ARCHIVES OF SURGERY

VOL 129 NO. 1, JAN 1994

American Medical Association
Physicians dedicated to the health of America

ARCHIVES OF SURGERY

PAPER

Enhanced Release of Elastase Is Not Concomitant With Increased Secretion of Granulocyte-Activating Cytokines in Whole Blood From Patients With Sepsis

Wolfgang Ertel, MD; Doreid Jarrar; Marianne Jochum, PhD; Volker Thiele; John Kenney, MA; Eugen Faist, MD; Friedrich-Wilhelm Schildberg, MD

Background: The proteolytic enzyme elastase released by granulocytes (polymorphonuclear leukocytes [PMN]) in high concentrations during sepsis causes degradation of essential plasma proteins, endothelial damage, and tissue edema. This may result in organ dysfunction and organ failure during sepsis, since increased elastase plasma levels correlate with the mortality rate of patients with sepsis. In vitro studies demonstrated a regulatory role of inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin 1β [IL-1β], IL-8]) upregulating protease release by PMN. In this light, the interactions between cytokine release by macrophages and altered elastase secretion during sepsis remain to be determined.

Methods: An ex vivo model consisting of lipopolysaccharide stimulation of human whole blood as a relevant physiological milieu was used. Heparinized blood was obtained from 20 patients with sepsis syndrome (APACHE II [Acute Physiology and Chronic Health Evaluation II] score 28.5±1.2 points [mean±SD]) on days 0 through 3, 5, 7, and 10 after sepsis diagnosis and from 20 control patients without infection. Blood was incubated with lipopolysaccharide (1 mg/L) for 8 hours. Plasma levels of elastase, TNF-α, IL-1β, and IL-8 were determined using enzyme-linked immunosorbent assay or bioassay (TNF-α), respectively.

Results: Elastase plasma levels in whole blood from patients with sepsis were increased up to 188% ($P<.01$) above normal, while the release of TNF-α (−87%), IL-1β (−91%), and IL-8 (−51%) was markedly ($P<.01$) decreased compared with control patients. Neutralization of TNF-α or IL-1β did not attenuate the increased release of elastase.

Conclusions: These data indicate an increased release of elastase by PMN despite a reduced secretion of PMN-activating cytokines. Although priming effects of TNF-α, IL-1β, and IL-8 on protease secretion in vivo cannot be excluded completely, other mediators or mechanisms may be involved in the upregulation of detrimental protease release during sepsis.

(Arch Surg. 1994;129:90-98)

From the Department of Surgery, University Hospital Grosshadern, Ludwig-Maximilians-University, Munich, Germany (Messrs Jarrar and Thiele, and Drs Faist and Schildberg); the Department of Clinical Biochemistry, Department of Surgery, Munich City (Dr Jochum); and the Institute of Biological Sciences, Syntex Research, Palo Alto, Calif (Mr Kenney). Dr Ertel is now with the Klinik für Unfallchirurgie, Departement Chirurgie, Universitaetsspital Zuerich (Switzerland).

INFLAMMATORY CYTOKINES such as tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and IL-8 have been implicated as principal mediators in endotoxin shock.[1-5] Moreover, these inflammatory cytokines form a link between monocytes (MO)/macrophages and polymorphonuclear leukocytes (PMN). Previous studies[6-9] revealed a trigger function of TNF-α, IL-1β, and IL-8 for activation of PMN. Tumor necrosis factor-α increased PMN adherence to endothelial cells, phagocytosis, respiratory burst activity, and degranulation in isolated PMN cultures.[10,11] Tumor necrosis factor-α and IL-1β were also found to induce and increase the expression of adhesion receptors on the membrane of endothelial cells and integrins on PMN that are reciprocally involved in intracellular adhesion.[12-14] Interleukin 8 induces the full pattern of responses observed in chemotactically stimulated PMN with activation of the motile apparatus and directional migration, expression of surface adhesion molecules, release of stored enzymes, and production of reactive oxygen

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PATIENTS, MATERIALS, AND METHODS

PATIENT SELECTION

Patients eligible for this study were those with sepsis syndrome or septic shock defined by the following criteria.[31] Sepsis syndrome was characterized by fever or hypothermia (temperature >38.3°C or <35.6°C), tachycardia (>90 beats per minute in the absence of β-blockade), tachypnea (respiratory rate >20 breaths per minute or the requirement of mechanical ventilation), and altered organ perfusion resulting in mental disorientation, oliguria, or elevated lactate levels. Septic shock was defined by clinical diagnosis of sepsis syndrome plus hypotension (eg, systolic blood pressure <90 mm Hg) or the requirement of vasopressor drugs to maintain blood pressure.

Twenty patients who fulfilled these criteria were enrolled in this study (**Table 1**). The study was carried out in accord with the Ethical Committee of the Ludwig-Maximilians-University, Munich, Germany. On the day of enrollment, the mean APACHE II (Acute Physiology and Chronic Health Evaluation II) score was 28.5±1.5 points. The infection sites included pneumonia (n=8), peritonitis (n=7), pleura empyema (n=2), and catheter sepsis (n=1). Bacteremia was documented in three patients (15%), while endotoxemia was found in eight patients (40%). Nine (45%) of the 20 patients with sepsis died in the first 28 days after diagnosis of sepsis because of multiple organ failure (MOF). Control patients (n=20) who were admitted to our hospital for hernia repair or cholecystectomy were comparable to the patients with sepsis with regard to age and sex.

COLLECTION OF BLOOD

Blood from patients with sepsis was collected on the day of enrollment (D0) and on days 1 through 3, 5, 7, and 10 thereafter. Blood from control patients was obtained once before operation to exclude any influence of stress, anesthesia, and surgical trauma.

Blood was drawn into heparinized syringes (20 U of heparin sodium per milliliter; heparin was tested for endotoxin: <5 pg of endotoxin per milliliter of heparin), immediately placed on ice, and then transferred into sterile 10-mL polypropylene tubes (Falcon, Becton Dickinson, Lincoln Park, NJ). For each blood sample, total and differential white blood cell counts were obtained (Coulter Counter, Coulter Corp, Hialeah, Fla). The numbers of MO and PMN per milliliter of blood were calculated for each blood sample from the total and differential leukocyte count.

An aliquot of 5 mL of blood was removed and rapidly processed as described below to serve as the 0-hour time point. The remainder of each blood sample was adjusted to 1 mg/L of lipopolysaccharide (LPS) (*Escherichia coli* 055:B5; Difco Labs Inc, Detroit, Mich) which resulted in a maximum stimulation of MO to secret inflammatory cytokines (W.E., unpublished observations, 1992). The blood-containing tubes were placed on a rotator in a 5% carbon dioxide atmosphere at 37°C. Control blood samples without LPS were handled similarly. At 1, 2, 4, 8, and 24 hours of culture, 5 mL of blood was removed and processed as follows: each aliquot was centrifuged over Ficoll-Hypaque density gradient (density=1.077; Seromed, Berlin, Germany) at 680*g* for 20 minutes, and the plasma was removed and stored immediately at −70°C until assayed. The viability of peripheral blood mononuclear cells was evaluated throughout the time course using trypan blue exclusion and was not found to change significantly over the 24-hour incubation period.

In addition, a monoclonal antibody against human TNF-α (Centocor, Malvern, Pa) or a monoclonal antibody against human IL-1β (Genzyme, Boston, Mass) was added to whole blood prior to LPS challenge to neutralize biologically active TNF-α and IL-1β, respectively. A 1/50 dilution of the anti–TNF-α antibody was capable to neutralize 750 U/mL of TNF-α, while a 1/40 dilution of the anti–IL-1β antibody completely blocked 50 ng/mL of IL-1β.

ELASTASE AND CYTOKINE ASSAYS

Because most released PMN elastase in plasma can be detected only in complex with α_1-proteinase inhibitor (E-α_1PI), quantitative estimation of plasma levels of the E-α_1PI complex was carried out with a highly sensitive two-site enzyme-linked immunosorbent assay (ELISA) (Merck, Darmstadt, Germany).[32,33] Plasma TNF-α levels were measured as previously described[34] using the WEHI 164 subclone 13 cell line. The detection limit of the assay was 0.1 U/mL of recombinant TNF-α. Biological activity of TNF in plasma samples could be completely abolished by the addition of a rabbit monoclonal anti–human-TNF-α antibody (Genzyme) indicating the specificity of the WEHI 164 cytotoxicity assay. Levels of IL-1β in plasma were measured using ELISA as previously described.[35] To remove putative factors (IL-1 inhibitory factors[36]) present in plasma that interfere with IL-1β measurements, a chloroform extraction was performed.[37] Interleukin 8 plasma levels were determined using a commercially available ELISA kit (Amersham, Braunschweig, Germany) according to the manufacterer's guidelines. The sensitivities of the IL-1β and the IL-8 ELISA were 15 pg/mL and 5 pg/mL, respectively. The results of the cytokine and elastase assays were normalized to represent 1×10^6 MO or 1×10^6 PMN.

STATISTICS

Results are presented as mean±SEM. Data were analyzed by unpaired Wilcoxon Rank Sum Test with Bonferroni correction for multiple comparisons. Differences were considered significant at $P<.05$.

Table 1. Clinical Data From 20 Patients With Sepsis Syndrome or Septic Shock

Patient No./ Age, y	Infection	Isolated Bacteria*	Bacteremia	Endotoxemia	APACHE II Score†	Outcome‡
1/65	Catheter sepsis	Gram−	No	No	29	R
2/51	Origin unknown	*Legionella*	No	Yes	36	R
3/56	Pleura empyema	Gram−	No	No	25	D (day 70)
4/63	Origin unknown	None	No	No	40	D (day 8)
5/53	Peritonitis	Gram−	Yes	No	31	R
6/62	Pneumonia	Gram+	No	No	23	D (day 20)
7/61	Peritonitis	Gram−	No	Yes	20	R
8/64	Pneumonia	Gram−	No	Yes	32	D (day 17)
9/71	Peritonitis	Gram−	No	Yes	42	D (day 18)
10/33	Peritonitis	Gram−	No	Yes	26	R
11/58	Pleura empyema	Gram−	No	No	28	D (day 28)
12/80	Peritonitis	Gram±	No	No	23	D (day 3)
13/69	Pneumonia	Gram+	Yes	No	27	D (day 10)
14/60	Pneumonia	Gram−	No	No	22	D (day 18)
15/86	Peritonitis	Gram−	No	No	38	R
16/56	Pneumonia	Gram+	No	No	20	R
17/76	Pneumonia	Gram−	No	Yes	29	D (day 68)
18/62	Peritonitis	Gram±	Yes	Yes	29	D (day 1)
19/72	Pneumonia	Gram±	No	Yes	27	D (day 34)
20/41	Pneumonia	*Candida*	No	No	22	R

**Minus sign indicates negative; plus sign, positive; and plus-minus sign, positive and negative.*
†APACHE II indicates Acute Physiology and Chronic Health Evaluation II.
‡R indicates recovery; D, death (day after sepsis diagnosis).

metabolites.[6] The activation of PMN by TNF-α, IL-1β, and IL-8 may lead to adherence of PMN to endothelial cells with induction of vessel wall injury due to the release of toxic oxygen species and lysosomal proteinases.[15-17]

NEUTROPHIL ELASTASE, a powerful neutral serine proteinase released from the azurophil granules, is thought to play a central role in PMN-mediated endothelial injury.[15,16,18,19] Elastase cannot only degrade almost all components of the intracellular matrix, but it can also cleave a variety of key plasma proteins (eg, immunoglobulins, complement proteins, and clotting factors) and even attack intact cells of the host, thus leading to tissue damage.[20-24] Elevated plasma levels of elastase have been described in patients with sepsis and correlated with morbidity and mortality of these patients.[25-30]

Although in vitro studies suggest an upregulation and activation of PMN by TNF-α, IL-1β, and IL-8, the interactions between altered release of these cytokines and elevated elastase secretion by PMN during sepsis remain to be determined. Therefore, it was the objective of this study to investigate whether PMN activation determined by the release of elastase is dependent on increased synthesis and secretion of inflammatory cytokines in whole blood from patients with sepsis.

RESULTS

BASELINE VALUES

For baseline values, total amounts of elastase, TNF-α, IL-1β, and IL-8 detected at time point 0 hour in whole blood were normalized according to 1×10^6 PMN or 1×10^6 MO. The E-α_1PI levels were significantly ($P<.05$) increased in the group with sepsis (33.1±5.1 ng/mL) by 130% above normal values (14.4±2.1 ng/mL). In contrast, TNF-α (<4 U/mL) and IL-1β (<250 pg/mL) were found only in minimal levels in some plasma samples from patients with sepsis, while neither TNF-α nor IL-1β could be detected in plasma from control patients. Interleukin 8 was detectable in plasma levels at 0 hour in both groups with increased ($P<.01$) IL-8 levels in the group with sepsis (366±45 pg/mL) compared with the control group (144±17 pg/mL).

KINETICS OF ELASTASE AND CYTOKINE RELEASE IN WHOLE BLOOD

Kinetic studies were performed over a 24-hour incubation period in the presence or absence of 1 mg/L of LPS. The spontaneous release of E-α_1PI in whole blood from

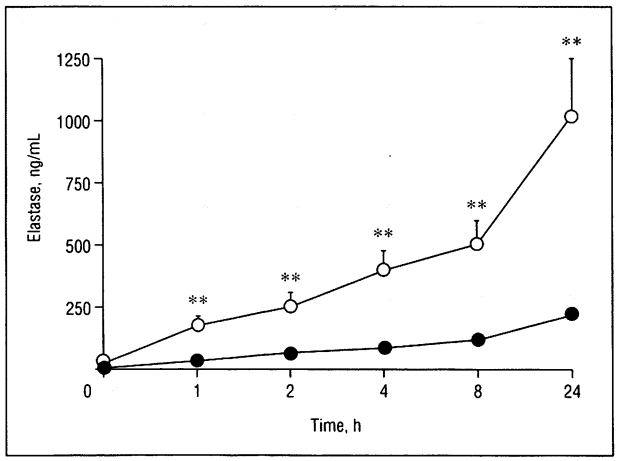

Figure 1. *Kinetics of elastase release (ng/mL per 1×10⁶ polymorphonuclear leukocytes) into whole blood from patients with sepsis (open circles) (n=12) and control patients (closed circles) (n=12). Whole blood was incubated for the indicated intervals in the presence of lipopolysaccharide (1 mg/L). Elastase plasma levels were determined with enzyme-linked immunosorbent assay as outlined in "Patients, Materials, and Methods" section. Values are mean±SEM; two asterisks indicate* P<.01 *sepsis vs control.*

patients with sepsis was significantly elevated at all examined time points with peak levels at 24 hours of culture (data not shown). Stimulation of whole blood with LPS resulted in a marked ($P<.01$) rise of E-α_1PI plasma concentrations in the group with sepsis with peak levels at 24 hours of culture (1019±236 ng/mL) in comparison to the control group (224±27 ng/mL) (**Figure 1**).

A spontaneous release of TNF-α or IL-1β was not observed in the two groups, while spontaneous secretion of IL-8 was significantly elevated in the control group compared with the group with sepsis (data not shown). At 2, 4, 8, and 24 hours after exposure to LPS, the release of TNF-α in whole blood from patients with sepsis was significantly decreased compared with the control group (**Figure 2**, top). In addition, secretion of IL-1β in LPS-stimulated whole blood from patients with sepsis was markedly ($P<.01$) depressed at 4, 8, and 24 hours of culture compared with controls (Figure 2, center). The LPS-induced release of IL-8 into whole blood from patients with sepsis was decreased ($P<.01$) at 4, 8, and 24 hours of culture in comparison to the control group (Figure 2, bottom).

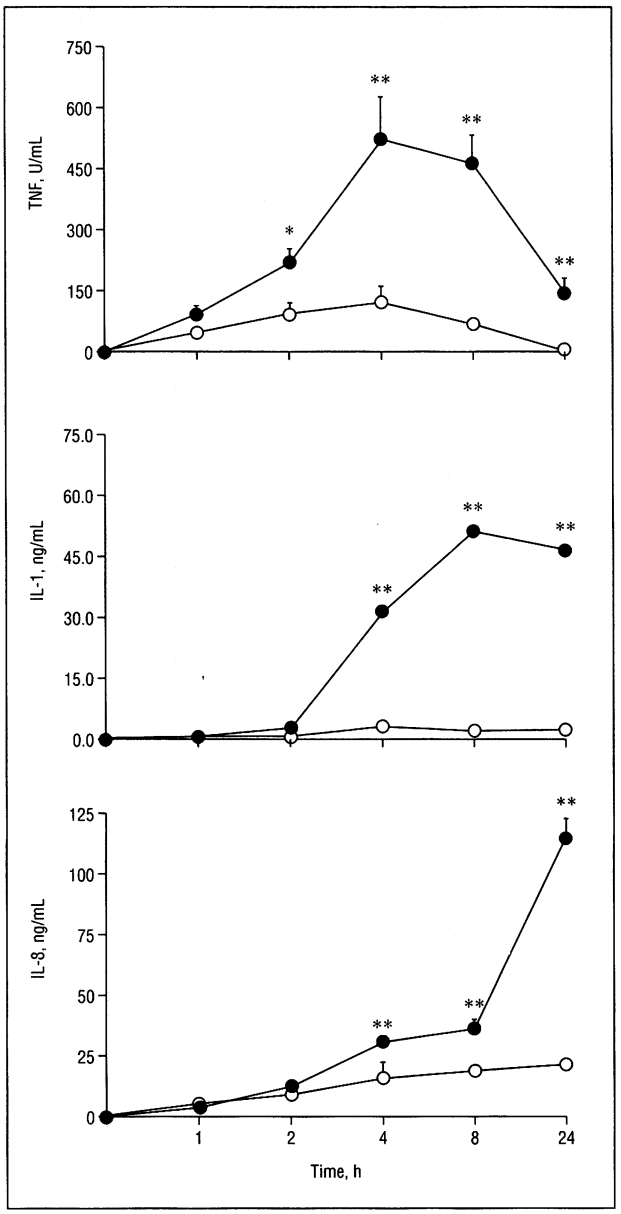

Figure 2. *Kinetics of tumor necrosis factor-α (TNF-α) (U/mL per 1×10⁶ monocytes) (top), interleukin 1β (IL-1β) (ng/mL per 1×10⁶ monocytes) (center), and interleukin 8 (IL-8) (ng/mL per 1×10⁶ monocytes) (bottom) release into whole blood from patients with sepsis (open circles) (n=12) and control patients (closed circles) (n=12). Whole blood was incubated for the indicated intervals in the presence of lipopolysaccharide (1 mg/L). Cytokine plasma levels were determined as outlined in "Patients, Materials, and Methods" section. Values are mean±SEM; one asterisk indicates* P<.05; *two asterisks,* P<.01 *sepsis vs control.*

RELEASE OF ELASTASE AND CYTOKINES IN WHOLE BLOOD ON CONSECUTIVE DAYS AFTER SEPSIS

Alterations of elastase and cytokine release in whole blood obtained from patients with sepsis were studied over a period of 10 consecutive days after study enrollment and compared with control patients. The plasma concentrations of E-α_1PI, TNF-α, IL-1β, and IL-8 were assessed at the 8-hour time point after stimulation of whole blood with LPS. The E-α_1PI concentrations in whole blood from patients with sepsis were significantly elevated ($P<.05$) on D0 and on days 0 through 3 after diagnosis of sepsis when compared with the control group (**Figure 3**). The E-α_1PI concentrations in the group with sepsis were similar to those of the control group on days 5, 7, and 10 (Figure 3).

The release of TNF-α and IL-1β in whole blood from patients with sepsis after exposure to LPS was markedly ($P<.01$) reduced on all days after diagnosis of sepsis compared with the control group (**Figure 4**, top and center). The LPS-induced release of IL-8 into whole blood from patients with sepsis was significantly reduced on

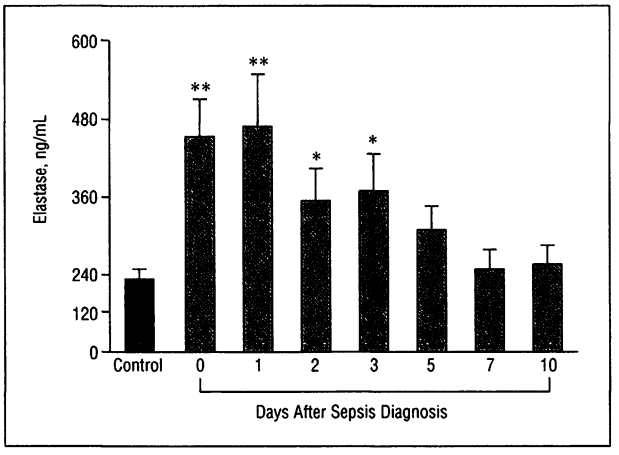

Figure 3. *Release of elastase (ng/mL per 1×10⁶ polymorphonuclear leukocytes) into whole blood obtained from patients with sepsis (shaded bars) (n=20) on day of sepsis diagnosis and days 1 through 3, 5, 7, and 10 thereafter in comparison with control patients (solid bar) (n=20). Whole blood was stimulated with lipopolysaccharide (1 mg/L) for 8 hours. Elastase plasma levels were determined with enzyme-linked immunosorbent assay as outlined in "Patients, Materials, and Methods" section. Values are mean± SEM; one asterisk indicates P<.05; two asterisks, P<.01 sepsis vs control.*

D0, day 1, and day 2, while similar amounts of IL-8 were found on days 3, 5, 7, and 10 after diagnosis of sepsis in comparison to the control group (Figure 4, bottom).

EFFECT OF ANTI–TNF-α AND ANTI–IL-1β ANTIBODIES ON ELASTASE RELEASE

The release of elastase into whole blood after exposure to LPS for 8 hours was examined in the presence of anti–TNF-α and anti–IL-1β antibodies (**Table 2**). Both antibodies were effective to neutralize TNF-α or IL-1β activity in whole blood as assessed by bioassay and ELISA. Examination of E-α_1PI concentrations in whole blood revealed that neither the anti–TNF-α nor the anti–IL-1β antibody reduced the release of elastase in the two groups (Table 2).

COMMENT

In this study, we demonstrate an enhanced release of PMN elastase into human whole blood from patients with sepsis in comparison to control patients without infection. In contrast, the secretion of PMN-activating cytokines (TNF-α, IL-1β, IL-8) in human whole blood from patients with sepsis after stimulation with LPS was decreased compared with the control group.

Multiple organ failure represents the major cause of death during and after sepsis and septic shock.[38] Besides a significant correlation between increased serum levels of the inflammatory cytokines TNF-α, IL-1β, and IL-6 and mortality of patients with sepsis,[39,40] the excessive release of proteolytic enzymes correlated with the incidence of adult respiratory distress syndrome and MOF following trauma, shock, and sepsis.[29,41-45] This may be due to the fact that proteolytic enzymes such as elastase

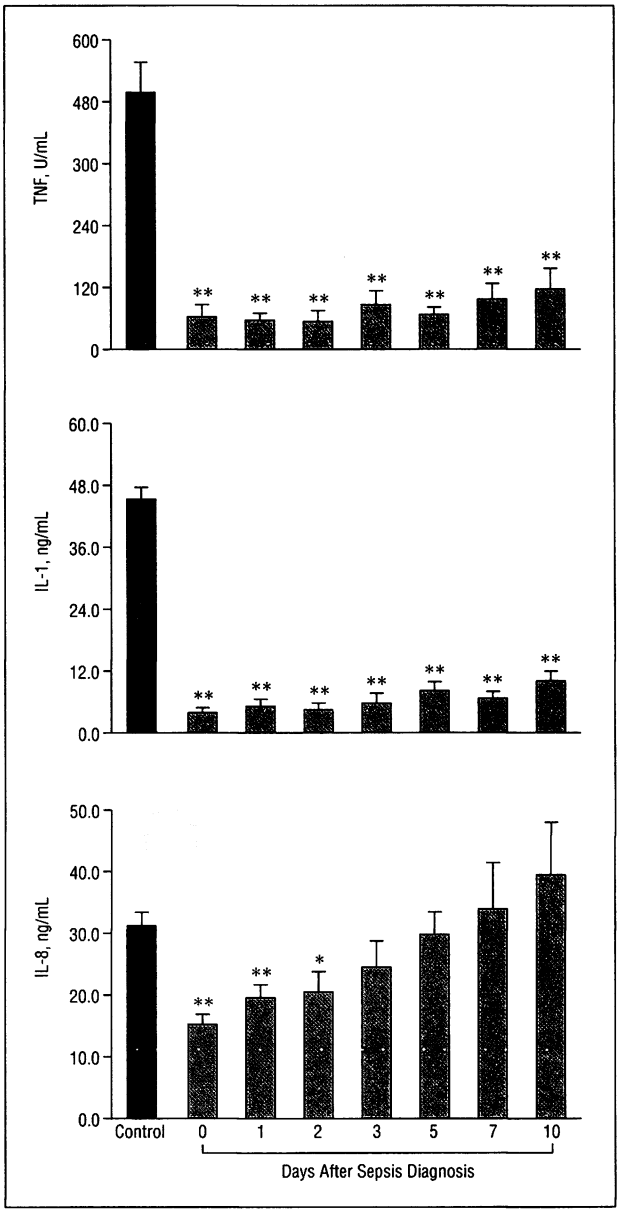

Figure 4. *Release of tumor necrosis factor-α (TNF-α) (U/mL per 1×10⁶ monocytes) (top), interleukin 1β (IL-1β) (ng/mL per 1×10⁶ monocytes) (center), and interleukin 8 (IL-8) (ng/mL per 1×10⁶ monocytes) (bottom) into whole blood obtained from patients with sepsis (shaded bars) (n=20) on day of sepsis diagnosis and days 1 through 3, 5, 7, and 10 thereafter in comparison with control patients (solid bars) (n=20). Whole blood was stimulated with lipopolysaccharide (1 mg/L) for 8 hours. Cytokine plasma levels were determined as outlined in "Patients, Materials, and Methods" section. Values are mean± SEM; one asterisk indicates P<.05; two asterisks, P<.01 sepsis vs control.*

contribute to progressive tissue destruction because of their proteolytic activity on essential structural proteins, thus leading to organ dysfunction and failure.[23] In addition, the enhanced release of lysosomal proteinases by PMN resulted in a significant consumption and degradation of extracellular substances in inflammatory diseases.[46]

Although previous studies revealed a correlation between the severity of sepsis and the incidence of MOF on

Table 2. Effect of Monoclonal Anti–TNF-α and Anti–IL-1β Antibodies on Release of Elastase (ng/mL per 1×10^6 PMN) Into Human Whole Blood From Patients With Sepsis (n=2) and Control Patients (n=2) After Exposure to LPS (1 mg/L) for 8 Hours*

Stimulus Antibody	No Stimulus –	+LPS –	No Stimulus +Anti-TNF	+LPS +Anti-TNF	No Stimulus +Anti-IL-1	+LPS +Anti-IL-1
Experiment 1						
Control	19	127	38	108	75	155
Sepsis	30	261	61	266	303	554
Experiment 2						
Control	13	137	26	98	68	165
Sepsis	54	309	61	325	246	419

**TNF-α indicates tumor necrosis factor-α; IL-1β, interleukin 1β; PMN, polymorphonuclear leukocytes; and LPS, lipopolysaccharide.*

one hand and the degree of elevated elastase plasma levels on the other hand, the interactions between PMN-activating cytokines such as TNF-α, IL-1β, or IL-8 and the enhanced release of elastase by PMN remained to be determined. Whole blood was used instead of purified cell cultures to eliminate confounding factors such as unspecific macrophage activation with increased messenger RNA expression, protein synthesis, and release of cytokines by isolation procedures.[47,48] Moreover, human whole blood stimulated with LPS represents an ex vivo model of sepsis to study cytokine interactions.[49] Although this experimental design cannot represent the immunologic processes in the whole individuum, it imitates the immunologic network in a localized area of inflammation. The cellular interactions as well as the influence of complement factors, mediators, or inhibitory peptides are preserved.

The results from this study reveal a significant increase of elastase in whole blood obtained from patients with sepsis in comparison with the control group in the presence or absence of LPS. The increased release of elastase by PMN in whole blood was observed early after diagnosis of sepsis with a decreasing tendency on days 5, 7, and 10. These data agree with results obtained from previous experimental[50] and clinical[27,29,30] studies. Moreover, in kinetic studies, elastase release into whole blood was observed to continuously increase over the 24-hour time course. It could be argued that the increasing release of elastase up to 24 hours may be due to cell death in whole blood. This could be excluded by the fact that viability of leukocytes was identical in both groups at all time points studied. Furthermore, subpopulations of leukocytes were similar at different time points of incubation using the direct immunofluorescence technique. Because the absolute numbers of neutrophils were significantly higher in patients with sepsis, elastase plasma levels were normalized to represent 1×10^6 PMN in both groups.

Although data from previous clinical investigations[41-45] and from this study demonstrate an excessive release of elastase into plasma, the regulation of PMN degranulation and enhanced release of proteolytic enzymes by PMN during sepsis is unclear. Because inflammatory cytokines have been found to activate PMN,[6-16] it could be hypothesized that these cytokines may also be involved in the increased release of proteolytic enzymes by PMN during sepsis, inasmuch elevated serum levels of the PMN-activating cytokines TNF-α, IL-1β, and IL-8 were described after injection of endotoxin or *E coli* in experimental and clinical models.[1-4] However, in contrast to the marked increase of released elastase observed in human whole blood from patients with sepsis, the release of the PMN-activating cytokines TNF-α and IL-1β was significantly depressed during the entire observation period. These data are in line with previous results by McCall et al[51] who demonstrated a reduced synthesis of IL-1β by PMN obtained from patients with sepsis.

Although depression of IL-8 release into whole blood from patients with sepsis after exposure to LPS for 8 hours was observed only until day 3, it can be assumed that IL-8 release in the group with sepsis may also be suppressed between day 3 and day 10, since peak levels of IL-8 occurred at 24 hours of culture. Moreover, while in the absence of LPS spontaneous release of elastase in whole blood from patients with sepsis was markedly elevated compared with control patients, only trace amounts of IL-1β and biologically active TNF-α were detected. These data lead us to conclude that TNF-α, IL-1β, and IL-8 may not be involved in the enhanced release of elastase by PMN. These conclusions are supported by control studies using neutralizing antibodies against biologically active TNF-α or IL-1β. In these experiments, neutralization of TNF-α or IL-1β did not inhibit spontaneous or LPS-induced elastase release into whole blood indicating the involvement of mechanisms other than PMN activation by TNF-α or IL-1β. These suggestions agree with results by Moore and collegues[52] who demonstrated an activation of PMN by endotoxin independent of TNF-α. The addition of the anti–IL-1β antibody even enhanced E-α_1PI concentrations in whole blood, which may be due to unspecific activation of PMN by IL-1β/anti–IL-1β complexes. Although additional studies using an anti–IL-8 antibody have to be carried out to investigate the precise role of IL-8 in PMN degradation in vivo, previous studies by Redl and collegues[50] comparing the kinetics of IL-8 and elastase plasma levels in a primate bacteremia model further support our results. The authors showed in their model that elastase

plasma levels already reached a plateau at 1 hour after injection of live *E coli*, while IL-8 was not detectable in the plasma at this time point. Although all these studies cannot completely rule out the possibility of priming effects of inflammatory cytokines on elastase release in vivo, it seems to be unlikely that the inflammatory cytokines TNF-α, IL-1β, and IL-8 predominantly contribute to the dramatic increase of elastase release into whole blood from patients with sepsis.

Neutrophil elastase causes endothelial injury, destruction of circulating proteins, and tissue damage leading to organ dysfunction and MOF. Elevated plasma levels of elastase have been found in patients with sepsis and septic shock. The activation and degranulation of PMN correlated with the severity and the outcome of sepsis. Using LPS-stimulated whole blood as an ex vivo model of sepsis, the results of this study reveal an excessive release of elastase into whole blood from patients with sepsis compared with control patients without infection. However, the release of PMN-activating cytokines in whole blood from patients with sepsis was dramatically reduced and neutralization of TNF-α or IL-1β with monoclonal antibodies did not attenuate elastase release. Although activation of monocytes/macrophages/PMN with an excessive synthesis and secretion of inflammatory cytokines may be inhibited by autoprotective mechanisms inducing endotoxin tolerance,[51] the increased release of elastase seems to be uncontrolled during persisting sepsis. These data imply that the inflammatory cytokines TNF-α, IL-1β, and IL-8 may not be responsible for PMN activation and degranulation observed in patients with sepsis. Thus, alternative pathways other than stimulation of PMN by these inflammatory cytokines may be involved in PMN activation. Moreover, therapy with neutralizing monoclonal antibodies directed against inflammatory cytokines or blockade of cytokine receptors may not attenuate PMN degranulation and excessive release of proteases in patients with sepsis.

Accepted for publication July 11, 1993.

This investigation was supported by grant ER 165/1-1 from the Deutsch Forschungsgemeinschaft (Gerhard-Hess Programm), Bonn, Germany.

Presented at the 13th Annual Meeting of the Surgical Infection Society, Baltimore, Md, April 30, 1993.

The WEHI 164 subclone 13 cell line used in this study was provided by Steven Kunkel, PhD, Ann Arbor, Mich.

Mrs Judith Eymann and Mrs Claudia Seidl provided technical assistance.

Reprint requests to Klinik für Unfallchirurgie, Departement Chirurgie, Universitaetsspital Zuerich, Raemistrasse 100, CH-8091 Zuerich, Switzerland (Dr Ertel).

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DISCUSSION