Effects of Recombinant Soluble Type I Interleukin-1 Receptor on Human Inflammatory Responses to Endotoxin

By Hugh L. Preas II, Debra Reda, Margaret Tropea, R. William Vandivier, Steven M. Banks, Jan M. Agosti, and Anthony F. Suffredini

Effects of soluble recombinant human type I interleukin-1 receptor (sIL-1R) were evaluated in 18 volunteers given intravenous endotoxin and randomized to placebo (n = 6), low-dose (n = 6), or high-dose (n = 6) sIL-1R. Soluble IL-1R decreased IL-1β (P = .001), but decreased IL-1α (P = .0001), and resulted in 10-fold and 43-fold dose-related increases in sIL-1R-IL-1a complexes compared with placebo (P ≤ .001). High-dose sIL-1R, was associated with increased levels of immunoreactive tumor necrosis factor-α (P = .02), IL-8 (P = .0001), and cell-associated IL-1β (P = .047). C-reactive protein levels were higher after sIL-1R, than placebo (P = .035). Soluble IL-1R, decreased the severity of chills (P = .03), but did not alter other symptoms, changes in temperature, systemic hemodynamic responses, or changes in leucocyte and platelet number. Thus, sIL-1R, had no discernable antiinflammatory effect following endotoxin administration due in part to low levels of circulating IL-1β and neutralization of IL-1a inhibitory function. This latter interaction represents an indirect mechanism of agonist activity elicited by sIL-1R, and may contribute to increases in inflammatory mediators, limiting therapy with sIL-1R during endotoxemia. This is a US government work. There are no restrictions on its use.

INTERLEUKIN-1 (IL-1) has a central role in the regulation of inflammatory responses to infection. Patients with sepsis and animals with experimental infection have elevated blood levels of IL-117,8 and when administered to animals or humans, IL-1 causes a syndrome resembling sepsis that includes fever, hypotension, leukocytosis, and increased vascular permeability.5,6 Blocking cell-associated IL-1 receptors with IL-1 receptor antagonist (IL-1ra) can neutralize IL-1 effects and result in improvement of hypotension and organ failure in septic animals.7,9 A clinical trial of IL-1 receptor blockade using IL-1ra in human sepsis, however, found no beneficial effects on outcome.10 Moreover, excessive inhibition of IL-1 is harmful in some models of infection, confirming an essential role for the IL-1 response during infection.11,12

Soluble IL-1 receptors are regulatory proteins that alter the biological effects of IL-1. Type I IL-1 receptor (IL-1R), is found on most cells and appears to mediate all IL-1-related cell signaling.13,14 Soluble recombinant human IL-1R1 (sIL-1R1), developed from the extracellular portion of human IL-1R1, has binding characteristics for IL-1 indistinguishable from those of membrane-associated IL-1R1.13,15,16 This recombinant protein inhibits a wide variety of inflammatory responses including in vivo alloreactivity to mouse heterotopic allografts and local injection of allogeneic cells,16 inflammation and articular damage in antigen-induced arthritis,17 and the severity of autoimmune encephalomyelitis.18 Subcutaneous administration of sIL-1R1 to humans inhibits late-phase cutaneous allergic responses.19

The administration of endotoxin to normal humans reproduces many of the cardiovascular and inflammatory responses of sepsis.20-22 We used this model to evaluate whether the administration of sIL-1R1 would alter the clinical and inflammatory manifestations of acute endotoxemia. Our results demonstrate an interaction between sIL-1R1, and circulating IL-1ra in vivo that may limit the antiinflammatory effects of sIL-1R1, as well as IL-1ra and result in enhancement of some inflammatory responses.

MATERIALS AND METHODS

Subjects and study plan. Thirteen men and five women, 19 to 35 years of age (mean, 26 years) were evaluated. With the exception of one woman taking oral contraceptives, none were taking any medications, and all had normal physical examinations, electrocardiograms, chest radiographs, and blood and urine analyses. The study was approved by and performed in accordance with the ethical standards of our institutional review board on human experimentation. Written consent was obtained from all the subjects. Study participants were admitted to the medical intensive care unit after fasting overnight and given maintenance intravenous (IV) fluids.

The subjects were randomized to receive a 30-minute infusion of either placebo (sIL-1R1, vehicle, n = 6), 1 mg/m2 (n = 6), or 10 mg/m2 (n = 6) of sIL-1R1 (Immunex Corp, Seattle, WA). A prior study in normal volunteers14 had shown that a dose of 1 mg/m2 resulted in a mean maximum concentration of 0.48 μg/mL of sIL-1R1 with a mean T1/2 of 17.3 hours. A dose of 10 mg/m2 was associated with a mean maximum concentration of 5.02 μg/mL of sIL-1R1 and a mean T1/2 of 15.3 hours. These doses were not associated with any significant clinical or laboratory abnormalities including induction of serum antibodies directed against sIL-1R1. Higher doses (>20 mg/m2) administered in this previous pharmacokinetic study were associated with allergic reactions.23

The recombinant human IL-1R1 was produced in a mammalian expression system (NS1 cells).14 The truncated soluble form of the IL-1R1 was engineered by isolating the cDNA, which encoded the N-terminal 312 amino acids of full-length IL-1R1 protein. A CAV-neo-S-ru IL-1R1 expression cassette, introduced into a murine myeloma cell line (NS1), produced sIL-1R1 as a glycoprotein with a molecular weight of 59 ± 2 kD. The receptor is a monomer in solution and binds one mole of IL-1 per mole of receptor and forms a stable complex between a single truncated receptor molecule and a single IL-1 molecule. The soluble IL-1R1 binds IL-1 with high-affinity, equal to the binding affinity of the intact, membrane form of IL-1R1.25

The sIL-1R1 was supplied as a sterile lyophilized preparation of

From the Critical Care Medicine Department, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD; and Immunex Corporation, Seattle, WA.

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Address reprint requests to Hugh L. Preas II, MD, Bldg 10, Room 7D-43, National Institutes of Health, Bethesda, MD 20892-1662.

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0.5 mg or 2.5 mg recombinant human protein per vial with 40 mg mannitol, USP; 10 mg sucrose, NF; and 1.2 mg tromethamine (Tris), USP. The placebo contained only the inactive excipients. The sIL-1R1 or placebo was reconstituted with 1 mL of Sterile Water for Injection (USP), diluted in 100 mL of 0.9% saline and infused for 30 minutes. Following the infusion, purified lipopolysaccharide prepared from Escherichia coli 0113 (U.S. Standard Reference Endotoxin, Lot EC-5, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD) was administered IV (4 ng/kg of body weight, n = 18) over 1 minute and flushed with 10 mL of 0.9% saline.

Oral temperature (Diatek, San Diego, CA), respiratory rate, heart rate, and blood pressure were measured at 15-minute intervals. Blood pressure was continuously monitored with radial artery catheters (Arrow International, Redding, PA). The number and severity of symptoms (chills, headache, arthralgia, myalgia, nausea) were assessed at the end of each study day based on a scale of absent—0, mild—1, moderate—2, or severe—3.

Systemic hemodynamic responses were evaluated further in the placebo subjects and those given high-dose sIL-1R1. Central pressures and cardiac output were assessed with pulmonary artery catheters (VIP, Baxter Healthcare, Edwards Critical Care Division, Irvine, CA) and ventricular function was evaluated with radionuclide (technetium-99m) heart scans as previously described.11 Systemic hemodynamics were measured at baseline (0 hour), 3 hours, 5 hours, and 8 hours after endotoxin administration. To assess ventricular function at different preloads, fluid loading was performed using 0.9% saline with nonpyrogenic heparin 10 U/mL blood, Abbott, North Chicago, IL and blood samples were clotted on ice. All samples were centrifuged at the bedside (4 to 10°C) and then frozen at −70°C until assayed.

Blood levels of cytokines were measured in duplicate by quantitative immunoassays. Tumor necrosis factor (TNF) bioactivity was estimated using immunoassays (all R&D Systems) with a detection limit of 9.4 mg/mL. Lactoferrin was assayed by ELISA as previously described.14 The detection limit of the ELISA was 1 ng/mL plasma.

Endotoxin-stimulated whole blood was used to assess the effects of sIL-1R1 on cytokine production in vitro. Blood from four additional normal subjects was collected in heparinized syringes (nonpyrogenic heparin 10 U/mL blood, Abbott, North Chicago, IL) and plated in 12-well culture plates (Costar, Cambridge, MA). Blood samples were stimulated with lipopolysaccharide 1 μg/mL (S minnesota; Sigma, St. Louis, MO) following the addition of sIL-1R1 (final concentration 0.5, 5, 15 μg/mL). These concentrations of sIL-1R1 were based on levels achieved in earlier clinical trials following the administration of 1, 10, or 30 mg/m², respectively. The plates were incubated on a rocking platform at 37°C with 5% CO₂. Samples for cytokines were assayed after 0, 1, 4, 8, 16, and 24 hours of incubation using immunoassays (all R & D Systems).

Statistics. Data were analyzed using a three-way analysis of variance (ANOVA) model.22 The three-way ANOVA included the following factors: (1) dose of sIL-1R1 (placebo, low, and high), (2) subject nested with dose of sIL-1R1, and (3) time. In addition, a dose-time interaction was included in the model to test the differences in the time course among the three doses. For cytokine levels, the dose-time interaction was decomposed as suggested by the IL-1α response, ie, high-dose was compared with the average of low and placebo, and then the similarity of low-dose and placebo was tested. Interactions involving subject-time were pooled into a residual term and used as the source of variability to test the time and dose-time interactions. When tests involved multiple comparisons, the P-values were corrected using a Tukey procedure.23 Ordered dose effects were tested using a Bartholomew test.24 Residuals from the ANOVA were assessed by a Shapiro-Wilk test.25 When residuals failed the test of normality, data (eg, cytokine levels) were logarithmically transformed to improve residuals. Spearman correlations were performed to assess the relation between maximum IL-1α and maximum TNF or IL-8. In two instances, data were judged as outliers by the test of Dixon and were removed from the specific analysis (C-reactive protein, IL-1α after incubation with M1 antibody).22 Data are reported as mean and standard error of mean (SEM).

RESULTS

Systemic and clinical responses. All 18 subjects given endotoxin developed fever, constitutional symptoms, and re-
lease of acute phase proteins (Table 1). The maximum temperature occurred at 6 hours and the magnitude and pattern of the fever did not differ among the groups. Chills were less severe in subjects given sIL-1R. Nadir (1 hour) and peak (8 hours) leukocyte responses were similar among all subjects. Increases in neutrophil band forms and decreases in lymphocytes, monocytes, and platelets were similar among the groups. The rise in plasma lactoferrin, a marker of neutrophil activation, was not altered by sIL-1R. Levels of C-reactive protein at 24 hours were significantly greater in subjects given sIL-1R compared with placebo, while serum amyloid A levels were similar among the three groups.

All subjects developed tachycardia (maximum, placebo 100 ± 4 beats/min, P = NS) and a decrease in mean arterial pressure (minimum, placebo 76 ± 3 mmHg, P = NS). Hyperdynamic cardiovascular responses occurred both in the placebo and in the high-dose sIL-1R subjects, characterized by significant increases in cardiac index and heart rate and decreases in mean arterial pressure and systemic vascular resistance index (Table 2). These responses were not altered by high-dose sIL-1R; no significant differences were found between the placebo and high-dose group in cardiac index, mean arterial pressure, systemic vascular resistance index, heart rate, oxygen consumption or delivery, or left ventricular ejection fraction.

Cytokine and acute phase protein responses. In subjects given endotoxin and placebo, low levels of IL-1β were present at baseline and a small increase occurred at 3 hours in the placebo subjects (10.6 ± 2.8 pg/mL). No increase occurred in subjects given sIL-1R, and this was significantly different than placebo (P = .0001, Fig 1A). Cell-associated IL-1β rose significantly at 3 hours (P = .0001) and was greater in high-dose sIL-1R subjects than placebo and low-dose sIL-1R subjects (P = .047, Fig 1B). IL-1ra was significantly lower in subjects given high-dose sIL-1R compared with low-dose sIL-1R or placebo (P = .0001) and these changes were maximum at 3 hours (Fig 1C). At 3 hours, complexes of IL-1Rα-IL-1ra in subjects given high-dose sIL-1R were 43-fold greater (P = .0001) and in low-dose 10-fold greater than placebo (P = .001) (Fig 2). Incubation of blood samples from all subjects given endotoxin (with and without sIL-1R) with anti-sIL-1R antibody (M1, 5μg/mL) resulted in a significant increase in measurable IL-1ra at 3 hours in all three groups (amount of increase in IL-1ra above 3-hour IL-1ra levels; placebo 110.2 ± 11.3, low-dose 122.0 ± 17.3, high-dose 138.0 ± 12.7 ng/mL, dose ordered P < .001). Increased amounts of the neutralizing M1 antibody (50 μg/mL) did not increase the amount of dissociable IL-1ra (data not shown).

Elevated levels of TNF-α cytotoxicity were present at 1.5 to 2 hours in all three groups (P = .0001) and were statistically similar among the groups (maximum, placebo 202 ± 94, low-dose 217 ± 133, high-dose 364 ± 196 pg/mL, P = NS). TNF-α immunoactivity rose with maximum values at 1.5 to 2 hours in all three groups (P = .001). Subjects given high-dose sIL-1R had higher levels than subjects given low-dose sIL-1R or placebo (P = .02, Fig 3A). IL-8 levels were maximum at 2 to 3 hours in all three groups and were greater in subjects given high-dose sIL-1R than in subjects given low-dose sIL-1R and placebo (P = .0001, Fig 3B). IL-6 levels rose (P = .0001) with maximum values at 3 hours and were statistically similar among the three groups (placebo 2,477 ± 1,653, low-dose 1514 ± 516, high-dose 3,124 ± 1,550 pg/mL, P = NS). G-CSF levels rose (P = .0001) with maximum values at 3 to 6 hours and were statistically similar among the three groups (placebo 2.761 ± 1,824, low-dose 2.045 ± 577, high-dose 2.057 ± 1,488 pg/mL, P = NS). IL-1ra levels correlated positively with both TNF-α immunoactivity (r values similar for all three groups, P = .001, average r = 0.81, P < .001) and IL-8 (r values similar for all three groups, P > .02, average r = 0.81, P < .001).

In vitro, the effects of adding sIL-1R to normal donor serum treated with EDTA and spiked with 20 pg/mL of
of sIL-1RI (20 ng/mL) resulted in 93% ± 7% recovery of IL-1β, while 100-fold excess of sIL-1R1 (20 μg/mL) resulted in 77% ± 5% recovery of IL-1β, while 106-fold excess of sIL-1RI administered resulted in the binding of IL-1β and IL-1ra and enhancement of some inflammatory responses during endotoxemia.

Soluble cytokine receptors may antagonize cell signaling by competing with membrane-associated receptors for their respective ligands. Examples include soluble IL-4 receptor and TNF-α receptor. Alternatively, other soluble cytokine receptors, after associating with their ligands such as IL-6 receptor or ciliary neurotrophic factor receptor, can initiate cell signaling by associating with a dimeric form of the gp130 signal transducing receptor. Examples include soluble IL-4 receptor on IL-4ra binding more avidly than IL-la or IL-lp to soluble IL-1RI. Because of a slow dissociation rate, IL-1ra binds more avidly than IL-la or IL-lp to soluble IL-1RI and enhances the binding of IL-1p and IL-1ra and enhancement of some inflammatory responses during endotoxemia.

In the current study, sIL-1RI administration resulted in decreased IL-1ra levels and increased sIL-1RI-sIL-1ra complexes. The increased levels of TNF-α, IL-8, and cell associ-
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Fig 1. Serum IL-1β (A), cell-associated IL-1β (B), and plasma IL-1ra (C) after endotoxin administration in subjects randomized to receive placebo, low-dose, or high-dose sIL-1R. Data presented as mean ± SEM and analyzed by three way ANOVA. Levels of complexes after high-dose sIL-1RI were 43-fold greater (P = .0001) and after low-dose sIL-1RI were 10-fold greater than in placebo subjects (P = .001).

Fig 2. IL-1R-IL-1ra complexes 3 hours after endotoxin administration in subjects randomized to receive placebo, low-dose, or high-dose sIL-1R. Data presented as mean ± SEM and analyzed by three way ANOVA. Levels of complexes after high-dose sIL-1RI were 43-fold greater (P = .0001) and after low-dose sIL-1RI were 10-fold greater than in placebo subjects (P = .001).

Fig 3. TNF-α immunoactivity (A) and IL-8 (B) after endotoxin administration in subjects randomized to receive placebo, low-dose, or high-dose sIL-1R. Data presented as mean ± SEM and analyzed by three way ANOVA. Subjects given high-dose sIL-1R had higher levels of TNF-α than placebo subjects given low-dose sIL-1R, or placebo (P = .02). IL-8 levels were greater in subjects given high-dose sIL-1R, than in subjects given low-dose sIL-1R, and placebo (P = .0001).
duction of other cytokines. Concentrations of IL-1ra similar to those generated in vivo with IL-2 infusions are sufficient to downmodulate production of IL-1β and TNF-α by IL-2-stimulated peripheral blood mononuclear cells. In the current study, the greatest reduction in IL-1ra levels occurred 1 to 2 hours after the augmented release of immunoreactive TNF-α and IL-8. It is likely that changes in circulating IL-1ra are detected later than those in the immediate cell environment. Antigenic TNF levels were increased, but the observed increase in TNF cytotoxicity was not statistically significant. This may be due to greater variability observed in the TNF bioassay compared with the immunoassay. It is unknown whether the increased levels of antigenic TNF were due to increased TNF receptor shedding. IL-1ra infusions during experimental human endotoxemia did not decrease TNF type I receptor levels. The effect of decreased IL-1ra levels, as observed in this study, on TNF receptor shedding is unknown. Correlations of maximum IL-1ra with TNF-α and IL-8 levels were positive and did not differ among the placebo and sIL-1Rα subjects. Therefore, in vivo, it appears that IL-1ra depletion alone did not account for the augmented levels of TNF-α and IL-8. Presumably other mediators contributed to the increased TNF-α and IL-8 levels.

Other studies that have attempted to limit IL-1 effects during experimental human endotoxemia have had only limited success. A continuous infusion of IL-1ra in subjects challenged with 2 ng/kg of endotoxin resulted in a decrease in symptom severity but not decreases in fever or heart rate. Other studies with IL-1ra infusion have described a reduction in neutrophilia without changes in symptoms or vital signs after 3 ng/kg of endotoxin. The use of sIL-1Rα in our study was associated solely with a decreased severity of chills. Although IL-1 may contribute to the development of symptoms, the results above describing the effects of a pure IL-1 antagonist (IL-1ra) demonstrate the redundancy of inflammatory pathways during initial responses to endotoxin. IL-1 produced in extravascular sites may limit the effects of circulating inhibitors. Alternatively, IL-1 may have a relatively minor role in the acute manifestations of endotoxemia. Some investigators have found IL-1β only at low levels in extracted plasma, while others have not found increases in circulating levels in human subjects, following endotoxin administration. In the current study, high levels of sIL-1Rα in vitro blunted the detection of IL-1β in spiked samples. Thus, the absence of a rise in IL-1β after sIL-1Rα infusion may be due to binding of IL-1β or in part to the effects of sIL-1Rα excess on the immunoassay.

Soluble cytokine receptors, including type I and II TNF receptors, as well as IL-1Rα, are detectable in the circulation during the acute inflammatory response to endotoxin as well as sepsis. An IL-1 binding protein with similarities to IL-1Rα has been described in normal serum and may be a fragment of IL-1Rα. We found low levels of complexed sIL-1Rα-IL-1α in the placebo subjects at 3 hours postendotoxin, and IL-1α could be dissociated from the complex by an anti–IL-1Rα neutralizing antibody. No increase in IL-1ra was found in preendotoxin baseline samples treated with the antibody. These results suggest that, during the peak inflammatory response to endotoxin, low levels of IL-1Rα are released into the circulation.

Soluble IL-1Rα has been effective in experimental models of inflammation including arthritis, transplantation, encephalomyelitis, and human allergic response, although its interactions with IL-1ra in these specific models has not been described. The beneficial effects observed in these models could be related to the dose of sIL-1Rα. Consistent with this possibility is the observation that both low- and high-dose sIL-1Rα decreased IL-1β levels, but, unlike high-dose sIL-1Rα, low-dose sIL-1Rα did not decrease IL-1ra levels. Increasing the dose of sIL-1Rα during acute endotoxemia would likely lead to larger reductions in IL-1ra and result in greater unopposed IL-1 effects. This might exacerbate inflammatory responses in a manner similar to the use of anti–IL-1ra antibodies in models of acute and chronic inflammation. Our data show that during the acute phase response to endotoxin, when there is a 100-fold greater release of IL-1ra than of IL-1β into the circulation, sIL-1Rα will have essentially no antiinflammatory effect due to low levels of circulating IL-1β and neutralization of IL-1ra inhibitory function. These results emphasize that the dosing of soluble cytokine receptors can result in unexpected agonist interactions during the acute phase response to endotoxin, and these may limit some of their therapeutic applications.

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