

Interleukin-1 Receptor Blockade Improves Survival and Hemodynamic Performance in *Escherichia coli* Septic Shock, but Fails to Alter Host Responses to Sublethal Endotoxemia

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Abstract

The present study was undertaken to evaluate the extent to which an endogenous interleukin-1 (IL-1) response contributes to the hemodynamic and metabolic consequences of sublethal endotoxemia or lethal Gram-negative septic shock. Young, healthy baboons received either a sublethal dose of lipopolysaccharide (LPS) or an LD₁₀₀ of live *Escherichia coli* bacteria, and one half of the animals in each group were continuously infused with IL-1 receptor antagonist (IL-1ra). Plasma IL-1 β was not detected in this model of endotoxemia. Administration of IL-1ra had only minimal effects on the modest hemodynamic and metabolic responses to sublethal endotoxemia, and did not attenuate the plasma cytokine response. In contrast, high circulating levels of IL-1 β (range 300–800 pg/ml) were seen during lethal *E. coli* septic shock. IL-1ra treatment significantly attenuated the decrease in mean arterial blood pressure (MAP) (from -72 ± 8 to -43 ± 6 mm Hg; $P < 0.05$) and cardiac output (from -0.81 ± 0.17 to -0.48 ± 0.15 liter/min; $P < 0.05$), and significantly improved survival from 43 to 100% at 24 h ($P < 0.05$). The plasma IL-1 β and IL-6 responses to lethal *E. coli* septic shock were also significantly diminished by IL-1ra treatment ($P < 0.05$), whereas tumor necrosis factor- α (TNF α) concentrations were unaffected. We conclude that an exaggerated systemic IL-1 β response is characteristic of lethal *E. coli* septic shock, and contributes significantly to the hemodynamic and metabolic consequences of *E. coli* septic shock. IL-1ra can significantly attenuate the cytokine cascade and improve survival. (*J. Clin. Invest.* 1992. 89:1551–1557.) Key words: tumor necrosis factor • interleukin-6 • interleukin-8 • lipopolysaccharide

Introduction

IL-1 is a pleiotropic cytokine presumed to participate in the initiation and regulation of the acute-phase response to injury and infection (1). Endogenous IL-1 production contributes to the febrile and hepatic acute-phase protein responses, as well as to the anorexia and skeletal protein wasting seen during an acute inflammatory response (2, 3).

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Earlier investigations of the cytokine cascade initiated during Gram-negative septic shock have emphasized a proximal role for tumor necrosis factor- α (TNF α).¹ Administration of TNF α to otherwise healthy animals induces cardiovascular collapse, multiorgan system failure, and death, similar to that seen in experimental lethal endotoxemia and in clinical sepsis (4). Passive immunization of primates with antibodies to TNF α improves survival in *E. coli* septic shock (5). However, an increasing body of evidence has also accumulated to suggest a role for IL-1 in the pathogenesis of Gram-negative septic shock.

We have recently reported that administration of recombinant human IL-1 α to healthy primates will result in hypotension and tachycardia similar to that seen in endotoxemia (6). Although quantities as low as 100 ng/kg body weight (BW) will produce significant tachycardia, 100 μ g/kg BW of IL-1 α decreases cardiac output and causes adrenal cortex hemorrhage. Okusawa and Dinarello also reported that administration of IL-1 β to rabbits induced hypotension, decreased systemic vascular resistance, and central venous pressures, as well as increased heart rate and cardiac output (7). Furthermore, IL-1 β potentiated the deleterious effects of TNF α (7, 8). Ohlsson and colleagues have recently reported that an endogenous IL-1 response contributes to endotoxin-induced lethality, as treatment of rabbits with a newly described IL-1 receptor antagonist (IL-1ra) improves survival (9). Similarly, Wakabayashi and Dinarello have observed that treatment of rabbits with IL-1ra blunts hypotension in sublethal *E. coli* shock (10).

The present study was designed to examine the role of endogenously produced IL-1 in the hemodynamic and metabolic responses to a mild endotoxemia and to a lethal Gram-negative bacterial (*E. coli*) infection in nonhuman primates. To inhibit the actions of endogenously produced IL-1, IL-1ra was administered continuously during the course of mild endotoxemia and in lethal *E. coli* septic shock. A primate model of sublethal endotoxemia and lethal septic shock was chosen for several reasons. First, this *E. coli* septic shock model is well controlled, reproducible, and manifests many of the clinical responses seen in decompensated septic shock, such as a transient increase and subsequent decrease in cardiac output, hypotension, and tachycardia. Although the animals are provided crystalloid resuscitation, inotropic agents or ventilatory support are not employed. Second, similarities between human and *Papio* cytokines allow a full characterization of the cytokine response

1. Abbreviations used in this paper: BW, body weight; IL-1ra, IL-1 receptor antagonist; MAP, mean arterial pressure; TNF- α , tumor necrosis factor- α .

in sepsis. Antibodies against human IL-1 β , TNF α , IL-8, and IL-1ra all crossreact with the corresponding *Papio* mediators, but do not crossreact with either mouse or rabbit proteins. Third, the pharmacokinetics of human recombinant IL-1ra in baboons would be expected to more closely approximate those in humans. Finally, this *E. coli* septic shock model has been used to explore the role of TNF α (5), allowing a direct comparison between the role of these two mediators.

In order to obtain a controlled and reproducible experimental septic shock model, a single bolus infusion of lethal quantities of *E. coli* was employed. However, this model differs from the pathogenesis of clinical septic shock which often originates from a nidus of infection. Furthermore, the interpretation of the results may be confounded to some degree as they are evaluated while the animal is in an anesthetized state, even though anesthetized control animals in previous studies have shown no significant hemodynamic changes (6).

The IL-1ra employed in the present study is a 17-kD recombinant human protein that shares 26% sequence homology with IL-1 β , binds to the IL-1 type I receptor with equal affinity, but has no agonist activity (11). When administered at a 1,000-M excess, IL-1ra will block the hemodynamic and metabolic responses produced by IL-1 α administration (6).

Methods

Reagents. IL-1ra was produced at Synergen Inc. Before intravenous administration, the IL-1ra was diluted with physiologic saline (0.9% sodium chloride; 0.5 ml/kg [BW]). Control animals received equivalent amounts of human serum albumin (HSA). *Salmonella typhosa* lipopolysaccharide (LPS) (phenol extracted; lot 126F-4020) was obtained from Sigma Chemical Co. (St. Louis, MO). Lyophilized *E. coli* 086:B7 (obtained from Dr. G. T. Shires, Texas Tech. Univ. Lubbock, TX) was used to grow cultures on tryptic soy broth agar slants; viability counts of the inoculum were determined by standard dilution techniques. *E. coli* bacteria were diluted with physiologic saline to obtain a concentration of 10¹⁰–10¹¹ CFU/ml.

Treatment of the animals. Young female baboons (*Papio anubis*) (10–15 kg) were housed and quarantined at the Research Animal Resource Center of Cornell University Medical College for 2–4 wk before study to confirm that they were in good health and free of transmissible diseases. Baboons were obtained through the National Primate Exchange by Buckshire Laboratories (Chelmsford, PA), and treated according to an experimental protocol approved by the Institutional Animal Care and Use Committee, Cornell University Medical College. The animal facility is registered with the U. S. Department of Agriculture and is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. The Laboratory of Surgical Metabolism adheres to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the American Physiological Society.

Before the experiment, baboons were fasted overnight and immobilized with intramuscular ketamine hydrochloride (10 mg/kg BW). During the study, anesthesia was maintained using sodium pentobarbital (5 mg/kg BW per h) intravenously to allow for spontaneous breathing. Control of the upper airway was obtained using an endotracheal tube (6.5 mm). Arterial and venous access were secured by sharply dissecting and bilaterally isolating the femoral vessels. Central pressures were monitored employing a balloon-tipped flow directed thermodilution pulmonary artery catheter (Swan-Ganz) placed via the femoral vein. One femoral artery was cannulated for blood pressure measurements and intermittent blood sampling. Continuous monitoring of the heart rate, systemic artery, pulmonary artery, and capillary wedge pressures was achieved with a Datascope (model 2000A; Datascope Corp., Paramus, NJ). Core temperature was recorded over the entire

length of the study and maintained using a Mylar® blanket. Hourly urine measurements were performed, using an urethral (Foley) catheter. Baseline hemodynamic parameters and blood collections were obtained over a 2-h period immediately before initiation of the experiment.

Sublethal endotoxemia and lethal *E. coli* bacteremia were induced in 21 experimental animals by administration of 500 μ g/kg BW *Salmonella typhosa* LPS ($n = 7$), or 10¹⁰–10¹¹ CFU/kg of live *E. coli* ($n = 14$), respectively. Before infusion of LPS or *E. coli*, the animals were assigned to receive either 10 mg/kg BW IL-1ra followed by a continuous infusion of 25 μ g/kg BW per min of IL-1ra ($n = 11$), or an equivalent amount of HSA ($n = 10$) for 8 h. The protein and/or LPS were delivered into a central vein as a single bolus over 30 s. The quantity of administered endotoxin was based on preliminary studies to produce a self-limited, mild endotoxemia associated with transient changes in heart rate and mean arterial blood pressures (MAP) (6). The live *E. coli* bacteria were infused over 30 min into the femoral vein. This quantity of bacteria represents an approximate LD₁₀₀ dose of live *E. coli* (5). The bacterial dose was administered to pairs of baboons that were studied concurrently. After randomization, the assigned treatment was started simultaneously with the bacterial infusion. All investigators involved in performing the study were blinded as to the experimental treatment regimen.

Continuous infusions of physiologic saline (0.9% sodium chloride; 3 ml/kg BW per h) were administered to all animals for 8 h. In addition, *E. coli* septic animals were resuscitated during the first 8 h with 10 ml/kg BW of physiologic saline every 15 min if they met two or more of the following criteria: (a) a 30% decrease in MAP; (b) a 30% increase in heart rate; (c) a pulmonary capillary wedge pressure (PCWP) of less than 2 torr; and (d) urine output of less than 1 ml/kg BW. At 8 h, fluid support was withdrawn from all animals and LPS baboons were killed by an intravenous injection of sodium pentobarbital (100 mg/kg BW), whereas animals receiving *E. coli* were observed for survival until 24 h. During the remaining 16 h, *E. coli*-treated animals received 1.0 ml/kg per h BW of physiologic saline containing the IL-1ra or placebo. Because of concerns regarding undue discomfort to nonhuman primates after induction of lethal *E. coli* septic shock, Institutional Animal Care and Use Committee guidelines required that the animals remained anesthetized for the duration of the study, and were euthanized at 24 h.

Hemodynamic parameters and urine output were monitored continuously. At 30-min intervals for the first 3 h, and at hourly intervals thereafter for 8 h, arterial blood was collected. Arterial blood samples were also obtained at 24 h immediately before death. The plasma was stored at –70°C until assays were performed.

Analytical methods. Plasma IL-1 β and TNF α were measured by standard ELISA techniques, as reported elsewhere (12, 13). The sensitivity of the assays are 30 and 34 pg/ml, respectively. Plasma IL-8, a recently described cytokine with neutrophil chemotactic properties, was also measured by ELISA with a sensitivity of 80 pg/ml (14). An ELISA was used to determine IL-1ra concentrations. A polyclonal rabbit antibody raised against recombinant human IL-1ra and purified by affinity chromatography was employed as the capture antibody. The affinity-purified antibody was also biotinylated, and a horse radish peroxidase conjugated to avidin was used to visualize the captured protein. The lower limit of detection for this assay is 600 pg/ml. IL-6 activity was determined using the B.9 hybridoma cell proliferation assay (15). One unit of activity is defined as the quantity of IL-6 required to produce half-maximal proliferation (lower limit of detection 10 B.9 U/ml).

Plasma glucose was measured using an automated glucose oxidase analyzer (Glucose Analyzer 2; Beckman Instruments Inc., Sunnyvale, CA). Lactate was measured enzymatically, according to the manufacturer's instructions (model 826-UV; Sigma Chemical Co.).

Prothrombin times, partial thromboplastin times, platelet counts, fibrinogen, fibrin split products, and complete blood chemistries were all measured at the clinical laboratories of the Animal Medical Center, New York. Arterial and mixed venous oxygen and carbon dioxide concentrations were assessed with a clinical blood gas analyzer (pH/Blood Gas Analyzer 1306; Instrumentation Laboratory, Inc., Lexington,

MA). Total white blood cell counts were performed using a Coulter counter (Coulter Electronics, Hialeah, FL).

Statistical analyses. Data were analyzed with a microcomputer-based commercial statistical package (StatPac®; Northwest Analytical, Portland, OR). Changes in hemodynamic, hematologic, and hormonal/metabolic responses due to time, and the individual treatment regimens were analyzed by one- and two-way analysis of variance. Comparisons between groups were performed using Newman-Keuls' multiple range test. Statistical significance was designated at the 95% confidence level.

Results

Sublethal endotoxemia. Baboons receiving 500 $\mu\text{g}/\text{kg}$ BW of *Salmonella typhosa* LPS showed only a 17% decrease in MAP, and a 44% increase in heart rate (both $P < 0.05$). Pulmonary capillary wedge pressures (PCWP) also declined (data not shown), whereas cardiac output was unaffected. IL-1ra treatment had no significant effect on changes in tachycardia, cardiac output, and MAP produced by this mild degree of endotoxemia (Fig. 1).

LPS induced a systemic $\text{TNF}\alpha$ and IL-6 response that were unaffected by IL-1ra treatment (Fig. 2). IL-8 levels were also unchanged by IL-1ra treatment. IL-1 β was only randomly detected in the plasma in either group at a sensitivity of 30 pg/ml.

After endotoxin administration, total leukocyte numbers declined, reaching a nadir at 2 h (Fig. 3), and rose again so that total leukocyte numbers were normal at 8 h. IL-1ra administration did not prevent the leukopenia or subsequent leukocytosis that developed after endotoxemia. Platelet counts, fibrinogen concentrations, and fibrin split products were unaffected by this degree of endotoxemia (data not shown) in all animals.

Arterial blood glucose and lactate levels (data not shown) increased in all endotoxin-treated baboons ($P < 0.05$), and the changes were unaffected by IL-1ra treatment.

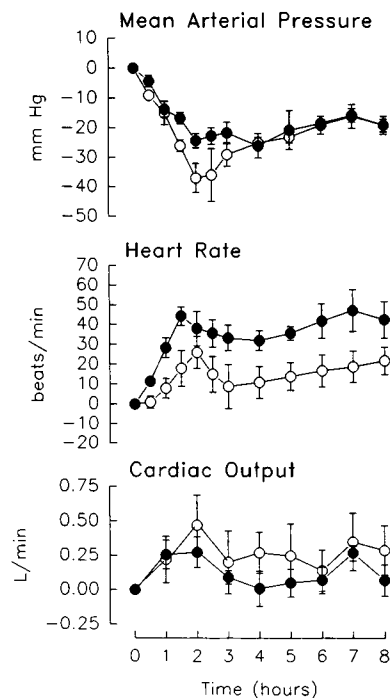


Figure 1. Changes in hemodynamic performance in baboons during sublethal endotoxemia and IL-1ra or placebo administration. Baboons were anesthetized and instrumented, as described in Methods. Animals received 500 $\mu\text{g}/\text{kg}$ BW of *Salmonella typhosa* LPS and were infused with either bolus of 10 mg/kg BW i.v. followed by 25 $\mu\text{g}/\text{kg}$ BW per min of IL-1ra (open circles), or placebo (closed circles). Sublethal endotoxemia produced a mild hypotension and tachycardia that were not significantly affected by IL-1ra treatment. Cardiac output was similarly unaffected by either endotoxemia or IL-1ra treatment.

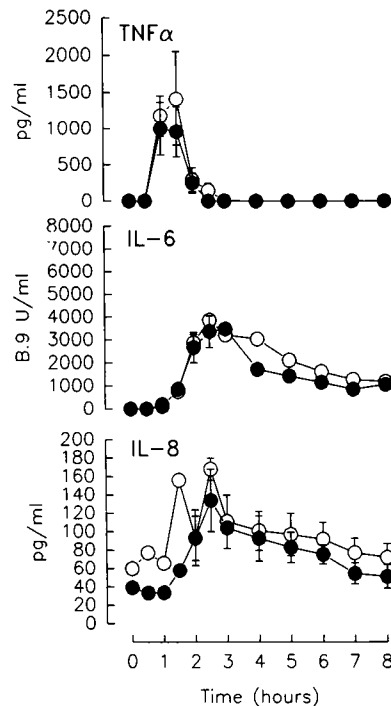


Figure 2. Cytokine production during endotoxemia and effect of IL-1ra treatment. $\text{TNF}\alpha$, IL-6, and IL-8 were measured, as described in Methods. $\text{TNF}\alpha$, IL-6, and IL-8 concentrations were unaffected by IL-1ra treatment. IL-1 β could not be detected at a sensitivity of 30 pg/ml in this model (closed circles represent placebo-treated animals, and open circles IL-1ra-treated animals).

Lethal *E. coli* septic shock. MAP and cardiac output declined precipitously in control baboons receiving *E. coli*, after a transient increase in cardiac output in the first 2 h. Baboons became significantly tachycardic within 45 min of administer-

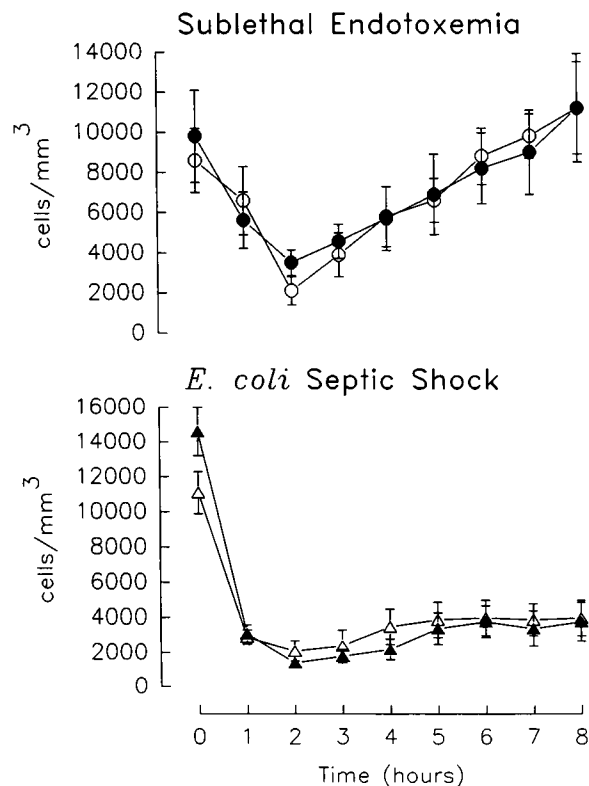


Figure 3. Leukocyte changes in baboons treated with IL-1ra or placebo during sublethal endotoxemia or lethal *E. coli* septic shock. Baboons were treated, as described in Methods. IL-1ra (open circles and open triangles, respectively) treatment had no effect on the leukocyte changes after sublethal endotoxemia and in lethal *E. coli* septic shock.

ing *E. coli*. Fluid resuscitation was instituted once predetermined hemodynamic criteria were met. During the first 8 h, 1,539±556 ml of 0.9% sodium chloride were administered in an effort to maintain hemodynamic performance. However, four of seven control baboons expired (57% mortality) within the first 24 h despite efforts to resuscitate the animals. One of the three remaining animals had significant organ dysfunction at death at 24 h; the animal was anuric and had a plasma creatinine of 5.2 mg/dl (baseline range < 1.0 mg/dl).

IL-1ra treatment. After the bolus infusion of 10 mg/kg BW IL-1ra and during the infusion of 25 µg/kg BW per min, plasma IL-1ra levels declined from a measured maximum of 50±4 µg/ml at ½ h to a plateau of 7.3±0.2 µg/ml beginning at about 2 h. This plateau continued for 6 to 8 h. Plasma IL-1ra clearances during this period ranged from 2.0 to 6.0 ml/min per kg BW (mean 3.3±0.3 ml/min per kg BW). In two of the baboons, however, plasma IL-1ra levels increased to 65 and to 120 µg/ml, respectively. Although these plasma IL-1ra levels are not steady-state levels, they do suggest decreased plasma IL-1ra clearances (0.40 and 0.23 ml/min per kg BW, respectively). The pronounced increases in plasma IL-1ra during the 8 to 24-h time period in two of the IL-1ra-treated animals were accompanied by increases in serum creatinine and BUN, suggesting a relationship between renal function and IL-1ra clearance. Across all baboons, plasma IL-1ra levels correlated well with simultaneously measured serum creatinine concentrations ($P < 0.0001$, $R^2 = 0.89$).

E. coli septic animals receiving IL-1ra also became hypotensive, but the transient increase and subsequent decline in cardiac output and MAP were not as precipitous as observed in baboons receiving placebo (Fig. 4). Fluid resuscitation was provided when the animals met predetermined criteria. As a result, crystalloid support during resuscitation tended to be lower in IL-1ra-treated baboons than in placebo-treated controls (771±259 vs 1539±556 ml). However, as shown in Fig. 5, these animals responded to fluid support by maintaining their MAP, whereas in placebo-treated animals, fluid support was unable to restore or maintain hemodynamic performance. Survival over the first 24 h was 100% (7 of 7) ($P = 0.03$ by Fisher's exact

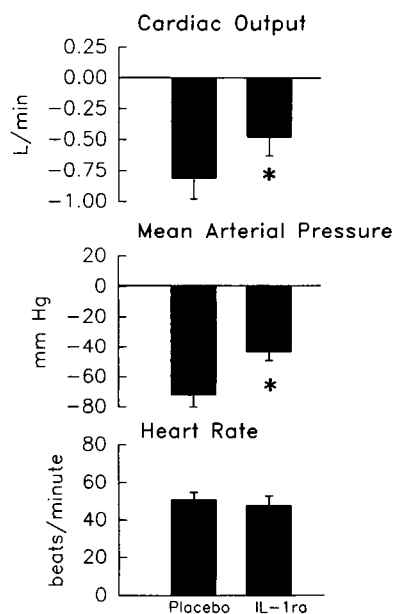


Figure 4. Changes in hemodynamic performance in *E. coli* septic shock baboons: IL-1ra treatment vs placebo. Maximal changes in MAP, cardiac output, and heart rate over the first 8 h in *E. coli* shock baboons treated with either IL-1ra or placebo. The differences in MAP and cardiac output in the two groups were statistically significant (* $P < 0.05$ by paired *t* test).

test vs placebo-treated baboons); however one of the IL-1ra-treated animals was anuric and had an elevated serum creatinine (3.5 mg/ml vs < 1.0 mg/ml at baseline).

Animal welfare concerns necessitated terminating the study after 24 h. However, six of the seven animals given IL-1ra would likely have survived the acute response to *E. coli*, as 24-h indices of renal, hepatic, and pulmonary function were all within normal limits. As previously stated, one of the IL-1ra-treated *E. coli* septic animals was anuric and was likely to have ultimately expired. This contrasts with the control group where four of seven expired within the first 24 h, and one of the survivors was anuric. In this case, only two of the seven placebo-treated animals were free of significant organ dysfunction and likely to have been long-term survivors, whereas in the IL-1ra group, the number of long-term survivors would likely have been six of seven ($P = 0.05$ by Fisher's exact test).

During *E. coli* septic shock, plasma TNFα concentrations peaked at 90 min and disappeared by 3 h (Fig. 6); IL-1ra infusions had no effect on these levels. In contrast, IL-1β appeared in the circulation within 2 h and remained elevated for the entire 24 h, and these concentrations were significantly reduced in the IL-1ra-treated baboons, compared with their sham-treated counterparts. IL-6 and IL-8 were also detectable

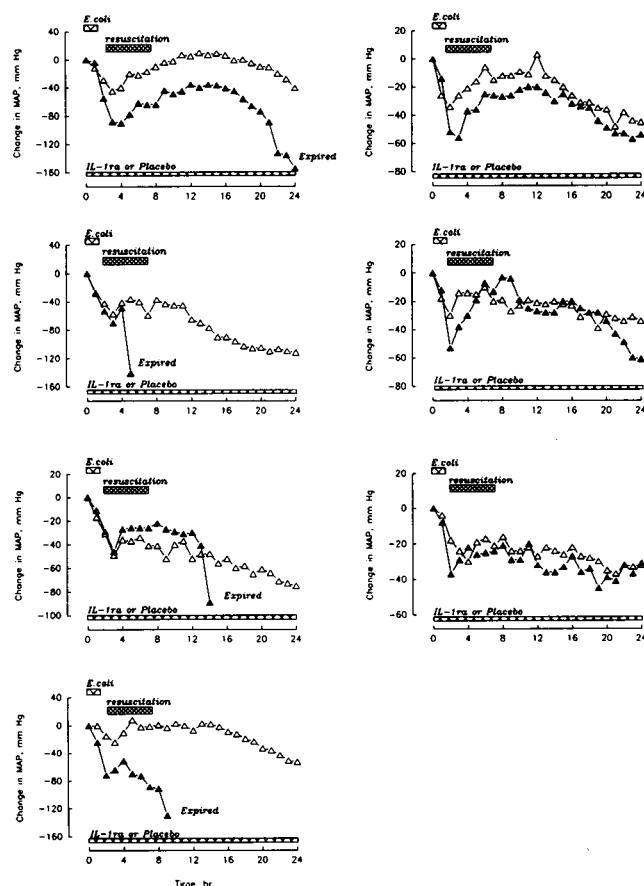


Figure 5. Changes in MAP in *E. coli* septic baboons: IL-1ra treatment vs placebo. *E. coli* septic shock was induced in baboons in a blinded and random fashion, one animal from each pair received IL-1ra at 10 mg/kg BW followed by 25 µg/kg BW per min (open triangles) while the other received placebo (closed triangles). MAP was evaluated continuously over the subsequent 24 h. Animals were resuscitated over the initial 8-h period with crystalloid according to preset criteria, as described in Methods.

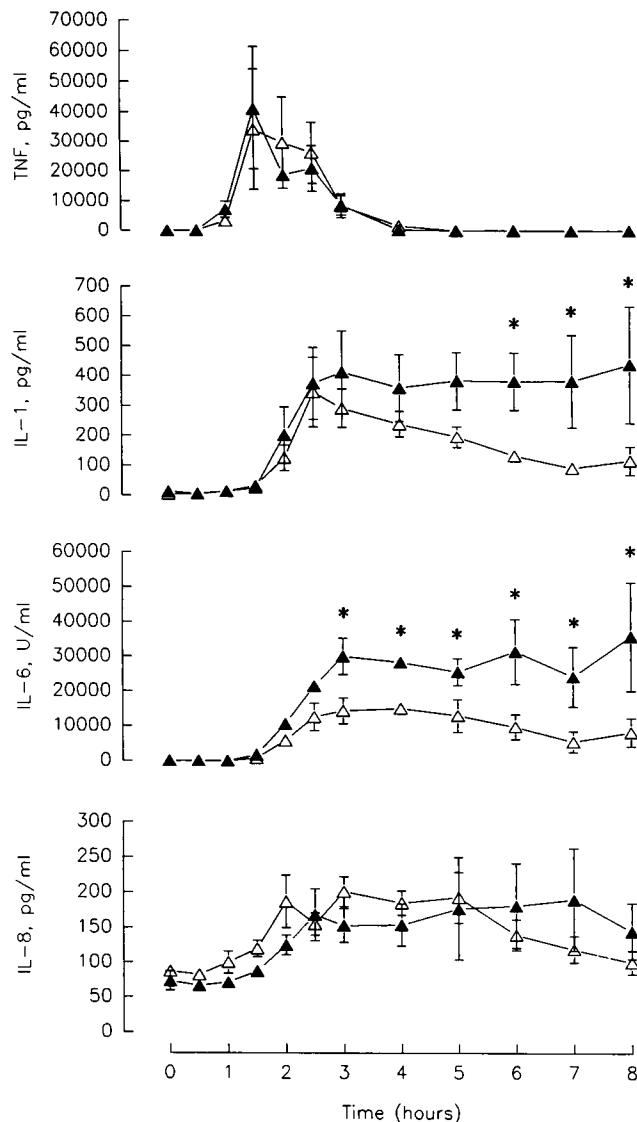


Figure 6. Cytokine levels during *E. coli* septic shock and effect of IL-1ra treatment. IL-1ra (open triangles) significantly decreased the plasma IL-6 response and attenuated the sustained IL-1 β response in *E. coli* shock when compared with placebo-treated animals (closed triangles) (* $P < 0.05$ by ANOVA and Newman-Keuls MRT). TNF α and IL-8 concentrations were unaffected by IL-1ra treatment.

within 3 h and remained elevated throughout the 24-h study period. The IL-6 levels were reduced, but the IL-8 levels were unaffected by IL-1ra treatment.

Blood glucose decreased transiently in *E. coli* septic baboons. Lactate levels increased continuously over the study period, reaching 44 ± 18 mg/dl, approximately two times that seen in sublethal endotoxemia. IL-1ra treatment had no significant effect on the lacticacidemia (Table I). However, IL-1ra treatment significantly minimized the nadir hypoglycemia seen during lethal *E. coli* septic shock (nadir 48 ± 4 vs 62 ± 6 mg/dl; $P < 0.05$). The other parameters were unaffected by IL-1ra (Table I).

Total white blood cell numbers declined in *E. coli* septic animals and remained suppressed over the course of the study (Fig. 3). IL-1ra treatment had no effect on the leukopenia. In both control and treatment groups, the leukopenia was asso-

ciated with decreased numbers of lymphocytes, polymorphonuclear cells, and monocytes, as well as increased number of immature polymorphonuclear leukocytes (data not shown). Control baboons receiving *E. coli* rapidly developed thrombocytopenia, fibrinogen levels declined, and by 8 h after *E. coli* administration, all animals had fibrinogen split products detectable in the blood (Table I). IL-1ra treatment did not prevent thrombocytopenia, but delayed the appearance of fibrin split products in the blood.

Discussion

This study used an IL-1 receptor antagonist to elucidate the role of endogenous IL-1 on the metabolic and hemodynamic responses to a sublethal endotoxemia and to lethal Gram-negative septic shock. IL-1ra is a 22–24-kD cytokine produced in vitro by blood monocytes and tissue macrophages. With an affinity similar to that of IL- β , IL-1ra binds preferentially to the IL-1 type I receptor that is found primarily on T lymphocytes, endothelial cells, tissue macrophages, and hepatocytes. However, IL-1ra has weaker affinity than IL-1 α for the type II receptor found principally on blood neutrophils and B cells (11, 16). IL-1ra has no known agonist activities, and administration of 10 mg/kg BW of IL-1ra to healthy primates is without any detectable biologic response (6). Because of the relatively rapid clearance of the protein in the circulation, IL-1ra was administered in the present study as a primed-continuous infusion that achieved circulating plasma concentrations at least 1,000–10,000-fold in molar excess to circulating IL-1 β . In previous primate studies, we observed that this molar excess of IL-1ra was sufficient to prevent the hemodynamic and physiologic responses to exogenously administered IL-1 α (6). The early rapid decline in plasma IL-1ra levels following the intravenous loading dose is consistent with rapid distribution and efficient plasma clearance of IL-1ra. The plateau plasma IL-1ra concentration during the initial 2–8-h period indicate constant plasma IL-1ra clearance over this time period. The estimated IL-1ra plasma clearance (2.0–6.0 ml/min per kg BW) reflect relatively efficient metabolic processing for IL-1ra in the baboon.

The findings presented here demonstrate that treatment of primate *E. coli* septic shock with an IL-1 receptor antagonist improves survival. The most profound improvements observed after *E. coli* administration and IL-1ra treatment were the preservation of cardiac output and MAP. During endotoxin-induced shock in rabbits, Ohlsson and colleagues were also able to demonstrate reduced lethality and significantly decreased tissue damage when animals were treated with IL-1ra (9). In the present study, the improvement in outcome with IL-1 receptor blockade occurred despite the absence of any significant attenuation in plasma TNF α levels. The present findings demonstrate that endogenous TNF α production can not by itself explain the pathophysiologic consequences of lethal *E. coli* septic shock. Rather, they suggest that IL-1, or mediators induced by IL-1, contribute significantly to this toxicity. However, in earlier primate studies, exogenous IL-1 was unable to produce irreversible shock and widespread tissue damage (6), arguing that endogenous IL-1 by itself cannot explain the lethal effects of Gram-negative or endotoxemic shock. Rather, the data imply that IL-1 contributes to and potentiates TNF α -induced cardiovascular collapse.

IL-1ra administration significantly decreased the plasma

Table I. Hematologic and Metabolic Parameters in *E. coli* Septic Shock Baboons

Time (h)	<i>E. coli</i> septic				<i>E. coli</i> + IL-1ra			
	0 (n = 7)	8 (n = 6)*	16 (n = 4)*	24 (n = 3) [§]	0 (n = 7)	8 (n = 7)	16 (n = 7)	24 (n = 7)
Metabolic indices								
Glucose, mg/dl	70±3	57±7	83±6	94±11	75±5	74±6	88±5	84±4
Lactate, mg/dl	10±1	35±12	27±3	44±18	9±1	22±3	37±6	40±9
Hematologic indices								
Thrombocyte, cells/mm ³ (×10 ³)	372±39	151±51	71±11	53±5	379±67	122±30	83±20	43±10
Fibrinogen, mg/dl	10±2	17±3	15±5	13±5	10±3	17±4	17±4	17±5
Fibrin split products, No. positive per total No.	0/7	4/6	3/4	2/3	0/7	1/7	5/7	6/7
Prothrombin time, s	15±1	24±2	22±3	18±3	18±1	18±1	19±3	19±3
Partial thromboplastin time, s	52±7	42±5	53±7	55±8	46±8	49±5	55±7	57±9
Hematocrit	36±1	28±3	26±3	23±4	37±1	31±1	27±2	23±2

* The mean is calculated among six animals who survived at this time point. [‡] Similarly, there are four baboons alive and the mean is calculated among them. [§] At this time point there are only three animals that survived and the mean represents them. ^{||} $P < 0.05$.

IL-6 response during lethal *E. coli* bacteremia. Similarly, pretreatment with anti-TNF α antibody leads to a diminished IL-6 response in *E. coli* septic shock (15). In a recent report, Starnes and colleagues reported that treatment of rats with a monoclonal antibody against murine IL-6 improved survival in *E. coli* shock (17). The authors have argued that IL-6 may be the terminal mediator of the pathologic responses to *E. coli* septic shock, and TNF α or IL-1 toxicity may be attributed to their capacity to induce a circulating IL-6 response. The present findings are not in disagreement with this conclusion, but are difficult to reconcile with the observations that IL-1 is equivalent to TNF α as an in vivo inducer of IL-6 (18), yet IL-1 is much less toxic than TNF α when exogenously administered, and that IL-6 has no known toxicity when infused into healthy animals. We have administered quantities exceeding 10 mg/kg BW to mice and have observed no evidence of toxicity (unpublished data). Similarly, Preiser and colleagues have also administered IL-6 to dogs and have reported no untoward hemodynamic effects (19).

Rather surprisingly, IL-1ra administration attenuated the plasma IL-1 β response to *E. coli* bacteremia in this model. These findings are in partial disagreement with those from similar studies in *E. coli* shock in rabbits. Although Wakabayashi and Dinarello were able to show that IL-1 receptor blockade could attenuate hypotension in a sublethal *E. coli* shock model in rabbits (10), the authors were unable to demonstrate a significant IL-1 β decline in IL-1ra-treated animals. The discrepancy may be explained by differences in the severity of the two *E. coli* septic shock models. In both studies, the early hemodynamic changes are similar and most likely due to the direct effect of the injurious stimulus, as well as mediated by TNF α . Mortality in the Wakabayashi study was 40% after administration of killed *E. coli* bacteria; with the present model, early mortality was 57% after infusion of live *E. coli*, and long-term mortality approached 75%. Furthermore, the magnitude of the IL-1 β response was markedly higher in the present study. In untreated *E. coli* septic baboons, the IL-1 β response lasted 24 h, whereas in rabbit studies, the IL-1 β response had returned to baseline within 5 h (10). In the present investigation, IL-1ra treatment did not block the initial production of IL-1 β , but prevented a sustained response. This initial spike in IL-1 β re-

fractory to IL-1ra treatment is likely the direct result of *E. coli* bacteremia, endotoxemia, and the subsequent endogenous TNF α response, all of which can induce IL-1 production (20). The resulting decline in IL-1 β levels may be explained by IL-1ra inhibition of IL-1 β synthesis, as in vitro studies have shown that IL-1 β can induce IL-1 β gene transcription in an autocrine manner (21).

The differences in severity between this and the rabbit model of Wakabayashi can also explain the failure of IL-1ra to attenuate the leukopenia observed in the present study. In rabbits receiving sublethal *E. coli* infections, total white cell numbers declined by only 60%, and IL-1ra treatment attenuated this response by ~ 30% (10). In the present study, *E. coli* septic shock produced greater than 90% reduction in circulating white cell numbers, and the numbers were unaffected by IL-1ra treatment.

In comparison to *E. coli* shock, IL-1ra treatment had only minimal effects on the hemodynamic, substrate, and white cell responses to a sublethal endotoxemia. The absence of a systemic response to IL-1ra during sublethal endotoxemia was surprising and unanticipated. Whereas IL-1ra treatment attenuated the hemodynamic changes and the plasma IL-6 response in lethal *E. coli* septic shock, it had no apparent effect during endotoxemia, suggesting that these responses to sublethal endotoxin administration are relatively IL-1 independent. The failure of IL-1ra to block the hypotension and IL-6 responses during endotoxemia cannot be easily explained by an inability of the receptor antagonist to block IL-1 mediated responses. It is unlikely that preservation of these responses can be explained by IL-1's actions on the type II receptor, as previous baboon studies have shown that IL-1 receptor antagonist will block the hypotension and IL-6 responses to exogenous IL-1 α administration (6).

Controversy exists whether a systemic IL-1 response occurs at all during sublethal endotoxemia. Previous studies from this laboratory have failed to show a circulating plasma IL-1 β response to endotoxemia in human volunteers (22). Michie et al. were also unable to detect circulating IL-1 β in volunteers receiving endotoxin (12). However, Cannon et al. have recently reported increased IL-1 β at a single time point following endotoxin administration (23). The present results would suggest

that circulating IL-1 is not likely to occur, or responsible for, most of the hemodynamic changes observed in sublethal endotoxemia.

In contrast, the severity of the infectious stimulus in the septic shock model correlates with the magnitude of the exaggerated IL-1 response, and hence its detection in the circulation. Therefore, we conclude that IL-1 is not the principal mediator of the hemodynamic and pathophysiologic responses to sublethal endotoxemia. However, excessive IL-1 production contributes to the pathophysiologic responses to lethal *E. coli* infection. Furthermore, IL-1 receptor antagonist may be beneficial in the treatment of hospitalized patients at risk of developing septic shock.

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References

1. Fong, Y., L. L. Moldawer, G. T. Shires, and S. F. Lowry. 1990. The biologic characteristics of cytokines and their implication in surgical injury. *Surg. Gynecol. Obstet.*, 170:363-378.
2. Long, N. C., I. Otterness, S. L. Kunkel, A. J. Vander, and M. J. Kluger. 1990. Roles of interleukin-1 β and tumor necrosis factor in lipopolysaccharide fever in rats. *Am. J. Physiol.* 259:R724-R728.
3. Gershenswald, J. E., Y. Fong, T. J. Fahey III, S. E. Calvano, R. Chizzonite, P. L. Kilian, S. F. Lowry, and L. L. Moldawer. 1990. Interleukin-1 receptor blockade attenuates the host inflammatory response. *Proc. Natl. Acad. Sci. USA.* 87:4966-4970.
4. Tracey, K. J., B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Milsark, R. J. Hariri, T. J. Fahey III, A. Zentella, J. D. Albert, et al. 1986. Shock and tissue injury induced by recombinant human cachectin. *Science (Wash. DC).* 234:470-474.
5. Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry, and A. Cerami. 1987. Anticachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. *Nature (Lond.)*. 330:662-664.
6. Fischer, E., M. A. Marano, A. Barber, A. A. Hudson, K. Lee, C. Rock, A. S. Hawes, R. C. Thompson, T. V. Hayes, T. D. Anderson, et al. 1991. A comparison between the effects of interleukin-1 α administration and sublethal endotoxemia in primates. *Am. J. Physiol.* 261:R442-452.
7. Okusawa, S., J. A. Gelfand, T. Ikejima, R. J. Connolly, and C. A. Dinarello. 1988. Interleukin 1 induces a shock-like state in rabbits. *J. Clin. Invest.* 81:1162-1172.
8. Waage, A., and T. Espevik. 1988. Interleukin-1 potentiates the lethal effect of tumor necrosis factor alpha/cachectin in mice. *J. Exp. Med.* 167:1987-1992.
9. Ohlsson, K., P. Bjork, M. Bergenfeldt, R. Hageman, and R. C. Thompson. 1990. An interleukin-1 receptor antagonist reduces mortality in endotoxin shock. *Nature (Lond.)*. 348:550-552.
10. Wakabayashi, G., J. A. Gelfand, J. F. Burke, and C. A. Dinarello. 1991. A specific receptor antagonist for interleukin-1 prevents *E. coli* induced septic shock in rabbits. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 5:338-343.
11. Hannum, C. H., C. J. Wilcox, W. P. Arend, F. G. Joslin, D. J. Dripps, P. L. Heimdal, L. G. Armes, A. Sommer, S. P. Eisenberg, and R. C. Thompson. 1990. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature (Lond.)*. 343:336-340.
12. Kenney, J. S., M. P. Masada, E. M. Engui, B. M. Delustro, M. A. Mulkins, and A. C. Allison. 1987. Monoclonal antibodies to human recombinant interleukin 1 (IL-1) beta: quantitation of IL-1 beta and inhibition of biological activity. *J. Immunol.* 138:4236-4240.
13. Michie, H. R., K. R. Manogue, D. R. Spriggs, A. Revhaug, S. O'Dwyer, C. A. Dinarello, A. Cerami, S. Wolff, and D. W. Wilmore. 1988. Detection of circulating tumor necrosis factor after endotoxin administration. *N. Engl. J. Med.* 318:1481-1486.
14. Van Zee, K. J., L. E. DeForge, E. Fischer, M. A. Marano, J. S. Kenney, D. G. Remick, S. F. Lowry, and L. L. Moldawer. 1991. IL-8 in septic shock, endotoxemia, and following IL-1 administration. *J. Immunol.* 146:3478-3482.
15. Fong, Y., K. J. Tracey, L. L. Moldawer, D. G. Hesse, K. B. Manogue, J. S. Kenney, A. T. Lee, G. C. Kuo, A. C. Allison, S. F. Lowry, and A. Cerami. 1989. Antibodies to cachectin/tumor necrosis factor reduce interleukin 1 β and interleukin 6 appearance during lethal bacteremia. *J. Exp. Med.* 170:1627-1633.
16. Chizzonite, R., T. Truitt, P. L. Kilian, A. S. Stern, P. Nunes, K. P. Parker, K. L. Kaffka, A. O. Chua, D. K. Lugg, and U. Gubler. 1989. Two high-affinity interleukin 1 receptors represent separate gene products. *Proc. Natl. Acad. Sci. USA.* 86:8029-8033.
17. Starnes, H. F., Jr., M. K. Pearce, A. Tewari, J. H. Yim, J. C. Zou, and J. S. Abrams. 1990. Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor- α challenge in mice. *J. Immunol.* 145:4185-4191.
18. McIntosh, J. K., D. M. Jablons, J. J. Mulé, R. P. Nordan, S. Rudikoff, M. T. Lotze, and S. A. Rosenberg. 1989. In vivo induction of IL-6 by administration of exogenous cytokines and detection of de novo serum levels of IL-6 in tumor-bearing mice. *J. Immunol.* 143:162-167.
19. Preiser, J. C., D. Schmartz, P. Van der Linden, J. Content, P. V. Bussche, W. Buurman, W. Sebald, E. Dupont, M. R. Pinsky, and J. L. Vincent. 1991. Interleukin-6 administration has no acute hemodynamic or hematologic effect in the dog. *Cytokine.* 3:1-4.
20. Dinarello, C. A., J. G. Cannon, S. M. Wolff, H. A. Bernheim, B. A. Beutler, A. Cerami, I. Figari, M. A. Jr. Palladino, and J. V. O'Connor. 1986. Tumour necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J. Exp. Med.* 163:1433-1450.
21. Dinarello, C. A., T. Ikejima, S. J. C. Warner, S. F. Orencole, G. Lonnemann, J. G. Cannon, and P. Libby. 1987. Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells in vitro. *J. Immunol.* 139:1902-1910.
22. Fong, Y., M. A. Marano, L. L. Moldawer, H. Wei, S. E. Calvano, J. S. Kenney, A. S. Allison, A. Cerami, G. T. Shires, and S. F. Lowry. 1990. The acute splanchnic and peripheral tissue metabolic response to endotoxin in man. *J. Clin. Invest.* 85:1896-1904.
23. Cannon, J. G., R. G. Tompkins, J. A. Gelfand, H. R. Michie, G. G. Stanford, J. W. M. Van der Meer, S. Endres, G. Lonnemann, J. Corsetti, B. Chernow, et al. 1990. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J. Infect. Dis.* 161:79-84.