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The Interleukin-1 Receptor Antagonist Can either Reduce or Enhance the Lethality of *Klebsiella pneumoniae* Sepsis in Newborn Rats

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***Klebsiella pneumoniae*, a worldwide cause of nosocomial infections, is one of the most common causes of death in newborns in nurseries. In this study, we investigated the role of interleukin-1 (IL-1) in an experimental animal model of neonatal sepsis, using a natural antagonist of IL-1 receptors, the IL-1 receptor antagonist (IL-1Ra), to block IL-1's effects in neonatal *Klebsiella* sepsis in the absence of antibiotic treatment. Newborn Wistar-Kyoto rats were injected intraperitoneally with a single dose (10 mg/kg) of either IL-1Ra ($n = 43$) or human serum albumin as a control ($n = 40$). At the same time, a 50% lethal dose of *K. pneumoniae* was injected subcutaneously. No antibiotics were given at any time. After 10 days, survival was 60% for the albumin group and 80% for the IL-1Ra group ($P < 0.01$). IL-1Ra treatment also afforded protection when the dose of bacteria was increased sixfold ($P < 0.01$). There were two episodes of leukopenia in the control group, which were suppressed by IL-1Ra ($P < 0.01$ and $P < 0.001$). IL-1 and IL-6 levels were lower in the IL-1Ra-treated group ($P < 0.05$ and $P < 0.001$, respectively). No differences between the two groups were observed in the number of bacteria in cultures of the blood, lungs, liver, or spleen. When IL-1Ra (10 mg/kg) was given both at time zero and 24 h after bacterial challenge, lethality was significantly increased ($P < 0.01$). Single doses of IL-1Ra of from 20 to 40 mg/kg progressively increased lethality compared with controls ($P < 0.01$) in both Wistar-Kyoto and Sprague-Dawley strain rats. In the same model, low doses of IL-1 itself (0.4 ng per rat), given 24 h prior to bacterial challenge, afforded protection ($P < 0.001$). These studies suggest that, in the absence of antibiotics, partial blockade of IL-1 receptors improves survival, whereas a longer or greater blockade increases lethality in newborn rats infected with *K. pneumoniae*.**

Interleukin-1 (IL-1) and tumor necrosis factor (TNF) have been implicated as mediators of hemodynamic, hematologic, and metabolic alterations in septic shock (30, 36). Infusions of antibodies raised against TNF have afforded protection against septic shock in mice, baboons, and humans (35). Similarly, blocking IL-1 has also afforded protection in models of septic shock. The latter has been accomplished by using the IL-1 receptor antagonist (IL-1Ra), a member of the IL-1 gene family which binds to IL-1 receptors, preventing IL-1 from triggering a biological response (7, 11). IL-1Ra possesses no known IL-1 agonist activities (reviewed in references 4 and 10). Treatment with IL-1Ra has reduced lethality from acute shock induced by endotoxin or by heat-killed *Escherichia coli* in rabbits (29, 40), live *E. coli* in baboons (14), and heat-killed *Staphylococcus epidermidis* in rabbits (2) and improved survival of mice to lethal doses of lipopolysaccharide (3). Patients with the sepsis syndrome who received a 3-day infusion of IL-1Ra had significantly lower mortality than patients who received a placebo (16).

Newborns are highly susceptible to gram-negative bacterial infections, and septicemia is a major cause of death at this age. In a previous article (23), we described a model in which newborn rats are infected at 24 h of age with live *Klebsiella pneumoniae*. This model has been used to test the effects of intravenous immunoglobulin preparations on survival and changes in leukocyte counts during bacterial infections (23). We developed this model because it is based on a progressive infection over days, whereas other models are based on death from an acute response to lipopolysaccharide or to large numbers of organisms. We investigated the role of IL-1 in this model of neonatal sepsis by blocking IL-1 receptors with the naturally occurring IL-1Ra.

MATERIALS AND METHODS

Reagents and cell lines. Human recombinant IL-1Ra was kindly provided by Robert C. Thompson (Synergen Inc., Boulder, Colo.) and Daniel E. Tracey (Upjohn Co., Kalamazoo, Mich.). Human recombinant IL-1β (10^8 U/mg) was donated by Aldo Tagliabue (Sclavo Research Centre, Siena, Italy). IL-6 expressed in CHO cells was obtained from the Genetics Institute (Cambridge, Mass.). The recombinant proteins used in these studies did not contain measurable endotoxin (<100 pg/mg), as determined by a *Limulus* amoebocyte lysate assay with a sensitivity of 20 pg/ml (Associates of Cape Cod, Woods Hole, Mass.). Five percent human serum albumin (HSA) was of clinical grade (Hyland Laboratories, Duarte, Calif.). RPMI 1640, HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 2-mercaptoethanol, and 3,(4,5-dimethylthiazol)2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma Co., St. Louis, Mo. Fetal calf serum was from Hyclone, Logan, Utah. D10S cells were derived from the murine T-helper cell line D10.G4.1 and used in a bioassay to measure IL-1 activity as described previously (31). B9 hybridoma cells were kindly

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provided by Lucien A. Aarden and used to measure IL-6 bioactivity (1).

Bacteria. A *K. pneumoniae* (capsule serotype K8) strain isolated from the blood of a septic human newborn was used for these studies. Bacteria were cultured in Trypticase soy broth at 37°C overnight, and 0.5 ml of bacterial suspension was incubated in 10 ml of new broth for 2.5 h at 37°C in order to reach the logarithmic phase of bacterial growth, as described previously (23). Bacteria were washed twice in sterile phosphate-buffered saline (PBS, pH 7.2), adjusted to 10^9 CFU in 1 ml of PBS, and kept on ice for no more than 20 min before use.

Animals. Wistar-Kyoto and Sprague-Dawley rats were purchased from Taconic Farms (Germantown, N.Y.) as litters of less than 24 h of age, with a wet-nurse rat for each eight suckling rats. The experimental protocol was approved by the Animal Research Committee of the New England Medical Center (Boston, Mass.). Newborn rats were used at 24 h of age, with an average weight of 7.5 g. Lethal doses were determined by survival rates at 21 days in groups of 20 newborn Wistar-Kyoto rats challenged with increasing concentrations of *K. pneumoniae* by a subcutaneous (s.c.) route, just cephalad to the tail (23). The 50% lethal dose (LD_{50}) of *K. pneumoniae* was 10^7 CFU per rat. All animals in this study had positive blood cultures between 6 and 12 h after the injection of an LD_{50}. This experimental model of neonatal septicemia has been described previously for newborn rats infected with *K. pneumoniae* (23) or with group B streptococci (15). It offers the advantage of a free peritoneal cavity for therapeutic interventions.

Survival after *Klebsiella* sepsis. Newborn Wistar-Kyoto rats were inoculated by the intraperitoneal (i.p.) route with a single 10-mg/kg dose of IL-1Ra (n = 43) or HSA (n = 40). Previous experiments established a dose-response scale (1.25, 2.5, 5, 10, and 20 mg/kg) for IL-1Ra. IL-1Ra at 10 mg/kg was the most effective, improving survival to *Klebsiella* sepsis. An LD_{50} of *K. pneumoniae* was inoculated s.c. into each animal immediately after the i.p. injection of either IL-1Ra or HSA. Survival was recorded every 6 h for 21 days. Similar experiments were performed with newborn Sprague-Dawley rats. In those experiments, we used a single i.p. dose of either IL-1Ra or HSA and 5×10^7 CFU of *K. pneumoniae* inoculated s.c. at time zero. Survival was monitored for 21 days.

Blood and organ tissue bacterial cultures. Another group of newborn Wistar-Kyoto rats were inoculated with IL-1Ra or HSA and infected with an LD_{50} of *K. pneumoniae* at time zero. Eight to 11 rats from each group were killed by CO_2 inhalation at 0, 6, 12, or 24 h and every 24 h thereafter up to 144 h after the infection. After blood was drawn by aseptic cardiac puncture, the lungs, liver, and spleen were removed by aseptic dissection. Ten microliters of blood was diluted in 1 ml of sterile distilled water and mixed thoroughly; 10 μl of diluted blood samples was incubated in 1 ml of Trypticase soy broth and on Trypticase soy agar plates at 37°C for 24 h. Organs were washed in sterile PBS, weighed, and then separately homogenized in 5 ml of sterile distilled water. Samples (10 and 100 μl) were incubated in broth and on agar plates for 24 h at 37°C. The number of bacterial colonies in the agar plates was counted, and the results are expressed as CFU per milliliter of blood or CFU per gram of organ. Broth cultures were used to show positive or negative results.

Leukocyte counts. Total and differential leukocyte counts were performed on coded blood samples by two different examiners with non-automated standard techniques. Results were recorded as the mean ± standard error of the mean (SEM) number of leukocytes (WBC) and polymorphonuclear cells (PMNs) at each time.

IL-1 and IL-6 bioassays. The bioactivity of IL-1 and IL-6 was measured in heparinized blood samples by capacity to induce cell proliferation in two specific bioassays. The D10S cell line was used to measure bioactive IL-1 as described previously (31), and the B9 hybridoma cell line was used to determine IL-6 activity (1). Cells were cultured in RPMI 1640 supplemented with 5% fetal calf serum, 50 μM 2-mercaptoethanol, 10 mM HEPES, and either IL-1α (10 pg/ml) for D10S or IL-6 (50 pg/ml) for B9 cells. Cells were harvested 6 days after splitting for D10S cells or 3 days after splitting for B9 cells. Cells were washed in sterile saline solution and adjusted to 10^4 cells per 100 μl of RPMI 1640 with 2% fetal calf serum. Four serially diluted plasma samples (100 μl each) were added in quadruplicate to 96-well flat-bottomed microtiter plates. Standard curves for IL-1 and IL-6 were made simultaneously. Plates were incubated at 37°C for 72 h in a 5% CO_2 atmosphere. During the last 4 h, 250 μg of MTT per ml in sterile PBS was added to each well. The medium was carefully removed, and the cells were lysed by adding 100 μl of isopropanol in order to expose and dissolve the crystals of reduced formazan from viable proliferative cells (26). Optical density was measured at 570 nm with a reference wavelength of 655 nm in an ELISA reader (Bio-Rad 3550). One unit of IL-1 (half-maximal cell proliferation) varied from 0.3 to 1.0 pg/ml (mean ± SEM, 0.7 ± 0.21 pg/ml), whereas 1 U of IL-6 varied from 0.3 to 0.7 pg/ml (0.5 ± 0.15 pg/ml). Anti-IL-1 and anti-IL-6 polyclonal antibodies neutralized the respective samples, confirming that the bioassays were actually measuring the biological activity of IL-1 and IL-6.

IL-1 protection in newborn rats. Log concentrations of IL-1β from 0.4 to 400 ng were administered i.p. to groups of 16 newborn Wistar-Kyoto rats. *K. pneumoniae* (3×10^7 CFU) was injected s.c. 24 h later. Survival was monitored for 21 days.

Statistical analysis. Survival curves were analyzed by the Kaplan-Meier method with the log rank test, calculating the χ^2 value to assess significance. Student's t test for unpaired samples was used to compare individual points between control and IL-1Ra groups for WBC changes, bacterial clearance, and cytokine levels in plasma.

RESULTS

A single dose of IL-1Ra improves survival to *Klebsiella* sepsis. Ten days after an LD_{50} of *K. pneumoniae* was administered, 24 of 40 (60%) animals injected with the HSA control were alive, whereas 35 of 43 (81%) rats that received a single dose of IL-1Ra (10 mg/kg) survived the infection ($P < 0.01$) (Fig. 1A). No further change in survival was observed after 10 days. When the bacterial challenge was increased from 10^7 CFU (LD_{50}) to 6×10^7 CFU of *K. pneumoniae*, the protective effect of IL-1Ra was still observed. Five of nine (55%) rats survived, but only one of nine (11%) from the HSA-treated group remained alive 5 days after the infection ($P < 0.01$) (Fig. 1B).

IL-1Ra prevents leukopenia in *Klebsiella* sepsis. There were two episodes of leukopenia in control rats 6 and 72 h after *Klebsiella* infection. In contrast, IL-1Ra-treated rats did not have significant changes in WBC counts in response to the infection (Fig. 2A). Differences between the two groups were significant at both 6 h ($P < 0.01$) and 72 h ($P < 0.001$). The number of PMNs was not significantly different between the two groups. Maximal neutrophilia was seen at 24 h in

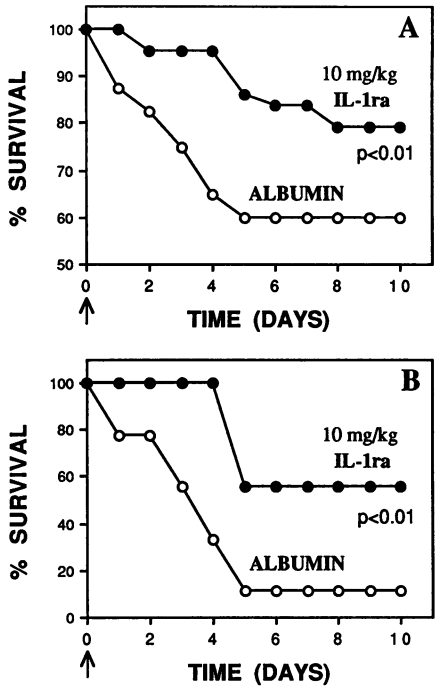

FIG. 1. IL-1Ra treatment enhances survival in *Klebsiella* sepsis. (A) Newborn Wistar-Kyoto rats were injected i.p. with a 10-mg/kg dose of IL-1Ra (n = 43) or HSA (albumin) (n = 40) and then inoculated s.c. with 10^7 CFU of *K. pneumoniae*. Survival after 10 days was 80% in the IL-1Ra group and 60% in the HSA control group ($P < 0.01$). (B) Bacterial challenge was increased to a mid-log dose of 6×10^7 CFU of *K. pneumoniae*. Five of nine (55%) IL-1Ra-treated rats survived, but only one of nine (11%) from the HSA-treated group remained alive ($P < 0.01$).

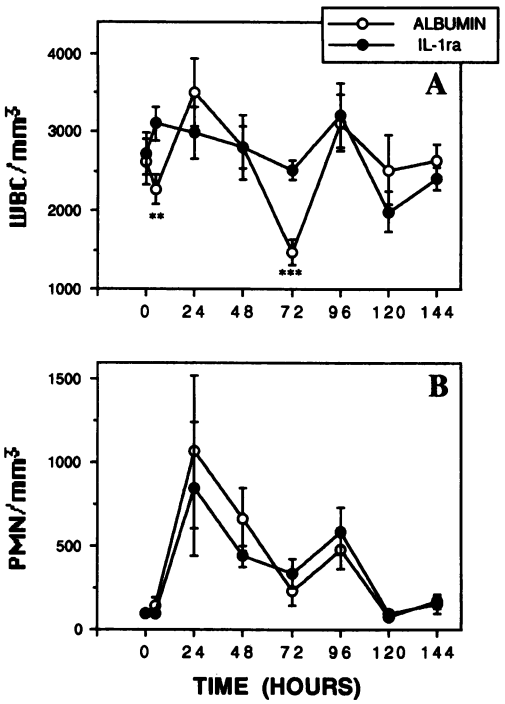

FIG. 2. Treatment with IL-1Ra prevents leukopenia in *Klebsiella* sepsis. Newborn Wistar-Kyoto rats were injected i.p. with a 10-mg/kg dose of IL-1Ra or HSA and then inoculated s.c. with 6×10^7 CFU of *K. pneumoniae*. Heparinized blood was obtained from 8 to 11 rats from each group at the times indicated. (A) Compared with time zero values, HSA-injected control rats experienced two episodes of leukopenia, at 6 h (**, $P < 0.01$) and at 72 h (***, $P < 0.001$) following *Klebsiella* challenge. IL-1Ra-treated rats did not experience leukopenia (change from time zero values is not significant). (B) Changes in PMN numbers were not different between the two groups. Maximal neutrophilia was seen at 24 h in both groups. Values are means ± SEM.

both groups, and neutropenia was not observed in either group (Fig. 2B).

IL-1Ra decreases circulating levels of IL-1 and IL-6 in *Klebsiella* sepsis. IL-1 and IL-6 were not detectable in circulating blood at time zero. A dramatic increase in the levels of both cytokines in the plasma was observed in the two groups at 3 and 6 h after *K. pneumoniae* challenge. IL-1 levels decreased gradually (Fig. 3A), whereas IL-6 levels decreased rapidly (Fig. 3B) to baseline levels. IL-1 levels were lower in the IL-1Ra-treated group at 48 h ($P < 0.05$), consistent with the peak of bacteremia. The initial increase in IL-6 levels was also lower in the IL-1Ra group at 6 h ($P < 0.01$). These findings suggest that IL-1Ra causes a decrease in IL-1-induced IL-6 levels at a very early phase of the acute response to newborn *Klebsiella* sepsis and also modifies IL-1 levels in that early response.

IL-1Ra does not affect bacterial clearance. Newborn rats had positive blood cultures from 6 h after bacterial inoculation. The initial counts ranged from a minimum of 100 CFU/ml to a maximum of 20,000 CFU/ml of blood, and in some animals, this increased to 400,000 CFU/ml at 48 h, during the time of maximal bacteremia. Although the peak of bacteremia lasted for 72 h in the HSA-treated group, this prolongation did not reach statistical significance between the IL-1Ra-treated and the control group during the study (Fig. 4A). Bacterial cultures of lungs, liver, and spleen did not show significant differences between the two groups (Fig. 4B, C, and D). A trend to greater hepatic clearance (Fig. 4C) and to lower splenic bacterial uptake in the IL-1Ra-treated rats was observed at 24 and 48 h following infection (Fig. 4D). However, these differences were not statistically significant.

IL-1Ra increases lethality of *Klebsiella* sepsis. In an effort to augment the protective effect of IL-1Ra, we increased the dose of IL-1Ra. One dose of 5 or 10 mg of IL-1Ra per kg was administered at time zero and 24 h after *K. pneumoniae* challenge. Surprisingly, as shown in Fig. 5, the two injections of IL-1Ra failed to protect septic rats from death. In fact, the administration of a second dose of IL-1Ra increased lethality. This reached statistical significance when two 10-mg/kg doses of IL-1Ra were injected ($P < 0.01$).

Low doses IL-1Ra protect but high doses increase lethality in newborn *Klebsiella* sepsis. In an attempt to elucidate why a double injection of IL-1Ra did not improve survival, increasing concentrations of the antagonist were administered at time zero as a single injection. Figure 6 displays the survival curves from four different experiments in which 5×10^7 CFU of *K. pneumoniae* were inoculated s.c. and twofold-increasing concentrations of IL-1Ra, ranging from 5 to 40 mg/kg, were administered i.p. to groups of 13 to 17 newborn Wistar-Kyoto or Sprague-Dawley rats. In accordance with the data shown in Fig. 1, both 5 and 10 mg of IL-1Ra per kg improved the survival of rats to *Klebsiella* sepsis (Fig. 6). In contrast, 20 and 40 mg of IL-1Ra per kg increased lethality in septic rats ($P < 0.01$). Mortality to sepsis in the HSA-treated

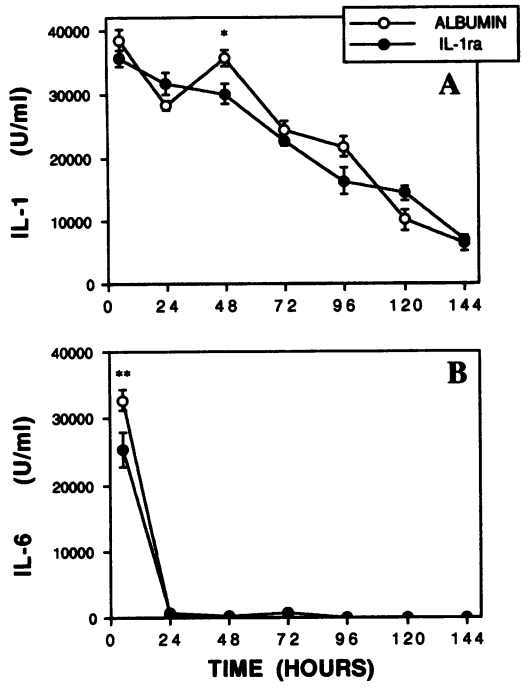

FIG. 3. Circulating levels of IL-1 and IL-6. Newborn Wistar-Kyoto rats were injected i.p. with a 10-mg/kg dose of IL-1Ra or HSA and then inoculated s.c. with 6×10^7 CFU of *K. pneumoniae*. Heparinized plasma was obtained from 8 to 11 rats from each group at the times indicated. (A) IL-1 levels. IL-1 levels were lower in the IL-1Ra-treated group at 48 h (*, $P < 0.05$). (B) IL-6 levels. IL-6 levels were lower in the IL-1Ra-treated group at 6 h than in the HSA-treated controls (**, $P < 0.01$). Values are means ± SEM.

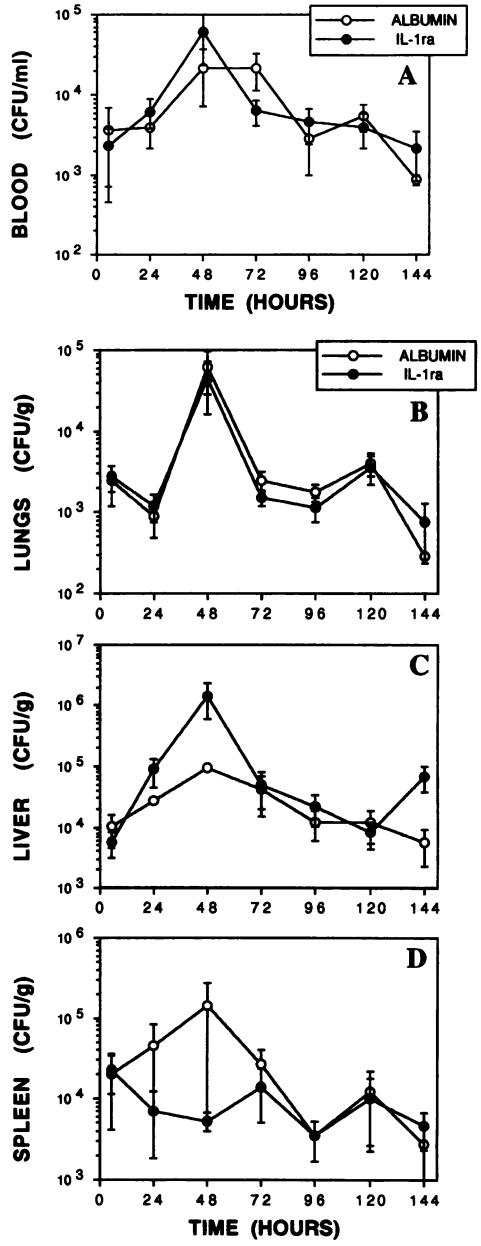

FIG. 4. Treatment with IL-1Ra does not alter bacterial clearance. Newborn Wistar-Kyoto rats were injected with a 10-mg/kg dose of IL-1Ra or HSA and infected with an LD_{50} of *K. pneumoniae* at time zero. (A) The difference in bacteremia was not significant between the two groups. Differences in bacterial cultures of lungs (B), liver (C), and spleen (D) did not reach significance between the two treatment groups. Values are means ± SEM.

group of Wistar-Kyoto rats was ~70%, whereas in the Sprague-Dawley strain, it was close to 40%. This suggests that the former rat strain is more susceptible to *K. pneumoniae*. The protective or detrimental effects of IL-1Ra treatment were dose dependent and observed consistently in both rat strains, independently of the apparent differences in susceptibility to the bacteria.

IL-1 protects newborn rats from *Klebsiella* sepsis. The data presented above suggest that increasing the blockade of IL-1 reduces host defense mechanisms against *Klebsiella* infection. Since several studies have demonstrated that low doses of IL-1 improve host defense to a variety of lethal challenges, we investigated whether IL-1 would similarly protect neonatal rats. As shown in Fig. 7, a single injection of IL-1β to newborn Wistar-Kyoto rats, given 24 h before bacterial challenge, resulted in a significant increase in survival ($P < 0.01$). This protection was observed for each dose of IL-1 tested; IL-1 was as effective at 400 ng as at 40 ng (not shown). At the lowest dose used, 0.4 ng (50 ng/kg), IL-1 significantly improved the survival of newborn rats with *Klebsiella* sepsis ($P < 0.001$).

DISCUSSION

The present studies offer new information concerning the role of IL-1 in the host processes leading to death as well as to survival. Animal studies have established that specific blockade of IL-1 during endotoxemia, bacterial sepsis, or acute inflammation affords protection and in most cases reduces lethality (reviewed in reference 10). This has been most dramatically demonstrated in a phase II trial with humans receiving a 3-day infusion of IL-1Ra for septic shock syndrome (16). The beneficial effects of blocking IL-1 are consistent with IL-1's ability to cause a shock-like syndrome in animals whether administered alone or with TNF (13, 30). In humans, an infusion of IL-1 at doses of 300 ng/kg or greater induces hypotension and a shock-like syndrome (34).

The beneficial effect of blocking IL-1 activity has been mostly studied in acute models, such as lethal endotoxemia

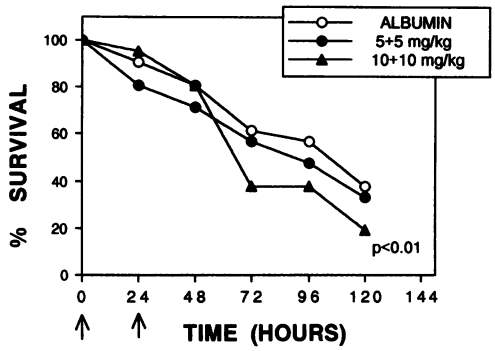

FIG. 5. IL-1Ra treatment increases lethality of *Klebsiella* sepsis. Low protective doses of IL-1Ra (5 or 10 mg/kg) were injected twice, at time zero and 24 h after injection of 5×10^7 CFU of *K. pneumoniae*, to Wistar-Kyoto rats ($n = 21$). Lethality was increased when a second dose of IL-1Ra was given ($P < 0.01$ for HSA versus two 10-mg/kg doses of IL-1Ra).

(3, 29) and gram-negative or gram-positive sepsis (2, 14, 40). In the present study, IL-1Ra reduced lethality in newborn rats with *Klebsiella* infection, even in the absence of antibiotic treatment, but we assumed that more than a single dose would be required. In this model, the peak of bacteremia takes place at 48 h (23), and 50% of the deaths occur between 48 and 72 h. Studies show that IL-1Ra is rapidly cleared from the circulation and that a therapeutic effect requires approximately 20 μg of IL-1Ra per ml in both animal and human sepsis (2, 29). It was of interest that a single i.p. injection of 10 mg of IL-1Ra per kg at the time of establishment of the *Klebsiella* infection in the s.c. tissue afforded significant protection from death regardless of the inoculum (1×10^7 or 6×10^7 CFU).

The mechanism of IL-1Ra protection in newborn rats is probably the result of several changes. There was a decrease in leukopenia after 6 and 72 h. This observation is consistent with other models, for which reduced leukopenia or infiltration of leukocytes into tissues in animals treated with IL-1Ra has been reported (24, 29, 37, 40). Since IL-1 increases the synthesis of endothelial cell adhesion molecules, blocking IL-1 may explain the differences in the two groups at 6 h. Blocking IL-1 may also explain the reduced leukocyte counts at 72 h, since IL-1Ra also reduces the production of circulating colony-stimulating factors during endotoxemia (20).

The reduced levels of IL-1 and IL-6 in rats treated with IL-1Ra are also consistent with the role of IL-1 in the production of other cytokines. For example, in mice treated with an antibody which blocks the type IL-1 receptor, IL-6 levels were reduced (17). Reduced circulating IL-1 has also been observed in baboons treated with IL-1Ra during acute sepsis with *E. coli* (14). In vitro, IL-1Ra blocks 50% of the IL-1, IL-6, and IL-8 synthesized by human mononuclear cells stimulated with endotoxin (18, 19, 32). We have also observed a reduction in the amount of TNF that circulates during staphylococcal bacteremia in rabbits treated with IL-1Ra (2). Therefore, one mechanism for the improved survival of rats treated with IL-1Ra may be reduced production of IL-1 itself as well as of other cytokines. Also, hypoglycemia associated with the infection may be reduced in IL-1Ra-treated rats, as it is in mice receiving IL-1Ra during endotoxemia (39).

Similar to other studies (38), there was no difference between the number of bacteria cultured from the circulating blood or organs of rats with improved survival due to the

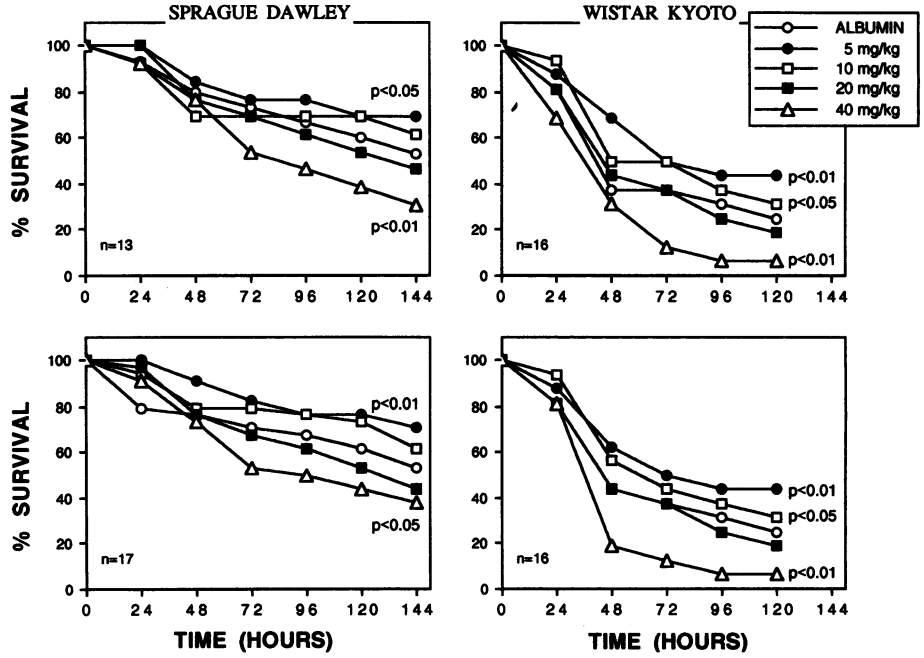

FIG. 6. IL-1Ra can either reduce or enhance lethality of *Klebsiella* sepsis. In four different experiments, increasing doses of IL-1Ra as a single i.p. injection and 5×10^7 CFU of *K. pneumoniae* were inoculated at time zero. The results for two strains of rats are reported. At 5 and 10 mg/kg, IL-1Ra treatment improved survival ($P < 0.05$ and $P < 0.01$, respectively) compared with the HSA-treated control groups. At higher doses of IL-1Ra (20 and 40 mg/kg), increased mortality was observed in both strains ($P < 0.01$ and $P < 0.05$, respectively).

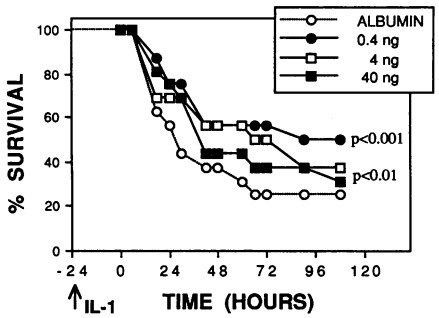

FIG. 7. IL-1 protects newborn rats from *Klebsiella* sepsis. A single i.p. injection of IL-1β was given 24 h before bacterial challenge to groups of 16 newborn Wistar-Kyoto rats. The dose of 0.4 ng induced a significant increase in survival ($P < 0.001$); the doses of 4 and 40 ng also increased survival ($P < 0.01$).

administration of IL-1Ra compared with the control group. In both groups, however, long-term survival of the infection is associated with eventual clearance of the organisms. These results support the concept that death due to this or other infections is not a direct effect of the microbe but rather of host factors triggering the lethal event. Among the many cytokines, TNF (35) and now IL-1 appear to play an essential role.

In an attempt to improve survival, we administered a second dose of IL-1Ra 24 h after establishment of the infection but did not expect a worsening of lethality. As we increased the amount of IL-1Ra at the time of infection (time zero) to 20 and 40 mg/kg, the number of deaths increased. At 40 mg/kg, there was a significant increase in lethality in both rat strains. This is in sharp contrast to studies with the rabbit, in which survival to lipopolysaccharide challenge was greatest with 100 mg of IL-1Ra per kg (29). One possible explanation for this difference is a requirement for small amounts of IL-1 to limit the infection in newborn rats, particularly since this is a model of progressive infection rather than an acute lethal event. Nevertheless, we conclude that increasing blockade of IL-1 reveals an essential role for this cytokine in host defense against *Klebsiella* infection in the absence of antibiotics.

In support of this concept, there is a large amount of data showing that in both normal and immunocompromised animals, an unusually small dose of IL-1 given between 24 and 1 h prior to a lethal challenge improves survival. In those studies, death is the result of endotoxemia, gram-negative, gram-positive, fungal, or malarial infections, radiation, hyperoxia, or hepatic failure (reviewed in reference 9). In the present studies, we also observed a reduction in lethality in newborn rats given a very low dose of IL-1 (50 ng/kg) 24 h prior to *Klebsiella* infection. We conclude that a partial blockade of IL-1 receptors improves survival in this model of *Klebsiella* sepsis but that a greater or longer blockade of these receptors might increase lethality. In the latter case, even small amounts of IL-1 produced during the infection could be prevented from exerting some critical beneficial effect on host defense. The protective effect of a single 10-mg/kg dose of IL-1Ra could be due to blockade of most but not all IL-1 receptors, leaving small amounts of IL-1 to trigger essential host defense mechanisms. Mechanisms for the improvement of host defenses by IL-1 include migration of neutrophils to the site of infection, reduction in the number of TNF receptors (21), increased hepatic acute-phase protein synthesis, improved bone marrow function, and induction of heat shock proteins (reviewed in reference 9). Although IL-1 can also act as an adjuvant and increase antibody formation (5), blockade of IL-1 receptors by IL-1Ra does not interfere with immune responses to antigens either in vitro (28) or in vivo (12).

How can a small amount of IL-1 improve survival while blocking larger amounts of IL-1 accomplishes the same end? An example of this duality in biology is well documented for trace elements: small amounts are essential for several metabolic pathways, whereas high doses are toxic (25). Death is seen at both ends of the scale. This model of neonatal sepsis in rats provides an example of this duality for IL-1. The clinical evidence supports the concept. In patients with sepsis, there is a statistically significant correlation between survival and production of IL-1, whereas failure to produce IL-1 is associated with death (6, 8, 22, 27). However, blocking high levels of IL-1, as occurs in disease, will reduce the severe consequences of this cytokine. It is presently unclear whether total blockade of IL-1 is advisable or even achievable in the clinical setting.

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