Rapid Communication

Interleukin-1 Receptor Antagonist Circulates in Experimental Inflammation and in Human Disease

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Interleukin-1 receptor antagonist (IL-1ra) is a 22-Kd protein that shares homology with IL-1β, binds to the IL-1 receptor, but has no known agonist properties. This inhibitor appears to be the first cytokine whose sole function is to block the actions of another cytokine. Exogenous IL-1ra administration has been shown to reduce mortality in experimental septic shock. We now report that IL-1ra is endogenously produced and circulates in experimental inflammation and in clinical disease. After experimental endotoxemia in human volunteers, IL-1ra concentrations increase from a baseline concentration of 460 ± 200 pg mL⁻¹ to 14,870 ± 290 pg mL⁻¹ at 3 hours (P < .05). IL-1ra is also detectable in all plasma samples from critically ill patients with a mean concentration of 8,680 ± 2,080 pg mL⁻¹ (range 320 to 55,370 pg mL⁻¹). In nonhuman primates, Escherichia coli septic shock induces elevated plasma levels of IL-1ra (P < .05). However, in animals that eventually succumb to septic shock, IL-1ra appears in quantities presumed inadequate to block the pathologic sequelae associated with high IL-1β levels. The findings suggest that IL-1ra may play a role in modulating the systemic host responses to a variety of nonlethal disease states by altering the balance between cytokines and their antagonists.

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Materials and Methods

Subjects. Four healthy males (ages 20 to 30) were admitted to the Adult Clinical Research Center at Cornell University Medical College. The study was approved by the Institutional Review Board of the New York Hospital-Cornell Medical Center and informed consent was obtained from all volunteers before enrollment. Under sterile conditions and with local anesthesia, arterial and venous catheters were placed percutaneously for continuous hemodynamic monitoring, blood sampling, and administration of endotoxin. After baseline venous blood samples were obtained, each subject received an intravenous (IV) injection of 20 IU kg⁻¹ body weight Food and Drug Administration (FDA) reference endotoxin (lot EC-5, from E coli 0113, provided by Dr H.D. Hochstein, FDA, Bethesda, MD). Repeated plasma samples were obtained over the following 15 hours for cytokine measurements.

Plasma samples from 12 critically ill surgical patients were obtained at various time points during their clinical course, and assayed for endogenous circulating IL-1ra (n = 50). All patients were hemodynamically monitored using invasive devices. Each patient’s clinical status was evaluated at blood sampling intervals using the APACHE II score, a physiologic severity of injury index. The patients were included in the study if their calculated APACHE score was 14 or greater on admission to the surgical intensive care unit, indicating an overall probability of mortality of at least 5% (Table 1). Blood samples were collected periodically corresponding to the development of sepsis as well as during its resolution.

Primates. Sixteen female Papio anubis baboons (10 to 15 kg), obtained through the National Primate Exchange by Buckshire Laboratories (Chelmsford, PA), were housed and quarantined for 4 weeks at the Research Animal Resource Center of Cornell University Medical College (CUMC) to confirm that they were in good health and free of transmissible disease. The experimental protocol was approved by the Institutional Animal Care and Use Committee at CUMC. Baboons were fasted overnight and immobilized with ketamine hydrochloride (10 mg kg⁻¹ intramuscularly [IM]) before the experiment. During the study, anesthesia was maintained using pentobarbital sodium (5 mg kg⁻¹ intravenously [IV]) at hourly intervals. After endotracheal intubation, arterial and venous access was secured by sharp dissection and bilateral isolation of the femoral vessels and invasive monitoring was
was collected at hourly intervals for further analysis. All animals received continuous infusion of physiologic saline. The bacterial infusion represents an approximate LD₅₀ dose of live Salmonella typhosa. The performance was supported with 10 mL kg⁻¹ crystalloid bolus every 15 minutes if they met two or more preset criteria, however, at 8 hours fluid support was withdrawn from all animals. Arterial blood pressure and cardiac output, and a significant lactic acidemia. The quantity of endotoxin given was based on preliminary studies to monitor the presence of equivalent quantities of control Pupio plasma. Samples were diluted 1:5 with phosphate-buffered saline (pH 7.4) containing 0.1% Tween 100, giving an overall sensitivity of the ELISA is 10 pg mL⁻¹.

The sensitivity of the ELISA is 64 pg mL⁻¹ and is log-linear from 0.064 to 500 ng mL⁻¹. Standards are determined in the presence of equivalent quantities of healthy, control Pupio plasma. Samples were diluted 1:5 with phosphate-buffered saline (pH 7.4) containing 0.1% Tween 100, giving an overall sensitivity for the assay of 350 pg mL⁻¹.

IL-1β was determined by ELISA, as published elsewhere. The sandwich ELISA uses two monoclonal antibodies, one biotinylated, and signal amplification is achieved through a biotin-streptavidin-conjugated horseradish peroxidase system. The sensitivity of the ELISA is 10 pg mL⁻¹. IL-6 levels were determined using a B.9 hybridoma proliferation assay. One unit is defined as the quantity necessary to produce one-half maximal proliferation, and the lower limit of detection is a 2 B.9 U/mL.

Statistics. Results obtained from critically ill patients, endotoxin-treated volunteers, and Pupio are presented as mean ± standard error of the mean. Differences in IL-1α levels among volunteers or Pupio after endotoxin, E. coli, or IL-1α administration were evaluated by one-way ANOVA. Differences from baseline were determined by Dunnett’s multiple range test. Differences in peak IL-1α and IL-1β levels in Pupio were assessed by one-way ANOVA. Post-hoc comparisons were performed with the Student’s t-test.
Correlations among continuous data were evaluated using least-squares. Significance was designated at the 95% confidence level.

RESULTS

Baseline IL-1ra concentrations ranged between 190 and 1,070 pg mL\(^{-1}\) (460 ± 200 pg mL\(^{-1}\)) in the healthy volunteers. A single IV bolus of endotoxin at 20 U kg\(^{-1}\) produced a monophasic peak of plasma IL-1ra at 3 hours (mean 14,870 ± 290 pg mL\(^{-1}\), \(P < .05\) v baseline by ANOVA and Dunnett’s) (Fig 1). Concentrations decreased thereafter. No IL-1β was detected at any timepoint before or after endotoxin administration. IL-1ra was detected in all 50 of the samples obtained from 12 critically ill patients in the surgical intensive unit with a mean concentration of 8,680 ± 2,060 pg mL\(^{-1}\) (Table 1). In contrast, IL-1β was detected in only 3 of the 50 samples (data not shown). IL-1α was not measured in the present study. However, IL-1α has not been previously detected in the plasma of baboons or human volunteers administered sublethal endotoxemia.\(^{10,12}\) Similarly, IL-1α has not been observed in the circulation of baboons expiring from lethal septic shock or in critically ill patients (data not shown).

While admission criteria to the intensive care unit varied, all of the patients were critically ill. All patients required ventilatory support during some period of their stay in the intensive care unit and 92% of the patients had undergone a surgical procedure before admission. Furthermore, all patients had at least one episode of sepsis, documented by positive pulmonary, peritoneal, or blood cultures. Plasma IL-6 levels were markedly elevated, consistent with the presence of metabolic stress.\(^{14}\)

Plasma IL-1α levels correlated with both IL-6 concentrations (\(R = .79, n = 50, P < .001\)) and repeated APACHE II scores (\(R = .67, n = 50, P < .001\)). However, peak IL-1α levels did not differ significantly among samples obtained from patients who survived or expired. Similarly, there were no significant differences in plasma IL-1α concentrations of samples obtained during intervals of sepsis or recovery (data not shown).

In Papio, IL-1α concentrations increased within 2 hours after anesthesia and instrumentation associated with the invasive monitoring. After LPS, IL-1α, and saline infusions, IL-1α levels became detectable within 2 hours, and peaked at 4 to 6 hours, decreasing thereafter. In contrast, IL-1α levels peaked 4 to 6 hours after a lethal \(E\) coli infusion, but concentrations were sustained for 18 to 24 hours (Fig 2). In control baboons that were instrumented and given physiologic saline only, IL-1α levels peaked at 550 ± 360 pg mL\(^{-1}\), whereas IL-1α induced a peak IL-1α of 3,070 ± 1,000 pg mL\(^{-1}\) (Table 2). Despite the fact that \(E\) coli, but not LPS, induced a systemic IL-1β response and produced death, maximal IL-1α levels were similar in both groups, with IL-1α peak levels of 7,550 ± 2,020 pg mL\(^{-1}\) in the \(E\) coli shock baboons and 7,300 ± 3,150 pg mL\(^{-1}\) in the LPS-treated animals. In contrast, IL-1β peaked at 876 ± 414 pg mL\(^{-1}\) in the circulation of the \(E\) coli septic baboons, and remained below the detection limit of the assay in the

| Table 2. Effect of IL-1α, LPS, and \(E\) coli Administration on Peak IL-1α and IL-1β Levels |
|-------------------|-------------------|-------------------|-------------------|
|                   | Peak IL-1α pg mL\(^{-1}\) (mean ± SEM) | Time (h) | Peak IL-1β pg/mL (mean ± SEM) | Time (h) |
| Sham infusion n = 4 | 550 ± 360 | 4-8 | <32 | 3,070 ± 1,100 | 4-8 | <32 |
| IL-1α (100 μg kg\(^{-1}\)) n = 3 | 7,300 ± 3,160* | 4-8 | <32 | 7,550 ± 2,020* | 4-12 | 876 ± 414* |
| LPS (500 μg kg\(^{-1}\)) n = 3 | 3,070 ± 1,100 | 4-8 | <32 | 7,550 ± 2,020 | 4-12 | 876 ± 414* |
| \(E\) coli (10\(^8\) cfu kg\(^{-1}\)) n = 6 | 7,550 ± 2,020* | 4-12 | 876 ± 414* |

\(*P < .05 v\) sham infusion.
endotoxin, saline, and IL-1α treated groups. The latter finding is consistent with previously published data.\textsuperscript{12,14}

**DISCUSSION**

The recent description of a naturally occurring IL-1 inhibitor synthesized by a variety of inflammatory cells prompted our investigation of whether IL-1ra can be measured in experimental models of sepsis or in clinical disease, and whether IL-1ra plays a role in modulating IL-1’s actions in these disease states. The present report confirms that IL-1ra is an inducible cytokine that circulates in experimental inflammation and in human disease.

During experimental endotoxemia in humans, the monophasic appearance of IL-1ra coincides with the previously described IL-1 inhibitory activity found in the plasma of human volunteers administered endotoxin,\textsuperscript{25} suggesting that IL-1ra may be a principal component of this inhibitory activity. IL-1ra levels from endotoxicemic volunteers were comparable with those seen in critically ill patients. There was also a significant correlation between plasma IL-1ra levels and APACHE II scores and IL-6 concentrations, indicating that IL-1ra concentrations do correlate to some extent with the magnitude of the inflammatory response. However, peak IL-1ra responses in patients expiring and those surviving did not differ. This latter finding is not surprising, given that in the nonhuman primates, sublethal endotoxemia and lethal \textit{E. coli} septic shock resulted in comparable IL-1ra levels, albeit significantly higher than concentrations seen in instrumented, sham-treated animals. Thus, these findings confirm that plasma IL-1ra levels are responsive to the magnitude of the inflammatory response, but in the present study IL-1ra levels could not discriminate among individuals that will survive or expire.

The data presented are also consistent with the conclusion that in lethal \textit{E. coli} septic shock, IL-1ra concentrations are inadequate to block the adverse tissue responses associated with exaggerated IL-1β production. Under in vitro conditions, 90% inhibition of IL-1α-mediated responses of T cells, synovial cells, and chondrocytes require 100- to 1,000-fold greater molar concentrations of IL-1ra.\textsuperscript{14,15} Previous primate studies have shown that a molar excess of 1,000 times as much IL-1ra as IL-1α is necessary to completely block the actions of IL-1 in vivo.\textsuperscript{12} The explanation for this observation is that relatively low receptor occupancy is required for IL-1 to elicit biologic responses. In baboons eventually dying of lethal \textit{E. coli} septic shock, the differences in peak IL-1β and IL-1ra concentrations are only 5- to 10-fold (Fig 2). Thus, during \textit{E. coli}-induced septic shock, IL-1ra appears in the circulation in insufficient quantities to prevent the adverse effects of an exaggerated IL-1 response. We have previously shown that when recombinant IL-1ra is administered in quantities of 13 to 46 mg kg\textsuperscript{-1} body weight in \textit{E. coli}-induced shock in rabbits or primates, mortality could be prevented.\textsuperscript{7,13}

If endogenous IL-1ra levels are inadequate to protect against IL-1-induced lethality in acute overwhelming septic shock, what then is the function of circulating IL-1ra after mild endotoxemia or in critically ill patients? Clearly, IL-1ra appears in the circulation more frequently than IL-1β and, with the exception of \textit{E. coli} septic shock, at molar concentrations of 100 to 1,000 fold higher. This finding is consistent with the fact that IL-1ra, but not IL-1β or IL-1α, possesses a classical signal sequence and is efficiently secreted.\textsuperscript{42} IL-1 levels are frequently higher at the local site of tissue injury than they are in the circulation\textsuperscript{16} and plasma IL-1β appearance is generally associated with an adverse outcome.\textsuperscript{17} Circulating IL-1ra during mild inflammatory stress may serve to reduce the systemic responses to localized IL-1 production when plasma appearance of IL-1β is minimal. However, during septic shock, the homeostatic mechanisms in place to offset the pathologic sequelae of exaggerated IL-1 production are insufficient to improve morbidity. It is in this situation that the possible therapeutic application of exogenous administration or endogenous stimulation of IL-1ra may be of benefit.

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