of unlabeled rhIL-1α. Cells were washed twice in the binding medium to remove unbound IL-1α and then placed at 37°C. Aliquots were removed at the indicated times, centrifuged, and resuspended in 50 mM glycine-HCl buffer (pH 3) containing 150 mM NaCl for 5 min at 4°C. The cells were then sepa-

rated from the buffer and released IL-1 was separated by centrifugation through silicone oil. Cell pellet and supernatant were recovered and counted.

Receptor sizing. PMN (2×10^7 cells) from patients and controls were incubated with 5×10^{-10} M ^{125}I-IL-1α in the presence or absence of 100-fold excess of unlabeled protein for 1 h at 4°C. The cells were then washed twice with ice-cold phosphate-buffered saline (PBS) containing 1 mM MgCl at pH 8.5 and then incubated at room temperature for 30 min with 0.15 mg/ml of the homobifunctional cross-linker, disuccidimidyl suberate (Pierce Chemical Co., Rockford, IL). The cells were then washed twice in PBS-1 mM MgCl at pH 8.5 buffer. After centrifugation, the entire cell pellet was resuspended in 10% SDS in 0.06 M Tris, pH 6.8, boiled for 2 min, and then centrifuged again. The cell supernatants were then stored overnight at −20°C. The following day the samples were suspended in 10% volume of treatment buffer containing 4% SDS in 0.06 M Tris buffer with glycerol and bromophenol blue, pH 6.8, in the presence or absence of 2-β-mercaptoethanol and boiled for an additional 2 min before being subjected to electrophoresis on a 7.5% polyacrylamide gel. Gels were then dried and exposed to film for up to 21 d at −70°C.

Flow cytometry. Cells were suspended in binding medium at a concentration of 2×10^6 cells/ml and incubated with 5×10^{-10} M unlabeled rhIL-1α for 4 h at 4°C. After three washes in binding medium, the cells were incubated sequentially with nonimmune goat IgG 1:100 dilution (a gift from Steve Kunkel, University of Michigan) for 30 min, goat anti–human IL-1α, 1:100 dilution, (a gift from Richard Chizzonite, Hoffmann-LaRoche, Inc.) for 60 min, and fluorescein isothiocyanate (FITC)-conjugated anti–goat IgG-F(ab')₂, 1:10 dilution (Cappel Laboratories, Cooper Biomedical, Inc., Malvern, PA) for 30 min. For evaluation of CR3, cells were incubated at 4°C for 30 min with a phycoerythrin conjugated anti–CR3 monoclonal antibody (Becton, Dickinson & Co., Mountain View, CA) which recognizes the CD11b γ subunit. Cells were washed twice in cold binding media between additions of the different antibody preparations. Cytofluorography was then performed on a FACS machine (Becton, Dickinson & Co.) with separate gating for red and green fluorescence.

Statistical analysis. The nonpaired or paired, two-tailed, Student's t test was employed for statistical analyses.

Results

Enhanced expression of the IL-1R on PMN from septic patients analyzed by ^{125}I-IL-1α saturation curves. The number of IL-1R sites per cell on human PMN from healthy volunteers and patients with the sepsis syndrome was quantitated using ^{125}I-IL-1 binding studies and Scatchard analysis of saturation curves. 25 patients and 20 normal controls were studied. Normal PMN had ~ 700 IL-1R per cell with a K_d of ~ 1.4 nM (Fig. 1), a finding similar to that reported by Rhyne et al. (7) and Paganelli-Parker et al. (8). In contrast, sepsis-PMN had a dramatic increase in the sites per cell of IL-1R compared to normal PMN (Fig. 1, *top*). A wide variation in IL-1R sites per sepsis-PMN (2,100–50,000) was found among the patients, but in all cases receptor expression was markedly increased above control levels, with a mean of 9,329±2,212 SE IL-1R per cell on sepsis-PMN and 716±42 SE IL-1R per cell on normal PMN ($P < 0.001$). No apparent difference in the K_d of IL-1R was noted between the sepsis-PMN (mean = 1.34 nM±0.25 SE) and normal PMN (mean = 1.43 nM±0.09 SE) (Fig. 1, *bottom*).

A patient with the sepsis syndrome followed over time is shown in Fig. 2 *A*. A marked elevation in the number of sites per PMN of IL-1R was present at the outset (~ 9,000). This patient had organ failure (brain and cardiovascular failure) only during the first 5 d of study. The increased expression of IL-1R returned to normal after organ failure subsided; the K_d
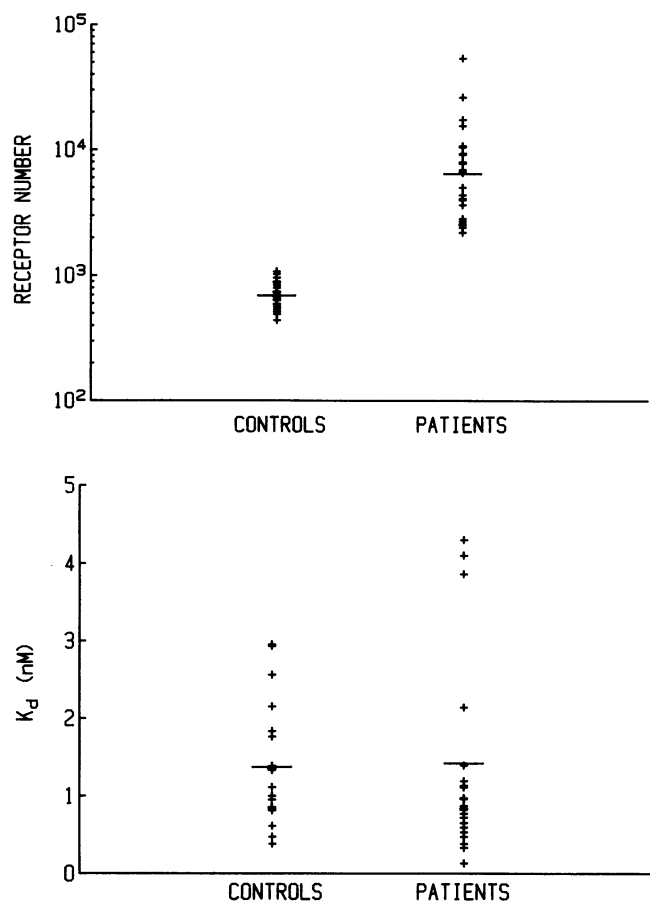
Figure 1. Expression of IL-1R on sepsis- vs. normal PMN. (Top) Scatter plot of sites per cell of IL-1R expressed on human PMN from 25 patients with the sepsis syndrome and from 20 controls. IL-1R sites per cell were determined by Scatchard analysis of saturation curves. Patient mean = 9,329±2,212 SE; control mean = 716±44 SE (P < 0.001). (Bottom) Scatter plot of IL-1R dissociation constant (K_d) on PMN from patients with the sepsis syndrome and controls. The K_d was determined from Scatchard analysis of saturation curves. Patient mean = 1.34 nM±0.25 SE; control mean = 1.43 nM±0.09 SE (P > 0.5).
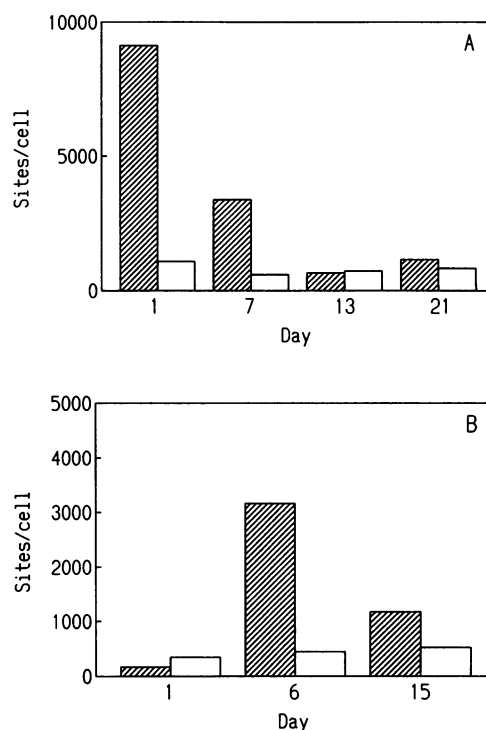

Figure 2. Longitudinal studies of IL-1R expression on PMN from patients with the sepsis syndrome. (A) A patient studied during and after recovery of the sepsis syndrome. Values are expressed as sites per cell of patient (hatched bars) or control (open bars) PMN. The patient had organ failure (brain and cardiovascular) only during the first 5 d of study. (B) A patient studied before, during, and after resolution of the sepsis syndrome. When first studied the patient had daily temperatures of 104°F without apparent infection. The second analysis was performed when the patient had the sepsis syndrome (hypotension and the adult respiratory distress syndrome). The third analysis was after recovery of hypotension and adult respiratory distress syndrome.

of IL-1R was always similar to that of normal PMN (not shown).

Another patient was studied before, during and after the sepsis syndrome. We first evaluated IL-1R in this patient when there was marked elevation in temperature (104°F) and no apparent infection (fever was felt to be due to a drug reaction or an alteration in thermal regulation). The patient then developed the sepsis syndrome with hypotension and the acute respiratory distress syndrome, and later recovered. IL-1R on PMN was evaluated at the three different phases of illness and the results are shown in Fig. 2 B. Normal expression of IL-1R was seen when there was high fever and no infection; IL-1R expression increased during the sepsis syndrome and rapidly returned to normal after recovery.

Analysis of patient data. In reviewing patient characteristics, no correlation could be found between the magnitude of the enhanced IL-1R expression and patient age (age range 15–101 yr), sex, race, the level of temperature, total white blood cells, absolute PMN count, presence of band-forms (range < 5% to > 30%), blood pressure, number of organs in failure,

organism responsible for infection (gram-positive or gram-negative bacteria, and fungi [Candida species] were causal), location of infection, culture of microbes from the blood, use of vasopressors, or patient outcome. There was no apparent relationship between sites per cell of IL-1R and the time the blood sample was obtained with respect to onset of the sepsis syndrome. The earliest that a patient was studied after onset of the sepsis syndrome was ~ 8 h, but virtually all patients were first studied within 72 h of onset.

Expression of IL-1R in other states of inflammation and myeloid stimulation. We next determined if amplified expression of IL-1R might discriminate the sepsis syndrome from other states of inflammation and myeloid stimulation. We divided patients into the following categories: (a) disseminated inflammation (fever, leukocytosis) without apparent infection but with organ failure; (b) disseminated inflammation with infection and without organ failure; (c) disseminated inflammation without infection or organ failure; (d) local inflammation without infection or fever. The results are shown in Table II. Of particular note: (a) the four patients with disseminated inflammation without infection but with organ failure had a mean of 1,467±308 SD IL-1R sites per PMN, a value significantly above normal (P < 0.01) but significantly below sepsis-PMN (P < 0.001); (b) the four patients with either active systemic lupus

Table II. IL-1 R Expression on PMN from Different States of Inflammation and Myeloid Stimulation

Condition	IL-1 R sites/cell
Normal	716 (42 SE)
Septic shock	9,329 (2,212 SE)
Disseminated inflammation without infection, with organ failure	
Systemic lupus: liver/renal failure	1,408
Acute pancreatitis: liver/renal failure	1,143
Dilantin syndrome: liver/renal failure	1,430
Hypersensitivity angiitis/renal failure	1,886
Disseminated inflammation with infection, but without septic shock	
Pneumonia, mixed gram-negative	1,000
Pneumonia, pneumococcal	460
Acute bacterial cellulitis	600
Disseminated inflammation without infection or organ failure	
Drug reaction, fever, and rash	250
Drug reaction, fever and rash	500
Drug reaction, fever, and rash	600
Local inflammation without infection or fever	
Systemic lupus, active arthritis	163
Systemic lupus, active glomerulonephritis	210
Rheumatoid arthritis, active	360
Rheumatoid arthritis, active	210
Psoriasis	1,279
Myeloid stimulation without inflammation	
Chronic granulocytic leukemia	569
Chronic granulocytic leukemia	109
Polycythemia vera	870

Figure 3. Rate of internalization of ^{125}I-IL-1α in sepsis-PMN. PMN were first incubated at 4°C for 1 h with ^{125}I-IL-1α with or without 100-fold excess of cold rhIL-1α. Cells were then warmed to 37°C for varying times, cooled to 4°C, and washed in glycine-HCl buffer (pH 3) to remove externally bound IL-1α before counting internalized ^{125}I-IL-1α.

Figure 4. Effect of in vitro administered rhGM-CSF on the expression of IL-1R of normal PMN. (*A*) PMN incubated with 100 U/ml rhGM-CSF for 18 h at 37°C. Controls were maintained at either 4°C or 37°C for 18 h in the absence of rhGM-CSF. Values are expressed as counts per minute specific binding±SE of *n* = 4 experiments performed in triplicate. The difference between the means of 4°C control and 37°C untreated control vs. rhGM-CSF are *P* < 0.01 and *P* < 0.01, respectively. The last bar is the mean of two experiments showing the effect of 5 μg/ml of cycloheximide on up-regulation of IL-1R by rhGM-CSF. (*B*) Scatchard analysis of a representative experiment showing control PMN or PMN incubated at 37°C for 18 h with 100 U/ml of rhGM-CSF. No specific binding was detected in the control PMN incubated for 18 h at 37°C in the absence of rhGM-CSF (not shown).

erythematosus (*n* = 2) or rheumatoid arthritis (*n* = 2) but without infection or fever had a mean of 236±86 SD IL-1R per cell, a value significantly lower than that of normal PMN (*P* = < 0.01); (*c*) no patient in any group exceeded 2,100 IL-1R sites per PMN of IL-1R, a value below the lowest value of the range of IL-1R observed on sepsis-PMN. One of the two patients with acute granulocytic leukemia (untreated and with ~ 20% blast forms and < 10% mature PMN) had a low number of IL-1R (109 sites per PMN).

Internalization of ^{125}*I-IL-1 on sepsis-PMN.* Fig. 3 shows the rate of internalization of ^{125}I-IL-1 bound to sepsis-PMN. 50% internalization of ligand was observed by 30 min, a value like that we reported for normal PMN (7).

Effects of granulocyte-macrophage/colony-stimulating factor (GM-CSF) on IL-1R expression on normal PMN. Since myeloid stimulation per se did not appear to be responsible for increased expression of IL-1R on sepsis-PMN and the rate of internalization of ^{125}I-IL-1α (and presumably IL-1R) was normal, we wondered whether normal blood PMN are capable of increasing the expression of IL-1R in vitro. PMN isolated from venous blood from healthy volunteers (*n* = 4) were incubated for 18 h at 4°C or 37°C with or without 100 U/ml of recombi-
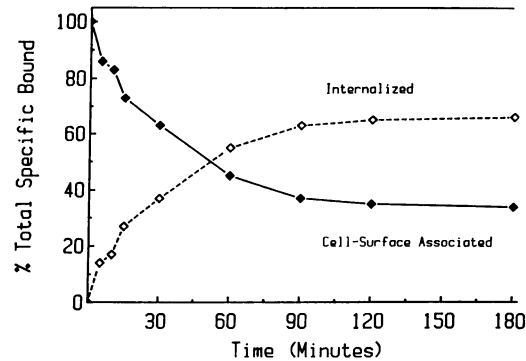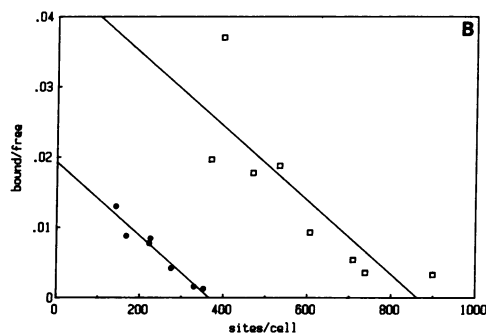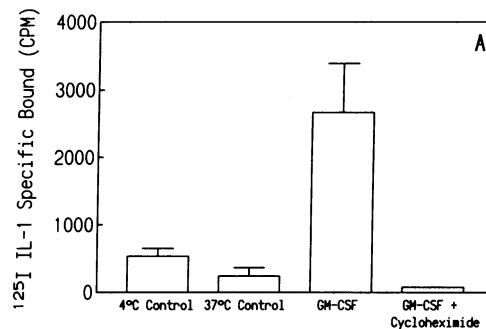
nant human GM-CSF (rhGM-CSF) (Cistron Corp, Pine Brook, NJ) and analyzed for binding of ^{125}I-IL-1α. As shown in Fig. 4 *A*, PMN incubated at 37°C for 18 h without rhGM-CSF had reduced specific binding of ^{125}I-IL-1α when compared with a 4°C control. In contrast, PMN incubated with rhGM-CSF had an increase in specific binding at 18 h ($P < 0.01$), and this increase could be almost totally inhibited by 5 μg/ml of cycloheximide, suggesting that synthesis of IL-1R occurred rather than alterations in recycling of previously synthesized receptors. Shown in Fig. 4 *B* is a Scatchard analysis. There was an increase in IL-1R induced by rhGM-CSF from 400 IL-1R sites per cell in control PMN held at 4°C for 18 h to ~ 800 sites per cell of IL-1R in PMN treated with rhGM-CSF for 18 h at 37°C; no change in K_d occurred. In this experiment, no specific binding was detected in the PMN incubated without rhGM-CSF for 18 h at 37°C.

Cytofluorographic analysis of IL-1R and CR3 expression on sepsis-PMN. Enhanced expression of the complement receptor CR3 on human blood PMN has been reported after burn injuries (22, 23) and in vitro after warming of cells to 37°C (24). To determine if increased expression of IL-1R is accompanied by an increase in CR3 expression, or if receptor up-regulation on sepsis-PMN is unimodal, we used flow cytometry to simultaneously analyze both CR3 and IL-1R on PMN from three septic patients and three controls. A unimodal increase in expression of IL-1R was observed on sepsis-PMN vs. normal PMN (Fig. 5 *A*). In contrast, no increase in the expression of CR3 was observed on sepsis-PMN with increases in expression of IL-1R (Fig. 5 *B*). As a further control, we determined that the antibody to the CD11b γ chain of CR3 could detect upregulation of CR3 on normal PMN stimulated by either N-formyl-methionyl-leucyl-phenylalanine or bacterial lipopolysaccharide (not shown).

An increased quantity of the low molecular weight form of IL-1R is expressed on sepsis-PMN. The size of the IL-1R was evaluated on PMN from three patients with the sepsis syndrome and three controls using crosslinking, solubilization, and electrophoresis. In all instances, a major band was observed with an apparent M_r of ~ 68,000 when corrected for bound IL-1α. An example is shown in Fig. 6. The bands observed were similar in M_r to those observed on normal PMN, but were of much stronger intensity in patients (*C*) than in controls (*A*). Bands with a higher M_r were always seen, perhaps representing aggregated IL-1R or IL-1R associated with other cellular proteins; lower M_r bands were not always detected and could represent proteolytic cleavage or a different species of IL-1R. Precise loading of a single lane was impossible so "overflow" of bands into adjacent lanes could not be avoided. All cross-linked bands could be completed by unlabeled IL-1α (*B* and *D*).

Discussion

PMN obtained from the peripheral blood of patients with the sepsis syndrome consistently have a unimodal increase in the number of IL-1R expressed on their surfaces with a normal K_d (Fig. 1). IL-1R expression on PMN returns to normal within a few days after resolution of the sepsis syndrome (Fig. 2). An increase in expression of IL-1R on sepsis-PMN was demonstrated by three methods, including saturation curves of ^{125}I-
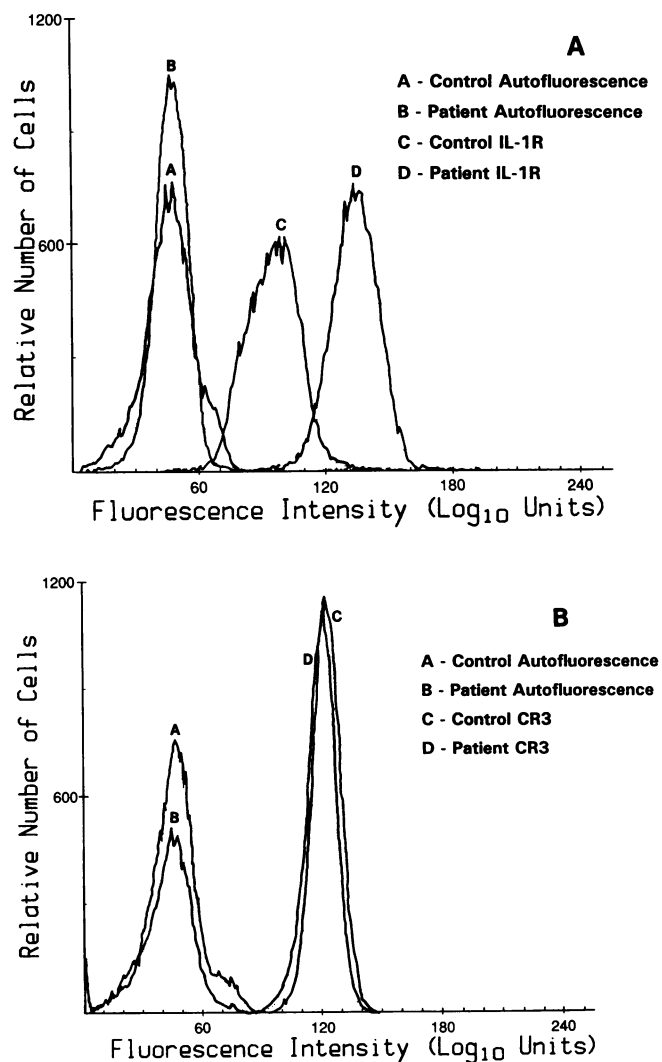

Figure 5. Cytofluorographic analysis of expression of IL-1R and CR3 on sepsis- and normal PMN. Fluorescence histograms of FACS analysis of PMN from a representative of three experiments of sepsis-PMN and normal PMN after staining with fluorescein or phycoerythrin-conjugated antibodies to detect IL-1R (*A*) and CR3 (*B*), respectively. All 3 patients studied had a unimodal increase in IL-1R with no detectable change in CR3.

IL-1α and Scatchard analysis, flow cytometry, and cross-linking of ^{125}I-IL-1α bound to IL-1R. Surprisingly, all 25 patients studied had an elevation in the number of IL-1R expressed per sepsis-PMN, suggesting that high levels of IL-1R expression of PMN might be a useful marker for the sepsis syndrome. Moreover, our investigation of IL-1R expression on blood PMN obtained from other states of inflammation and myeloid stimulation suggests that the level of expression of IL-1R may discriminate among various disease categories (Table II). Of particular note is our observation that PMN from patients with acute disseminated inflammation and organ failure not caused by infection do not express the high number of IL-1R sites per cell that are found on sepsis-PMN, although their values are higher than normal PMN. Furthermore, infections without organ failure but with signs of systemic inflammation such as fever and
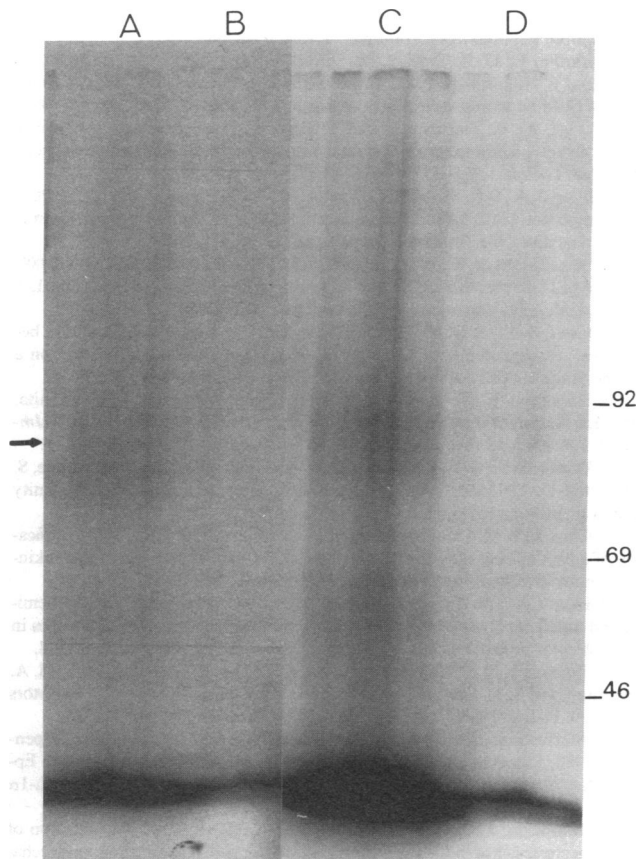
Figure 6. SDS-PAGE analysis of co-valently cross-linked IL-1R and ^{125}I-IL-1 on sepsis and normal PMN with and without competition by rhIL-1α. (*A*) Normal PMN without competition. (*B*) Normal PMN with competition. (*C*) Sepsis-PMN without competition. (*D*) Sepsis-PMN with competition. The molecular weight standards are shown in the right margin (in thousands). The arrow on the left is thought to represent the IL-1R with an M_r of ~ 68,000. Normal and sepsis-PMN were studied in parallel, but the film of the normal PMN was exposed for 30 d and that of sepsis-PMN for 7 d.

leukocytosis appear not to be associated with increases in IL-1R, and active inflammatory processes such as systemic lupus erythematosus and rheumatoid arthritis may be accompanied by a decrease in expression of IL-1R on circulating PMN. We emphasize that more patients among various types of inflammatory processes must be studied before the actual sensitivity and specificity of IL-1R expression on PMN can be established as the indicator for these diseases.

The increased expression of IL-1R on circulating PMN during the sepsis syndrome surprised us in that we had originally hypothesized that PMN from these patients would have a decrease in expression of IL-1R, because sepsis is often associated with circulation of IL-1 as well as other cytokines such as TNFα (5, 6, and reviewed in reference 25). Circulating IL-1 would be expected to homologously down-regulate the IL-1R of PMN, and circulation of TNFα might reduce IL-1R by heterologous down-regulation. Indeed, we have found that homologous down-regulation of IL-1 occurs in vitro after stimulation of normal PMN with IL-1 (7), and heterologous down-regula-

tion of IL-1R is induced by many stimuli, including endotoxin, FMLP, and TNFα (7, 26). We cannot explain the apparent paradox of increased expression of IL-1R on sepsis-PMN. Although we do not know if the patients we studied had circulating IL-1 or TNFα, elevations in plasma IL-1 and TNFα would be expected in some of our patients.

The M_r of IL-1R expressed on sepsis-PMN is the same as that found on normal PMN, further establishing that PMN express a lower M_r form of IL-1R. At least two forms of IL-1R are expressed on cells (1, 27). One has a M_r of 80,000 (7, 8, 14, 15) and is found on T-cells and cells derived from mesenchyme, such as fibroblasts and synovial cells (9–13); another is a lower M_r IL-1R (60,000–70,000) found on B cells, monocytes, and PMN (7, 8, 14, 15). The high and low M_r forms of IL-1R are now referred to as types 1 and 2 IL-1R, respectively, and they appear to be products of separate genes (14, 28). Types 1 and 2 IL-1R differ not only in M_r but in the transductional events linked to functional responses of cells stimulated by IL-1 (13).

Another surprising finding in this study is that the increased expression of IL-1R on sepsis-PMN is not associated with an increase in expression of complement receptor CR3. Increased expression of CR3 has been reported on blood PMN obtained from patients with the inflammation of burn injury (22, 23), on PMN in exudates (29, 30), and on blood PMN of patients with systemic lupus erythematosus (31). Although we observed no change in CR3 expression on sepsis-PMN, the increased expression of specific receptors on sepsis-PMN is not limited to IL-1R. We also have observed a unimodal increase in expression of the receptor for TNFα on sepsis-PMN in 11 of 11 patients with the sepsis syndrome (32); sepsis-PMN from these patients also had no change or a decrease in expression of the CR3 receptor. We have found that agents that enhance the expression of CR3 on normal PMN in vitro always concomitantly induce a rapid down-regulation of the IL-1R (26). A somewhat analogous finding of discordant expression of receptors on human PMN was reported for C5a and the receptors CR1, CR3, FcγR III, and FMLP (33). Apparently, the expressions of IL-1, TNFα, and C5a receptors on PMN are under different controls than the other receptors, and up-regulation of some receptors may be associated with down-regulation of others.

We have not clearly identified the mechanism responsible for enhanced expression of IL-1R on sepsis-PMN. Two anatomical sites might possibly regulate expression of IL-1R, the bone marrow and the peripheral circulation. Our data (Table II) suggest that myeloid stimulation in the presence or absence of severe systemic inflammation is not a sufficient stimulus to induce up-regulation of IL-1R. Our finding that the IL-1R expression increases in normal PMN incubated with rhGM-CSF in vitro, and that this increase is inhibited by cycloheximide, suggests that enhanced expression of IL-1R on sepsis-PMN could depend on events that take place in the peripheral circulation and that require protein synthesis. However, it is unlikely that GM-CSF alone would be the agonist responsible for increased expression of IL-1R, since the increase in number of IL-1R induced by rhGM-CSF on normal PMN was well below that found in sepsis-PMN and increases in circulating GM-CSF have not to our knowledge been reported in the sepsis syndrome. Other stimuli released during the sepsis syndrome,

either alone or acting together, might amplify expression of the IL-1R gene. For example, Spriggs et al. recently reported that the steroid dexamethasone and prostaglandin E_2 used together markedly enhance expression of the low M_r II-1 R expressed on human monocytic cells (34). Dr. Spriggs has also found that stimulation of normal human PMN in vitro with dexamethasone and prostaglandin E_2 causes a significant increase in the expression of IL-1R (personal communication).

The functional responses of normal PMN to stimulation of IL-1R by IL-1 appear to be tightly controlled. We (7, and unpublished observations) and others (35) have found that IL-1 stimulation of normal human PMN does not induce early responses of PMN such as NADPH oxidase-activation, degranulation, chemotaxis, translocation of protein kinase C, activation of phospholipase C for phosphatidylinositol or phospholipase D for choline-containing phosphoglycerides, or increases in free intracellular calcium. However, IL-1R of normal PMN responds to stimulation by IL-1 by inducing synthesis of both IL-1α and β proteins (19, 36), as well as TNFα (unpublished observations). We were unable to evaluate the function of the IL-1R on sepsis-PMN in this study because of limitations on blood procurement, so we do not know whether the increased expression of IL-R on sepsis-PMN is associated with an alteration in PMN responses which follow stimulation by IL-1.

In summary, the sepsis syndrome of humans is consistently associated with marked increases in the number of IL-1R expressed on circulating PMN, without a change in its M_r or K_d. The increase in IL-1R is unimodally distributed on all sepsis-PMN. In contrast, sepsis-PMN have no increase in expression of CR3. Our limited study of other states of inflammation and myeloid stimulation suggests that amplified expression of IL-1R on blood PMN might discriminate the sepsis syndrome from other diseases. The mechanism responsible for enhanced expression of IL-1R on sepsis-PMN is unknown, but we speculate that it follows an event or events which induce synthesis of IL-1R in PMN and that occurs, at least in part, in the peripheral circulation. The functional significance of this phenotypic change of PMN during the sepsis syndrome is unknown.

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