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Interleukin-1 (IL-1) Receptor Antagonist Prevents *Staphylococcus epidermidis*-Induced Hypotension and Reduces Circulating Levels of Tumor Necrosis Factor and IL-1β in Rabbits

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Similar to shock in gram-negative sepsis, shock from gram-positive organisms is mediated, in part, by tumor necrosis factor (TNF) and interleukin-1 (IL-1). In the present study, rabbits were infused with IL-1 receptor antagonist (IL-1ra) prior to and during *Staphylococcus epidermidis*-induced hypotension. After injection of bacteria, a maximal fall in mean arterial pressure to −42% below baseline occurred at 200 min in vehicle-treated animals compared with a nonsignificant decrease of only 7% in the IL-1ra-treated group ($P < 0.01$, vehicle versus IL-1ra). A similar attenuation was observed in the fall in systemic vascular resistance ($P < 0.05$). After the injection of *S. epidermidis*, TNF levels rose to a peak elevation of 475 ± 160 U/ml in vehicle-treated rabbits, but in rabbits receiving IL-1ra, maximal TNF levels rose only to 85 ± 23 U/ml ($P < 0.01$). Plasma IL-1β reached maximal concentrations at 180 min of 364 ± 71 pg/ml in vehicle-treated animals but only 145 ± 12 pg/ml in rabbits given IL-1ra ($P < 0.05$). The reductions in TNF and IL-1 were not due to interference by IL-1ra in the respective assays. In vitro, IL-1ra inhibited *S. epidermidis*-induced TNF from mononuclear cells by 31% ± 11%, from spleen cells by 17% ± 4% ($P < 0.05$), and from whole blood by 42% ± 17%. Despite the near reversal of the fall in mean arterial pressure and systemic vascular resistance in IL-1ra-treated rabbits, leukopenia and thrombocytopenia were unaffected. These results demonstrate that IL-1ra blocks shock-like hemodynamic parameters and reduces circulating IL-1 and TNF levels in a model of gram-positive sepsis.

Animal models of sepsis have usually employed lipopolysaccharide (LPS) or gram-negative bacteria as the inducer of a shock-like state. However, in humans, septic shock also occurs during gram-positive bacteremia (6, 9, 36), and the mortality for patients with gram-negative or gram-positive bacteremia does not differ greatly (5). By using a well-characterized canine model of septic shock, Natanson et al. (33) demonstrated that *Staphylococcus aureus*, in the absence of endotoxemia, induced the same cardiovascular abnormalities of septic shock as *Escherichia coli* did, suggesting that structurally and functionally distinct microorganisms could activate a common pathway resulting in similar cardiovascular injury and mortality. We recently demonstrated that heat-killed *Staphylococcus epidermidis* is also capable of inducing a shock-like state and tissue injury in rabbits (42).

In that model, we observed a similar degree of complement activation and generation of circulating levels of interleukin-1 (IL-1) and tumor necrosis factor (TNF) but in the absence of detectable endotoxemia. In addition, circulating IL-1β levels had a greater correlation coefficient ($r = 0.81$; $P < 0001$) with the degree of hypotension than TNF levels did ($r = 0.48$; $P < 0.02$). These findings are consistent with the observation that TNF and IL-1 can independently induce a shock-like state; moreover, these two cytokines act synergistically in inducing hypotension and tissue injury (35).

To distinguish the role of IL-1 from that of TNF in this model of gram-positive septic shock, we employed a specific blockade of IL-1 receptors. Specific blockade of IL-1, using the IL-1 receptor antagonist (IL-1ra), reduces the severity of disease in a variety of animal models (reviewed in references 3 and 13). Relevant to the present studies, IL-1ra blocks hypotension and death due to LPS in rabbits and mice (2, 34) and a shock-like state in rabbits and baboons after intravenous *E. coli* administration (15, 41). Because our previous studies demonstrated production of IL-1 and TNF during endotoxin-independent sepsis, we undertook the present experiments using specific IL-1 receptor blockade in *S. epidermidis*-induced hypotension in rabbits to determine the role of IL-1 in this model.

(This work was presented in abstract form at the International Cytokine Workshop, Stresa, Italy, November 1991.)

MATERIALS AND METHODS

Bacterial preparation. The *S. epidermidis* strain (a human blood culture isolate) used in these studies is encapsulated. *S. epidermidis* was inoculated in brain heart infusion broth (Bethesda Biological Laboratories, Cockeysville, Md.) prepared in pyrogen-free water. After being shaken overnight, cultures were centrifuged and washed three times in pyrogen-free saline and then boiled for 30 min with two subsequent washes. The concentration of bacteria was determined spectrophotometrically and confirmed by direct counting in a Petroff-Hausser bacterium counter. Aliquots of the bacte-

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rial suspensions were stored at −70°C until used in experiments. Bacterial suspensions were thawed, washed with sterile saline, and sonicated for 5 min (Sonifier 450; Branson, Danbury, Conn.) just prior to use in experiments. Before sonication, the bacteria consisted of clumps of approximately four organisms; after sonication, microscopic examination revealed that the bacteria were separated from each other but not broken into pieces. As described previously (42), the concentration of LPS in the supernatant from the last washing was less than 10 pg/ml on the basis of the U.S. Standard Endotoxin (EC-5; Bureau of Biology, Bethesda, Md.) as assessed by *Limulus* amebocyte lysate assay with a sensitivity of 10 pg/ml (Associates of Cape Cod, Woods Hole, Mass.).

Rabbit model. This study was approved by the Animal Review Committee of the New England Medical Center and Tufts University. Rabbits were prepared by the method described previously (35, 41, 42), with minor modifications. Female New Zealand White rabbits (4.3 ± 0.3 kg) were housed for a minimum of 1 week in the animal care facilities and were free of infection. On the day of the experiment, the rabbit was anesthetized with an initial intramuscular injection of 6 mg of xylazine per kg of body weight and 20 mg of ketamine per kg, followed by an intramuscular injection of 10 mg of ketamine per kg every hour. Catheters (American Edwards Laboratories, Irvine, Calif.) were placed in the ascending aorta (3French thermodilution probe) from the right carotid artery, the superior vena cava from the right jugular vein, and the abdominal aorta from the left femoral artery to continuously record mean arterial pressure (MAP) and central venous pressure (CVP) and to measure cardiac output (CO). CO was measured by the thermodilution method; systemic vascular resistance (SVR) was calculated as follows: [(MAP − CVP) × 80]/CO (in dynes per second per centimeter^{-5}). Hemodynamic parameters were recorded every 20 min, and blood sampling from the left femoral artery catheter was performed hourly. The volume of blood removed was replaced with an equal volume of saline. The total fluid (saline) administered to each rabbit during the experiment was 5.5 ml/kg/h.

After the catheters were inserted, the hemodynamic parameters were observed for 60 min before heat-killed *S. epidermidis* was injected. Two groups received different amounts of bacteria. We gave one group 6×10^{10} organisms per kg suspended in 10 ml of sterile saline; the other group received 3×10^{10} organisms per kg. All injections were administered through the right ear vein and infused over 20 min beginning at $t = 0$ min. On the basis of previous studies (41), 5 min before bacteria were injected ($t = -5$ min), 10 mg of recombinant human IL-1ra (14) per kg was given over 1 min in 2.5% human serum albumin (Hyland Laboratories, Duarte, Calif.), and then a continuous infusion of IL-1ra followed for 4 h. Vehicle control rabbits in each group received the bolus infusion of the same amount of human albumin or saline. We measured hemodynamic parameters from $t = -60$ min to $t = 300$ min. The data of the hemodynamic parameters are depicted as the mean percent changes ± standard error of the mean (SEM) from $t = 0$. At $t = 310$ min, the rabbits were euthanized with intravenous pentobarbital. There was a gross inspection of the organs at that time, and tissues were fixed in buffered formalin, embedded, sectioned, stained, and examined as described previously (42).

Rabbit PBMC, spleen cells, and whole blood stimulation in vitro. Rabbit peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation as described elsewhere (7, 10). Rabbit spleen cells were dispersed with a wire mesh. PBMC and spleen cells were suspended in RPMI 1640 culture medium (Whittaker M.A. Bioproducts, Walkersville, Md.) containing heat-inactivated 1% (vol/vol) normal rabbit serum. PBMC or spleen cells (5×10^5) were added to individual wells of 96-well flat-bottom plates with differing amounts of either heat-killed *S. epidermidis* (2 or 200 organisms per cell) or recombinant rabbit IL-1β (0.25 or 2.5 ng/ml) (7) in the absence or presence of IL-1ra. Cultures were incubated for 20 h at 37°C. Rabbit whole blood was drawn into a heparinized syringe (5 U/ml of blood). Whole blood was diluted twofold with RPMI 1640 medium in polypropylene tubes with 10^9 heat-killed *S. epidermidis* organisms per ml (final concentration) in the absence or presence of IL-1ra, and the tubes were rotated (4 rpm) for 20 h at 37°C. After incubation, the plasma in each sample was collected by centrifugation. All samples were stored at −70°C until assayed. In PBMC and spleen cell cultures, total TNF and IL-1α production was determined after three cycles of freeze-thawing.

TNF, IL-1, and IL-1ra assay. TNF activity was measured by cytotoxicity in the L929 murine fibroblast as described elsewhere (29). In brief, after 18 h of incubation of L929 cells in 96-well microtiter plates (4.0×10^4 cells per well), the culture medium was removed, and recombinant human TNF (kindly provided by Genentech, Inc., South San Francisco, Calif.) as the standard or serum samples were added with actinomycin D (5 μg per well). Serum samples were diluted 10- to 1,000-fold with RPMI 1640 medium, whereas in vitro TNF production by PBMC and spleen cells was determined by using serial dilutions of the samples. The cells were stained with 0.1% crystal violet in 100% methanol, and the optical density at 595 nm was measured with a Microplate Reader (Bio-Rad Laboratories, Richmond, Calif.). The units of TNF were calculated on the basis of the cytotoxicity of a standard curve by using known concentrations of TNF in the same assay.

Rabbit IL-1β was determined by specific radioimmunoassay (RIA) (10) using goat antibodies raised against recombinant rabbit IL-1β as described previously (7). Plasma samples collected in EDTA and aprotinin were assayed in the RIA after two chloroform extractions to optimize detection of circulating IL-1 as previously described (8). Spleen and PBMC cultures were not subjected to chloroform extraction. The sensitivity of rabbit IL-1β RIA was 60 to 120 pg/ml, and that for IL-1α was 20 to 40 pg/ml (95% confidence level).

Circulating levels of recombinant human IL-1ra in rabbits were also determined by specific RIA for human IL-1ra as described previously (38). Plasma samples were diluted 1,000- to 100,000-fold for measurements. The sensitivity of this assay was 180 pg/ml (95% confidence level).

Data analysis. Results were expressed as the mean ± SEM. The Mann-Whitney U test was used to evaluate whether values at the same time point were different for IL-1ra- and vehicle-infused rabbits. A paired *t*-test (two tailed) was used when the values at a particular time point were compared with the value at zero time (analysis of variance) in the same group, or paired values were compared with each other at the same time point.

RESULTS

Effect of IL-1ra on hemodynamic parameters after *S. epidermidis* injection. Rabbits were injected with 6×10^{10} killed *S. epidermidis* organisms per kg, and hemodynamic parameters were measured. The level of hypotension which oc-

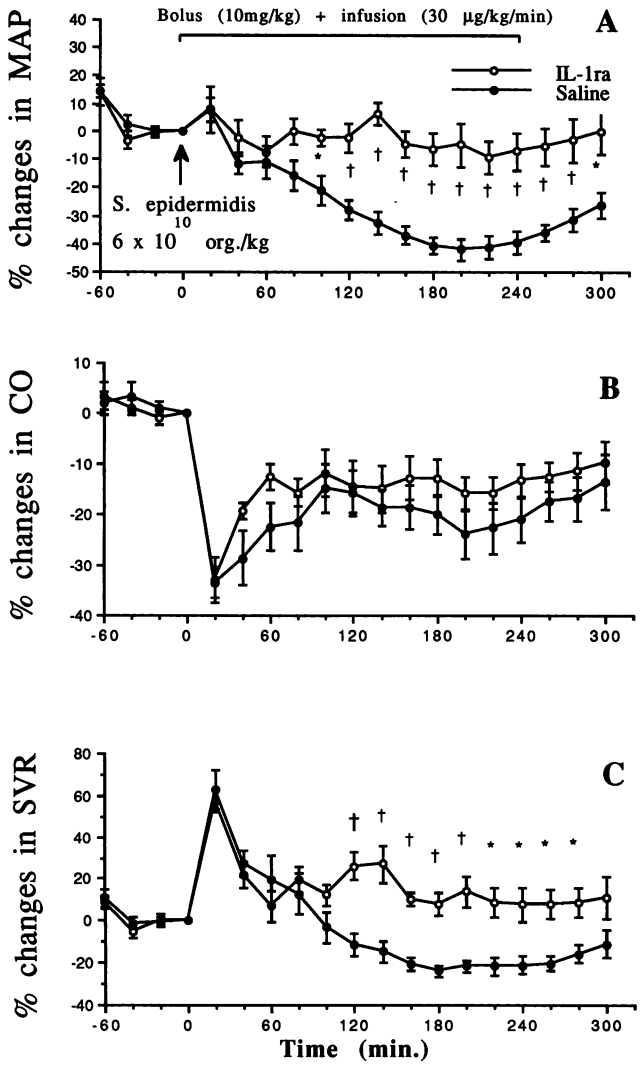

FIG. 1. Effect of IL-1ra on *S. epidermidis*-induced hypotension. The percent changes in MAP (A), CO (B), and SVR (C) at $t = 0$ are shown. IL-1ra-treated rabbits were given a bolus injection of 10 mg/kg at 5 min before *S. epidermidis* injection and then a continuous infusion of 30 μg/kg/min for 4 h. Control rabbits received a bolus injection of saline-albumin and then an infusion of an equal volume of saline-albumin. Both groups received 6×10^{10} *S. epidermidis* organisms per kg. Symbols: open circles, rabbits ($n = 5$) injected with IL-1ra; closed circles, rabbits ($n = 7$) injected with a saline-albumin control infusion. Data are presented as means ± SEM. Asterisks and daggers indicate the values at each time point which are significantly different between vehicle- and IL-1ra-treated rabbits (*, $P < 0.05$; †, $P < 0.01$; by Mann-Whitney test).

curred after this dose of bacteria was comparable to that previously reported (42). Twenty minutes after the injection of *S. epidermidis*, rabbits developed a transient increase in MAP and SVR as well as a brief fall in CO (Fig. 1). After 40 min, MAP decreased 12% below baseline levels and continued to fall until reaching a maximal decrease of −42% ± 3.7% at $t = 200$ min. Thereafter, MAP began to increase. Rabbits were pretreated with a bolus injection of IL-1ra, followed by a constant infusion. In contrast to rabbits treated with the vehicle, IL-1ra-treated rabbits did not develop hypotension. There was only a transient but statistically nonsignificant fall in blood pressure of 7% below baseline at 60 min. The difference in MAP between the IL-1ra-treated and vehicle control rabbits was significant at t of 100 and 300 min ($P < 0.05$) and from 120 to 280 min ($P < 0.01$; Fig. 1). Similar differences were observed in SVR. There were no significant differences between the CO in the two groups.

We then decreased the amount of bacteria by 50% from 6×10^{10} to 3×10^{10} organisms per kg to reduce the rapid but brief fall in CO which took place immediately after the injection of bacteria. The decrease in MAP (maximal decrease of 36%) was less than that observed with the higher dose of bacteria, but there was no significant decrease in CO immediately after the injection. For rabbits receiving 50% less bacteria, the amount of IL-1ra was also reduced from 30 to 20 μg/kg/min. Rabbits were still pretreated with the same bolus injection of 10 mg of IL-1ra per kg. There was, however, no statistically significant reduction in the hypotension in rabbits treated with IL-1ra when this lower dose of bacteria was used (Fig. 2). The increase in CO and fall in SVR were somewhat reduced in rabbits receiving IL-1ra, but these changes in hemodynamic parameters did not reach statistical significance.

Circulating levels of human IL-1ra in rabbits with or without *S. epidermidis* injection. Rabbits given a bolus (10 mg/kg) of IL-1ra and then a constant infusion at 30 μg/kg/min did not manifest changes in hemodynamic or hematological parameters (data not shown), similar to the results in human volunteers given intravenous 10-mg/kg doses of IL-1ra (21). Immediately after a 10-mg/kg bolus injection in rabbits, the circulating level of human IL-1ra in rabbits which received 6×10^{10} *S. epidermidis* organisms per kg was 160.0 ± 18.0 μg/ml. During the infusion, the mean plasma concentration of IL-1ra was 14.7 ± 3.0 μg/ml at 60 min and between 7 and 10 μg/ml at 120 and 240 min (Fig. 3). After discontinuing IL-1ra administration at $t = 240$ h, the levels fell to 1.8 ± 0.1 μg/ml at 300 min. In control rabbits not given *S. epidermidis* but injected with the same amount of IL-1ra, IL-1ra levels were similar to those in the bacteremic rabbits.

Effect of IL-1ra on hematologic parameters following *S. epidermidis* injection. The circulating numbers of leukocytes in both vehicle- and IL-1ra-treated rabbits immediately fell to −50.6% ± 10.6% and −48.5% ± 8.2% from baseline levels, respectively (Fig. 4A). Leukocytes in IL-1ra-treated rabbits reached a nadir of −66.6% ± 7.2% at $t = 180$ min and started to increase thereafter. In contrast, a decrease in leukocytes in vehicle-treated rabbits was sustained, with a maximal decrease at 240 min (−70.7% ± 5.4%). On the other hand, the circulating numbers of platelets in both groups dramatically dropped to −94.6% ± 3.2% in the vehicle-treated group and −95.7% ± 19% in the IL-1ra-treated group (Fig. 4B). In both groups, the numbers of platelets remained low at 240 min.

Pathological changes following *S. epidermidis* bacteremia were assessed by gross as well as microscopic examination of lung, liver, or kidneys. Gross examination did not reveal detectable differences between the vehicle- and IL-1ra-treated rabbits; histological examination showed changes similar to those reported previously following *S. epidermidis* bacteremia (42), but again no detectable differences between the vehicle- and IL-1ra-treated rabbits were observed.

IL-1ra reduces circulating levels of TNF and IL-1β. TNF levels in rabbits receiving *S. epidermidis* (6×10^{10} organisms per kg) rapidly rose to maximal concentrations at $t = 60$ min (350 U/ml) and $t = 120$ min (415 U/ml; Fig. 5A). However, in rabbits receiving *S. epidermidis* but also treated with IL-1ra,

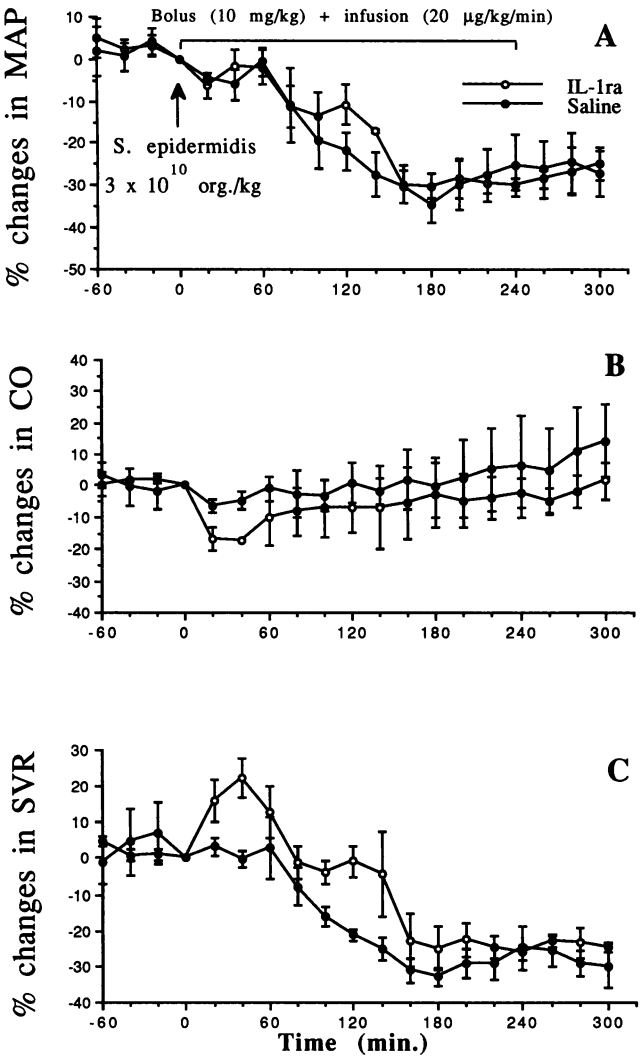

FIG. 2. Effect of IL-1ra on hemodynamic parameters after *S. epidermidis* injection. Percent changes in MAP (A), CO (B), and SVR (C) at $t = 0$ are shown. IL-1ra-treated rabbits were given a bolus injection of 10 mg/kg at 5 min before *S. epidermidis* injection and then a continuous infusion of 20 μg/kg/min for 4 h. Control rabbits received a bolus injection of saline and then an equal volume of saline in a constant infusion. Both groups received 3×10^{10} *S. epidermidis* organisms per kg. Symbols: open circles, rabbits ($n = 3$) injected with IL-1ra; closed circles, rabbits ($n = 3$) injected with a saline control infusion. Data are presented as means ± SEM.

TNF levels at 60 min were markedly (80%) reduced ($P < 0.01$). In rabbits receiving *S. epidermidis*, the mean peak elevation of IL-1β was 364 ± 71.3 pg/ml and occurred at 180 min after the injection. However, in IL-1ra-treated rabbits, the peak IL-1β level was reduced by more than 50% (145 ± 12.2 pg/ml; Fig. 5B). This reduction in IL-1β levels was nearly complete since prior to *S. epidermidis* injection, IL-1β levels were marginally higher, likely because of surgical trauma.

The sum of circulating TNF levels at $t = 60$, 120, and 180 min and IL-1β levels at $t = 120$, 180, 240, and 300 min in rabbits receiving *S. epidermidis* (6×10^{10} organisms per kg) and the TNF levels in the vehicle control was compared with that in rabbits treated with IL-1ra. This reflects the total

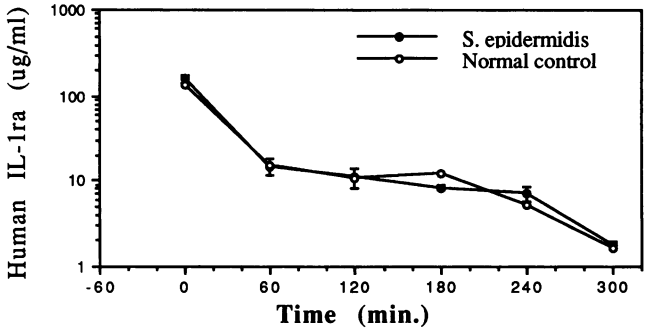

FIG. 3. Circulating levels of human IL-1ra during *S. epidermidis*-induced shock. Levels of human IL-1ra were measured by RIA (22, 38). Rabbits ($n = 4$) were given a bolus injection of 10 mg of IL-1ra per kg at $t = 5$ min and then a continuous infusion of IL-1ra at 30 μg/kg/min for 4 h with or without *S. epidermidis* (6×10^{10} organisms per kg). The plasma level of IL-1ra at the time point $t = 0$ represents the level immediately after the bolus injection and prior to the initiation of the continuous infusion.

circulating levels of TNF and IL-1β during the period of bacteremia. As depicted in Fig. 6, the cumulated amount of TNF and IL-1β in IL-1ra-treated rabbits was significantly less than that in vehicle-treated rabbits ($P < 0.01$ and $P < 0.05$, respectively).

TNF levels were also measured in rabbits receiving 9×10^{10} *S. epidermidis* organisms per kg. These rapidly rose to concentrations at $t = 60$ min of 405 ± 155 U/ml and at $t = 120$ min of 960 ± 225 U/ml ($n = 3$). In rabbits infused with IL-1ra (10-mg/kg bolus, followed by an infusion of 30 μg/kg/min),

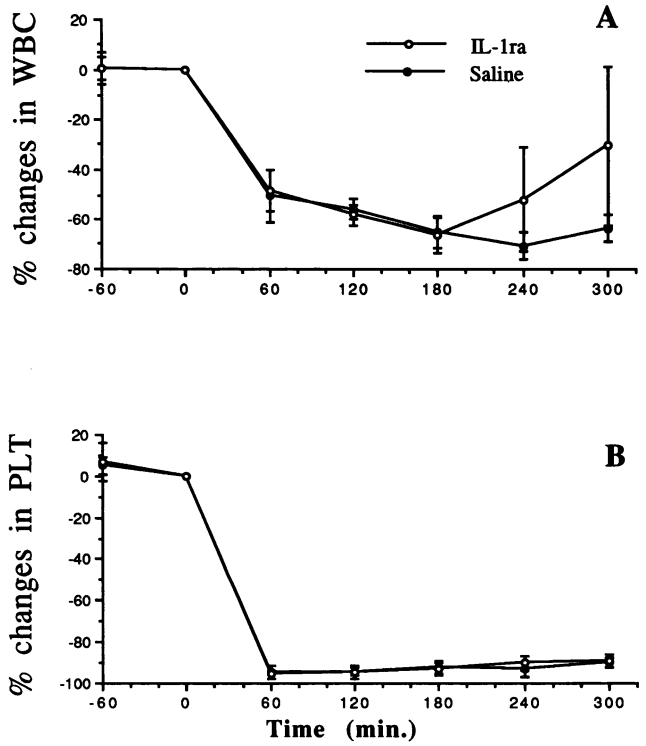

FIG. 4. Effect of IL-1ra on circulating leukocytes (WBC; A) and platelets (PLT; B). Rabbits were injected with 6×10^{10} organisms per kg, and data are taken from the experiments depicted in Fig. 1.

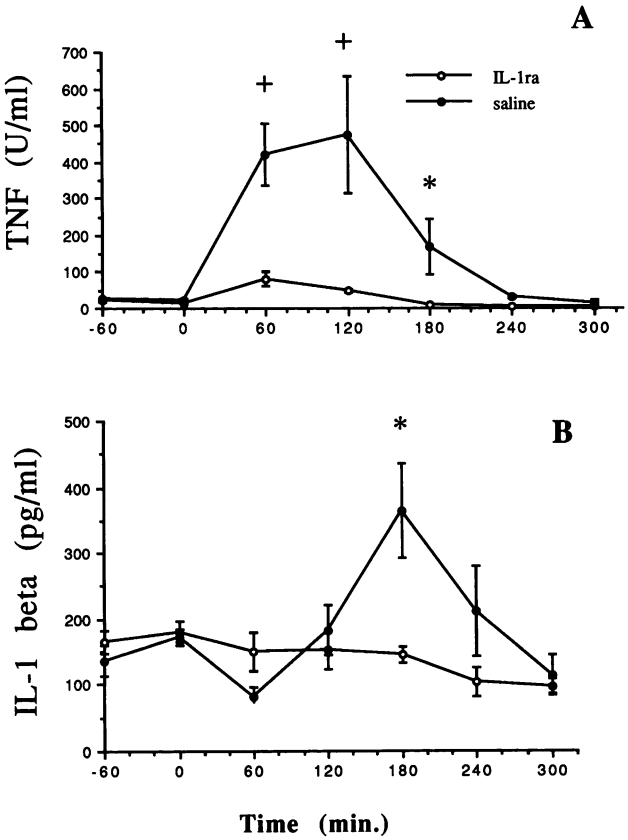

FIG. 5. Circulating levels of TNF and IL-1β during *S. epidermidis*-induced shock. (A) Levels of TNF in rabbit serum as measured by L929 assay (see Materials and Methods); (B) levels of IL-1β measured in the plasma by rabbit IL-1β RIA (10). Rabbits were injected with 6×10^{10} organisms per kg, and blood samples were taken from the same rabbits as those described in the legend to Fig. 1. Data are presented as means ± SEM. Asterisks and crosses indicate that the values at each time point are significantly different between vehicle- and IL-1ra-treated rabbits (*, $P < 0.05$; +, $P < 0.01$; by Mann-Whitney test).

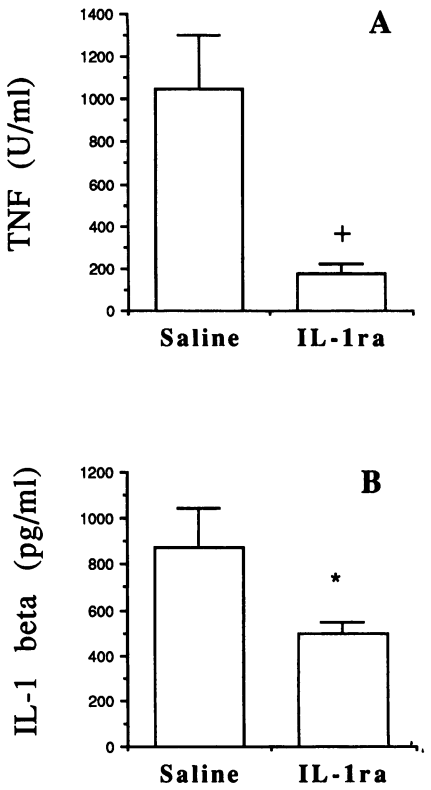

FIG. 6. Effect of IL-1ra on the sum of circulating TNF and IL-1β levels during *S. epidermidis*-induced shock. (A) Sum of values of TNF at 60, 120, and 180 min; (B) sum of values of IL-1β at 120, 180, 240, and 300 min. Rabbits were injected with 6×10^{10} organisms per kg, and blood samples were taken from the same rabbits described in the legend to Fig. 1. Data are presented as means ± SEM. Asterisks and crosses indicate significant differences between vehicle- and IL-1ra-treated rabbits (*, $P < 0.01$; +, $P < 0.05$ by Mann-Whitney test).

the TNF levels at these same time points were 96 and 385 U/ml, respectively ($P < 0.01$). The levels of IL-1β in these same rabbits peaked at 180 min with a level of 594 ± 156 pg/ml, but in rabbits receiving IL-1ra, the level at 180 min was 401 pg/ml ($P < 0.05$). In rabbits receiving 3×10^{10} *S. epidermidis* organisms per kg, IL-1β levels were 1,450 ± 1,050 pg/ml at 180 min ($n = 3$), whereas in three rabbits receiving IL-1ra (10-mg/kg bolus, followed by an infusion at 20 μg/kg/min), the level at 180 min was 501 ± 40 pg/ml.

Effect of IL-1ra on IL-1β RIA and TNF bioassay. To determine whether plasma concentrations of IL-1ra interfere with the RIA for IL-1β, we compared the competitive inhibition of rabbit IL-1β with ^{125}I-IL-1β for binding to anti-rabbit IL-1β in the presence of IL-1ra. As shown in Fig. 7A, there was no effect on the standard curve for rabbit IL-1β in the presence of either 1 or 10 μg of IL-1ra per ml, the latter concentration being in the range of in vivo levels at 180 min. We next compared the bioassay for TNF in the presence of increasing concentrations of IL-1ra. The TNF used in these experiments was a pool of serum samples from rabbits injected with *S. epidermidis* and then bled after 60 min. The source of natural rabbit TNF was diluted and used in the bioassay with and without IL-1ra. As shown in Fig. 7B, there was no statistically significant difference in the amount of TNF detected in the presence or absence of IL-1ra. We conclude from these experiments that the reduction of IL-1 and TNF in bacteremic rabbits receiving IL-1ra is not due to interference with the respective assays.

Effect of IL-1ra on the production of TNF in vitro. Since IL-1ra did not affect the detection assays for either IL-1 or TNF, we next investigated whether the reduction in circulating levels of TNF and IL-1β in IL-1ra-treated rabbits could be observed in vitro. For these experiments, we tested the effect of IL-1ra on TNF and IL-1 production by PBMC, spleen cells, and whole heparinized blood. Concentrations of IL-1ra used in vitro were higher than the concentration found in the circulation in these experiments (see above). IL-1ra significantly inhibited 70% of TNF synthesized by rabbit PBMC stimulated with 2.5 ng of rabbit IL-1β per ml (Fig. 8A). However, when these same PBMC cultures were measured for IL-1α, only a 10% reduction was observed (data not shown). These results are in contrast to those reported for the ability of human IL-1ra to block IL-1β-induced IL-1α production from human PBMC in which a 90% inhibition was observed (20). IL-1ra similarly reduced the production of TNF when either 2 or 200 organisms per cell was used, although the reductions (20 and 30%) did not reach statistical significance.

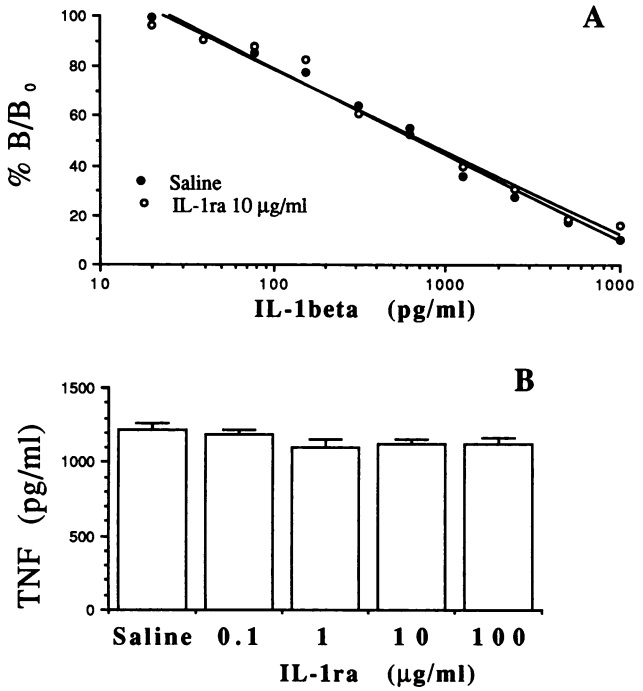

FIG. 7. Effect of IL-1ra on rabbit IL-1β RIA and TNF bioassay. (A) Rabbit IL-1β RIA standard curves with either 10 µg of IL-1ra per ml or saline coincubated with each concentration of rabbit IL-1β; (B) TNF bioassay with and without IL-1ra. The concentration of IL-1ra in the bioassay is indicated. Data are the means of three experiments ± SEM. B/B_0, sample bound/control bound.

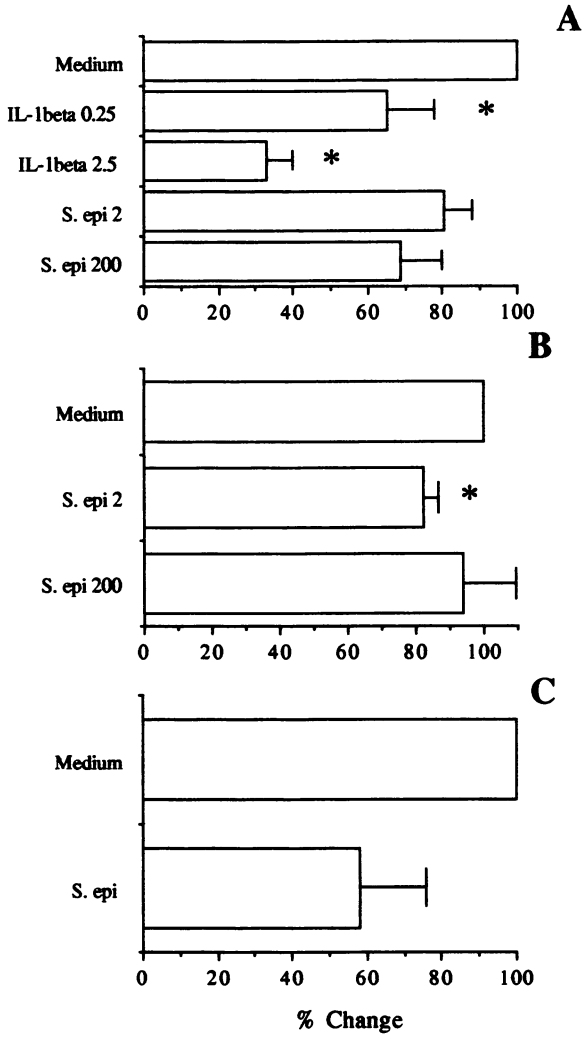

FIG. 8. Effect of IL-1ra on the production of TNF in vitro. (A) Rabbit PBMC (n = 6) stimulated with rabbit IL-1β (0.25 or 2.5 ng/ml) or *S. epidermidis* (2 or 200 organisms per cell); (B) rabbit spleen cells (n = 4) stimulated with *S. epidermidis* (2 or 200 organisms per cell); (C) rabbit whole blood (n = 3) stimulated with *S. epidermidis* (10^9 organisms per ml). The concentration of IL-1ra was 10 (A), 50 (B), or 30 (C) µg/ml. Data are presented as mean percent changes (± SEM) in cytokine concentration in the presence of IL-1ra compared with that of medium control (100%, no IL-1ra). Asterisks indicate significant differences from medium control (P < 0.05; by paired t test).

We next used rabbit spleen cells stimulated with heat-killed *S. epidermidis* and measured the amounts of TNF and IL-1α. As shown in Fig. 8B, there was a 20% reduction (P < 0.05) in the amount of TNF synthesized by stimulated rabbit spleen cells in the presence of IL-1ra. There was also a 20% reduction in the amount of IL-1α produced by spleen cells stimulated by *S. epidermidis* (data not shown).

In an attempt to mimic in vivo conditions, we investigated the effect of IL-1ra on the production of TNF using rabbit whole blood. Heparinized rabbit blood was rotated at 37°C in the absence or presence of IL-1ra with 10^9 *S. epidermidis* organisms per ml for 24 h. The supernatant plasma was removed, diluted, and assayed for TNF. Under these conditions, IL-1ra inhibited the production of *S. epidermidis*-induced TNF by 42% compared with the control (Fig. 8C). However, because of the variation in the production of rabbit TNF from whole blood, this reduction was not statistically significant.

DISCUSSION

Pretreatment with IL-1ra has reduced the severity of LPS- or *E. coli*-induced shock in rabbits (34, 41) and baboons (15); however, in the present study, the degree and kinetics of hypotension induced by *S. epidermidis* were nearly completely blocked by IL-1ra when the higher dose of bacteria was used. When 50% less bacteria was used, IL-1ra had no statistically significant effect. These results are similar to those reported in baboons given *E. coli*, where significant improvement was seen with IL-1ra pretreatment as the severity of the shock was increased (15). A recently completed phase III clinical trial of IL-1ra in 900 patients with septic shock syndrome has revealed similar findings in that there was no statistically significant decrease in mortality when all patients receiving IL-1ra were analyzed; however, a subgroup of patients entering the study with high risk of death showed a 22% reduction in mortality (P = 0.03) when treated with 10 g of IL-1ra over 3 days (16). Both clinical and animal studies are consistent with the previous observation that levels of IL-1β correlate with the degree of hypotension (42).

As Natanson et al. (33) and others (44) have shown, septic shock does not require the participation of endotoxin. In a recent study of humans with septic shock, Ahmed et al. reported that there are no differences in hemodynamic changes between patients with gram-negative bacteremia

and patients with gram-positive bacteremia (1). In the present model, IL-1 appears to play an essential role since blockade of IL-1 receptors prevented the fall in MAP associated with *S. epidermidis* bacteremia.

Although the purpose of the present study was to compare the effect of IL-1 receptor blockade in *S. epidermidis*-induced shock with that of *E. coli*-induced shock previously described (34, 41), we unexpectedly observed a dramatic reduction in the circulating levels of IL-1β and TNF during the *S. epidermidis* bacteremia which we did not observe during the *E. coli*-induced hypotension (41). In the case of IL-1β, this was a nearly complete suppression since the levels at 180 min were lower than those at $t = 0$. The elevated levels of IL-1β at $t = 0$ are likely due to the trauma of catheter placement. High concentrations of IL-1ra in the plasma of these animals (20 μg/ml) did not, however, interfere with the rabbit IL-1β RIA nor the TNF bioassay. These data are similar to those reported in baboons in which IL-1ra treatment was used for *E. coli* sepsis (15) where decreased circulating levels of IL-1β were reported. The present findings are also relevant to the studies of Fong et al., who demonstrated reduced IL-1β levels in the circulation of baboons treated with monoclonal antibody to human TNF (17). Therefore, it appears that blocking either IL-1 or TNF reduces the circulating level of IL-1. Although no reduction in TNF levels has been noted in studies with *E. coli* and IL-1ra (15, 41), the levels of TNF induced by LPS are significantly higher than those induced by staphylococci (42).

Several years ago, studies suggested that crude preparations of endogenous pyrogen injected into rabbits induced additional circulating endogenous pyrogens (4). Recently, recombinant TNF (11) or IL-1 (12, 28) injected into rabbits also induced IL-1 and TNF, thus confirming the initial observations made by using crude endogenous pyrogen preparations, that is, cytokines induce their own synthesis which can be detected in the circulation. In two studies, specific blockade of either TNF (17) or IL-1 (15) during sepsis results in a dramatic reduction in IL-1 and TNF. The present study is consistent with these studies. Thus, we conclude that the cytokine self-induction mechanism can be modulated by specific cytokine blockade in vivo.

In systemic, nonmicrobial inflammation, it is likely that TNF and IL-1 also induce the production of each other as well as that of IL-6, IL-8, and colony-stimulating factors. In fact, blocking IL-1 receptors in models of local inflammation or systemic LPS administration is associated with reduced levels of circulating colony-stimulating factors (26) and IL-6 (19). Blocking IL-1 receptors in LPS-stimulated human PBMC has reduced the synthesis of TNF, IL-1, IL-6, and IL-8 in vitro (20, 37). The blockade of IL-1 receptors in PBMC also reduces the amount of LPS-stimulated IL-1β mRNA (23). In vivo, rabbit expression of IL-1β mRNA and protein can be detected as soon as 30 min after an intravenous injection of LPS (7, 10), although there are no comparable studies using *S. epidermidis* injection in rabbits. Nevertheless, early expression of biologically active IL-1 must occur in vivo for IL-1ra to reduce circulating TNF levels at 60 min in this model of gram-positive shock.

The appearance of TNF appeared to be, in part, dependent on IL-1 activity despite the fact that IL-1β levels are not measurable at 60 min after the injection of *S. epidermidis*, whereas circulating TNF reached maximal levels at 60 and 120 min. There are two possible explanations for the ability of IL-1 to stimulate the production of TNF in the rabbit model of *S. epidermidis* bacteremia. (i) There is an early, local production of IL-1, although not measurable soon after the bacteremia, which is stimulating TNF. Or, (ii) there is a pool of preformed IL-1 which is released early after the injection of the *S. epidermidis*. In support of the first explanation, IL-1α may account for the unmeasurable compartment of IL-1 activity. In support of the latter possibility, platelets may be a source of preformed IL-1β which is biologically active, particularly on endothelial cells (24, 25). Human blood platelets will express biologically active IL-1 after treatment with ADP, thrombin, or epinephrine. Activated platelets can bind to endothelial cells or monocytes and neutrophils via adhesion molecules (31, 32). Within 5 min after activation, platelets express cell-associated IL-1 activity (24, 25), suggesting that a preformed pool of IL-1 exists. We have recently shown that activated human platelets induce IL-8 synthesis in endothelial cells and that this is blocked by IL-1ra (30).

Local IL-1, from activated platelets, for example, may play an important part in the development of an early phase of sepsis. The nearly immediate and dramatic fall in circulating platelets after the injection of *S. epidermidis* supports the rapid activation of platelets. Thus, the production of IL-1β and TNF in these rabbits during *S. epidermidis*-induced shock appears to be under the control of IL-1 itself.

The endothelium appears to be a logical site of IL-1 action in this model since we observed microemboli with platelets on injured endothelial cells (42). Therefore, the experiments using whole blood, spleen cells, or PBMC might reflect only a part of in vivo conditions. Although IL-1ra reduced the amount of *S. epidermidis*-induced TNF synthesized in PBMC, spleen cells, and whole blood in vitro, these reductions did not reach statistical significance. However, we have reported that IL-1ra causes significant (50%) reductions in in vitro cytokine production when human PBMC stimulated with LPS, *S. epidermidis*, toxic shock syndrome toxin-1, IL-2, or phorbol myristate acetate was used (20, 23, 37). Our in vitro results with rabbit tissue are consistent with the in vitro reduction in humans as well as the reduction in the circulating levels of TNF and IL-1β during *S. epidermidis* bacteremia in rabbits treated with IL-1ra. It is important to note that we assessed total cytokine synthesis in vitro whereas the effect of IL-1ra in vivo may have been on cytokine release.

We are cautious not to overinterpret these findings since circulating levels do not reflect the local production and activity of IL-1 and TNF. However, the reduction in hypotension may be due to both a direct blockade of IL-1 effects on the vasculature, such as reduced platelet-activating factor, eicosanoid, or nitric oxide syntheses, as well as decreased TNF and IL-1 release. Recent clinical trials in humans indicate that hypotension following the intravenous infusion of IL-1ra can be severe (43). Severe (stage-4) hypotension following a single intravenous injection of 1 μg of IL-1α per kg has been reported (40). Although TNF in humans also induces a shock-like syndrome, there is a report that anti-murine TNF antibodies, although reducing TNF levels in the circulation, did not reduce the mortality of *Streptococcus pyogenes* sepsis (44). The antibody used in this latter study had provided protection against lethal gram-negative shock (39). Other studies, however, have shown that anti-TNF antibodies provide protection in models of gram-positive shock (18, 27).

We believe our studies are relevant to the clinical treatment of shock induced by gram-positive organisms, particularly *S. aureus*, *Streptococcus pneumoniae*, and other gram-positive bacteria. In the present study, we reduced the

bacterial challenge by 50% and the infusion rate by 30%. However, it is unlikely that these reductions were related to the failure of IL-1ra to reduce the hypotension induced by a lower dose of staphylococci. This is the same dose (20 μg/kg/min) which was used in rabbits challenged with *E. coli* where IL-1ra reduced not only hypotension but also mortality (41). The effectiveness of IL-1ra appears to be related to the severity of disease. The concentration of IL-1 which can be measured in the circulation of humans and animals in septic shock is low (maximal, 0.5 to 1 ng/ml) compared with the 10- to 20-μg/ml concentration of IL-1ra. Therefore, even a 30% reduction in the infusion concentration is still a 1,000-fold molar excess. It has been characteristic of this and other studies of IL-1ra (15, 21, 34) that a 1,000-fold or greater molar excess of IL-1ra to IL-1 is needed to reduce the severity of the disease.

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