Brief Definitive Report

A Recombinant Human Receptor Antagonist to Interleukin 1 Improves Survival after Lethal Endotoxemia in Mice

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Summary

Interleukin 1 (IL-1) is an endogenously produced cytokine that mediates a variety of physiological effects that may be beneficial or deleterious to the host. C57Bl/6 mice treated intravenously with a recently characterized human recombinant receptor antagonist protein to IL-1 (IL-1ra) had improved survival when treated after a lethal *Escherichia coli* endotoxin (lipopolysaccharide [LPS]) challenge. IL-1ra was effective when treatment was initiated after LPS, and intravenous administration every 4 h for 24 h was required. Serum levels of tumor necrosis factor (TNF) activity after LPS and in vitro TNF cytotoxicity were not altered by treatment with IL-1ra. These experiments provide direct evidence that the lethal effects of LPS may be mediated through the action of IL-1 and that the IL-1ra can provide a new treatment strategy for disease processes mediated via this cytokine.

Recent evidence suggests that the administration of endotoxin or bacteremia initiates the production and release of endogenously produced cytokines such as TNF, IL-1, IL-6, and IFN-γ in a complex cascade through which the lethal effects of endotoxin or septicemia are mediated. The role of TNF as a central mediator of the lethal effects of endotoxin has been supported by the findings that: (*a*) infusion of TNF causes shock, tissue injury, and death characteristic of endotoxin shock; (*b*) passive immunization against TNF protects mice from a subsequent lethal endotoxin challenge and prevents hypotension during lethal bacteremia in the baboon; and (*c*) circulating plasma TNF levels peak very rapidly after endotoxin administration (1–3). However, after the administration of a lethal endotoxin challenge in mice, death typically occurs from 24 to 48 h later, during which time the deleterious effects of a variety of endogenous mediators may be contributing to the demise of the animal.

We hypothesized that since IL-1 shares many physiological activities with TNF and is produced and released in large quantities soon after TNF in response to endotoxin or bacteremia (4–7), it may also be a central comediator of endotoxin lethality. The repetitive administration of IL-1, in amounts comparable with physiologically produced concentrations, causes anorexia, inflammation, and fever (8–10), while higher doses induce hypotension, tissue injury, organ failure, and death characteristic of septic shock (11, 12). IL-1 and TNF act synergistically to enhance lethality when both cytokines are given in sublethal doses in vivo (13). A treatment strategy directed towards inhibiting the deleterious physiological effects of IL-1 may ameliorate or prevent tissue injury or death in inflammatory or infectious disease states.

Recently, a newly described receptor antagonist to IL-1 (IL-1ra) produced from IgG-adherent human monocytes has been purified, sequenced, and cDNA for the 18-kD protein expressed in *Escherichia coli* (14, 15). The protein binds to the IL-1 receptor but has no agonist activity, nor does it bind to the cytokine. The current experiments were performed to determine the in vivo effects of the IL-1ra against the lethality of endotoxin.

Materials and Methods

Animals. Female C57Bl/6 mice weighing 19–21 g were housed six per cage and kept in a controlled environment. All experiments were conducted in compliance with the Animal Care and Use Committee of the National Institutes of Health.

Reagents. IL-1ra (generously supplied by R.C. Thompson, Synergen, Inc., Boulder, CO) was brought to a final concentration in 0.5% fatty acid–poor endotoxin-free BSA (Calbiochem-Behring Corp., La Jolla, CA) and kept at 2–8°C. Control mice received an equal volume of protein carrier solution (vehicle). *E. coli* endotoxin (LPS, serotype 0127:B8) was purchased from Sigma Chemical Co. (St. Louis, MO) and reconstituted in saline to a standard concentration of 10 cc/kg.

In Vitro IL-1ra Activity. Thymocytes from 6-wk-old C3H/HeJ mice were incubated for 72 h with recombinant human (rh)IL-2 (Cetus Corp., Emeryville, CA) and, where indicated, 100,000 U/ml rhIL-1α (Hoffman-La Roche, Inc., Nutley, NJ) as described for the mouse thymocyte assay (16); 10^6 cells were placed in each well with 200 μl complete media (RPMI with 10% FCS). IL-1ra was

included in the media in serial dilutions in the inner 60 wells of a 96-well plate. [^{3}H]Thymidine was added to the media after 72 h (1 μCi per well) and incubated for 6 h. Cells were then harvested onto glass fiber filters and [^{3}H]thymidine uptake was measured in a Betaplate liquid scintillation counter (Pharmacia/LKB Biotechnology, Inc., Piscataway, NJ).

A confluent monolayer of L929 cells in each of the inner 60 wells of 96-well plates was incubated for 18 h with 200 μl of complete media containing dactinomycin (1.36 μg/ml) as well as rmuTNF (Genentech, Inc., South San Francisco, CA) and IL-1ra in the concentrations indicated, in a fashion similar to thoe L929 bioassay for TNF (17). The cells were washed, fixed, and stained with crystal violet; retained dye was solubilized; and absorbance (595 nm) was measured on a Titertek Multiscan plate reader (Flow Laboratories, McLean, VA).

In Vivo Circulating TNF Activity. Mice (n = 29) were treated with either IL-1ra (25 mg/kg) or vehicle intravenously followed by LPS (30 mg/kg) intravenously 10 min later. Mice were exsanguinated by retro-orbital puncture 90 min and 4 h after LPS. Serum was separated and frozen at −20°C until analyzed. TNF activity was analyzed by the L929 assay as described. Units of TNF were calculated as the reciprocal of the serum dilutions at half killing as determined by log transformation and linear regression analysis of dilutions vs. absorbance. All activity was blocked by a specific rabbit anti-mouse TNF antibody purified in our laboratory (18).

Survival Experiments. All survival experiments followed the same general format. In each experiment, mice were injected intraperitoneally with 40 mg/kg *E. coli* LPS. After 20 min, mice were randomly assigned (12/group) to receive IL-1ra or an equal volume of vehicle every 4–5 h for five doses intravenously. Survival was assessed every 6 h for 48 h and then daily for 6 d. Mice surviving 72 h appeared stable. To assess the effect of duration of treatment after LPS, mice (11–12/group) were randomized into one of three treatment groups: (*a*) IL-1ra (25 mg/kg) intravenously every 4 h for five doses; (*b*) IL-1ra (25 mg/kg) intravenously every 4 h for two doses followed by vehicle for three doses; or (*c*) an identical volume of vehicle intravenously every 4 h for five doses.

Statistics. Survival curves were constructed by the Kaplan-Meier method and analyzed for differences using the score test of the Cox proportional hazards model for grouped data. The in vitro effects of increasing concentrations of IL-1ra were tested using linear regression on the base 10 logarithm of the concentrations, with analysis of the residuals for normality and for absence of other trends. Other data were evaluated using Student's t test with unknown but assumed equal variance.

Results and Discussion

Initial in vitro experiments demonstrate that the IL-1ra inhibits the proliferation of IL-1-dependent C3H/HeJ mouse thymocytes in a dose-dependent fashion and has no biologically significant effect on TNF cytotoxicity against L929 cells (Fig. 1). In the mouse thymocyte experiment (Fig. 1 *A*), the slope of the regression line for data points with IL-1 is significantly different from control (p <0.0001). In the L929

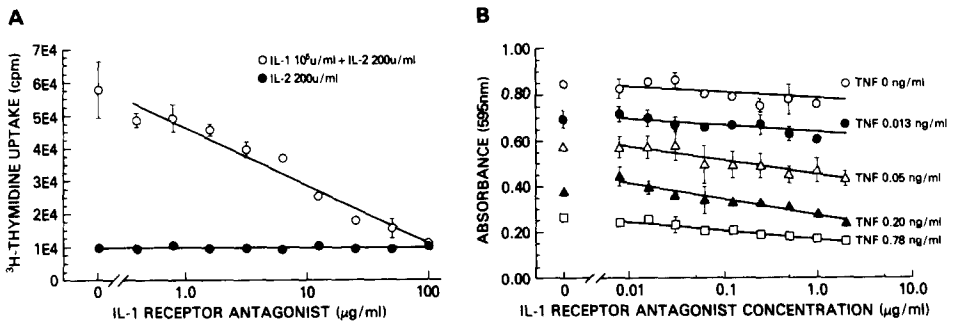

Figure 1. In vitro activity of the IL-1ra. (*A*) The addition of increasing doses of the IL-1ra inhibits the cellular proliferation ([^{3}H]-thymidine uptake) induced by the addition of IL-1. Cells exposed to IL-2 alone maintain a low level of [^{3}H]thymidine uptake, and the IL-1ra does not inhibit IL-2 activity. Each data point represents three separate wells incubated concurrently. (*B*) Increasing concentrations of the IL-1ra does not inhibit cellular cytotoxicity induced by increasing concentrations of rhTNF. Each data point represents three concurrently run experiments.

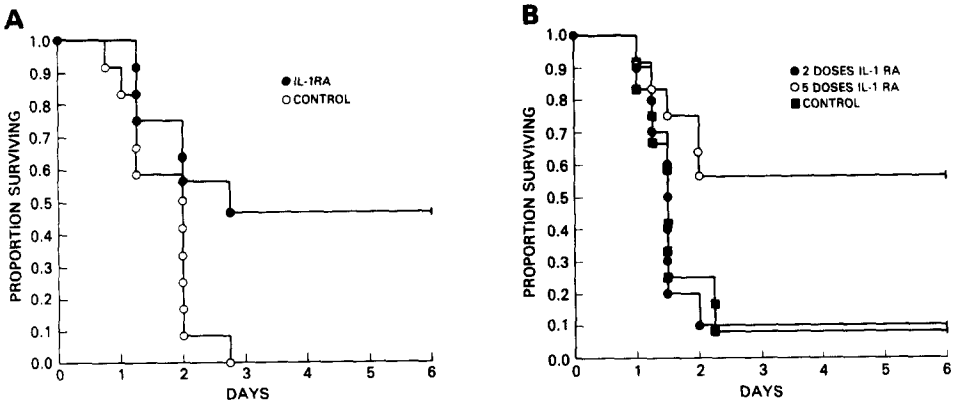

Figure 2. (*A*) Survival after administration of LPS in mice treated with IL-1ra or vehicle. Either IL-1ra (25 mg/kg) or vehicle was administered intravenously as described. Mice treated with IL-1ra beginning 20 min after administration of LPS had significantly improved survival compared with vehicle-treated controls (p = 0.004). (*B*) Effect of duration of treatment with IL-1ra on survival after LPS in mice. After LPS, mice were randomized into one of three treatment groups as described in Materials and Methods. Mice treated every 4 h with five doses of IL-1ra had significantly better survival than mice treated with either two doses of IL-1ra or vehicle (p = 0.01). Two doses of IL-1ra did not improve survival compared with vehicle alone.

Table 1. *Effect of IL-1ra on Circulating Levels of TNF Activity After LPS*

Treatment	TNF activity after: 0 h	1.5 h	4 h
IL-1ra	<4	1,733 ± 659	28 ± 6
	(3)*	(7)	(5)
Vehicle‡	<4	1,047 ± 267	21 ± 4
	(3)	(6)	(5)

* Figures in parentheses are *n*.
‡ NS vs. IL-1ra at each time point.

experiment (Fig. 1 *B*), the slope of the regression lines with varying doses of TNF are negative and, at TNF concentrations of 0.05 and 0.2 ng/ml, are significantly different from the other three. This does not represent, however, biologically important alterations in cell killing and does not show any inhibition of TNF cytotoxicity at any IL-1ra concentration.

In the in vivo experiments, female C57Bl/6 mice were injected intraperitoneally with 40 mg/kg of LPS; 20 min later, treatment with either IL-1ra or an equal volume of carrier solution was instituted every 4 h for 24 h. In this animal model, others have demonstrated that the receptor antagonist blocks IL-1-induced IL-6 production for up to 6 h, indicating that in its present formulation interval injections are necessary for a prolonged effect (W.R. Benjamin, personal communication). In initial experiments, doses between 5 to 25 mg/kg were not effective in improving survival when the IL-1ra was administered subcutaneously (data not shown). Mice treated with the IL-1ra (25 mg/kg) intravenously had a significantly improved survival compared with control animals (Fig. 2 *A*). When the dose of IL-1ra was doubled a similar survival was noted, implying that the lower dose had effectively blocked receptor sites on target cells and that a higher dose of receptor antagonist protein did not have any additional therapeutic value (data not shown). The receptor antagonist was not effective in improving survival after LPS when administered intravenously for only two rather than five doses (Fig. 2 *B*). After the administration of LPS, animals treated with IL-1ra appeared to have less piloerection and febrile shaking than animals treated with vehicle, suggesting that these were IL-1-mediated effects that had been effectively ameliorated.

To test whether the beneficial effects of IL-1ra are secondary to an alteration of TNF production after LPS, serum levels of TNF activity after an intravenous dose of LPS (30 mg/kg) were measured in mice treated simultaneously with IL-1ra or vehicle. At either 1.5 or 4 h after LPS, circulating levels of TNF activity were not altered by treatment with IL-1ra, indicating that the receptor antagonist protein did not exert its beneficial effects on the lethality of LPS by reducing circulating levels of TNF (Table 1).

The precise timing of IL-1ra treatment necessary to improve survival after LPS has not been determined in these experiments. It is possible that treatment with IL-1ra may be delayed for several hours after LPS as serum levels of IL-1 do not peak until approximately 3–5 h after LPS (7). While circulating levels of IL-1 after endotoxin are measurable for as long as 8 h (6, 7), physiologically active IL-1 may be present in tissues for hours or days. In this animal model, treatment for >8 h was necessary for a survival advantage.

The most significant feature of IL-1ra protection against LPS lethality is the fact that it is effective when treatment is initiated after the administration of endotoxin. Previous attempts to improve survival against LPS lethality using agents that either block the production or activity of endogenously produced mediators have only been effective when the agent was administered before LPS (19). For example, passive immunization against TNF improves survival against lethal endotoxemia when antibody is given 6 h before LPS (2). Treatment with anti-TNF antibody simultaneously or after LPS may not allow time for circulation and dispersion of the antibody into host tissues to counter the rapid deleterious effects of TNF. However, the deleterious effects of IL-1 may be of slower onset and more protracted, which may explain why treatment with IL-1ra can be initiated after the administration of LPS and is not effective when given for only two doses.

In conclusion, these experiments demonstrate that IL-1 is a central mediator in endotoxin lethality. Furthermore, an antagonist to the receptor for IL-1 can be used in vivo as an effective treatment strategy to improve survival after lethal endotoxemia in mice. A receptor antagonist to IL-1 may be a clinically applicable treatment strategy in disease processes mediated by this cytokine in humans.

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