A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal Gram-negative infection

(immunomodulation/natural resistance/macrophages)

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ABSTRACT Natural and synthetic immunomodulators that increase nonspecific resistance to infection induce interleukin 1 (IL-1) production. Therefore, we investigated the effect of the administration of IL-1 on the survival of lethally infected granulocytopenic mice. Mice with cyclophosphamide-induced granulocytopenia were injected with approximately 10⁷ Pseudomonas aeruginosa in the thigh muscle at time 0; gentamicin was administered 6 hr and 23 hr later. When recombinant human IL-1β (one of the two forms of IL-1) was given as a single i.p. injection 24 hr before the infection, survival was increased. Using 80 ng of IL-1β per mouse, survival compared to control animals was 98% vs. 71% at 24 hr, 98% vs. 60% at 30 hr, 86% vs. 36% at 36 hr, and 61% vs. 11% at 48 hr (P < 0.001) after the infection. No effect of IL-1β was observed when it was given 0.5 hr before or 6 hr after the infection. Animals not treated with gentamicin also benefited from the IL-1. Administration of the cyclooxygenase inhibitor ibuprofen did not affect the activity of IL-1. Numbers of bacteria cultured from the blood, thigh muscle, liver, spleen, and kidney were similar in IL-1-treated and control animals. Superoxide production by peritoneal macrophages was also similar in the two groups. These studies demonstrate that IL-1 pretreatment protects granulocytopenic mice against lethal pseudomonas infection and suggest that this protection occurs through a noncellular mechanism.

For a number of decades, investigators have sought methods to enhance nonspecific resistance to infection (1–4). Several substances have been found that are able to increase natural resistance mechanisms. Most of these substances, such as lipopolysaccharides (2), BCG (bacillus Calmette–Guérin), and muramyl peptides (4) are derived from bacteria, and their administration has led to considerable toxicity. Despite efforts to modify toxicity (e.g., by the preparation of analogues (4)), these immunomodulatory substances have not gained acceptance as a therapy in humans.

The increase in natural resistance to infection produced by these substances may be due to enhancement of humoral (5) and/or cellular defense mechanisms. With respect to humoral defense, hepatic acute-phase proteins and other nonspecific factors may play a role; with respect to cellular defenses, stimulation of mononuclear phagocytes is considered a major mechanism (6).

Substances that increase nonspecific resistance appear to be able to stimulate mononuclear phagocytes to synthesize and secrete interleukin 1 (IL-1) in vitro (7). IL-1 is the collective name for two 17-kDa proteins (IL-1β and IL-1α) that have a number of important biological effects (7, 8). Because IL-1 is a major mediator of fever and other acute-phase responses, it is possible that many of the effects of the immunomodulatory substances mentioned above are mediated by this molecule. Therefore, we investigated the ability of recombinant IL-1 to ameliorate the outcome of a lethal bacterial infection in mice. We chose to study granulocytopenic mice in order to simulate the situation in neutropenic patients, in whom Gram-negative bacteremia is relatively common and the prognosis is grave. A portion of this work has previously been presented.§

MATERIALS AND METHODS

Mice. Female, 25-g Swiss–Webster mice (Taconic Farms, Germantown, NY) were kept in cages (six mice per cage) with filter lids and were fed standard lab chow and water ad libitum.

IL-1. Recombinant human IL-1β (kindly supplied by Cis­tron Biotechnology, Pine Brook, NJ) that contained less than 60 pg of endotoxin per mg of protein was used in the majority of the experiments. Recombinant IL-1β from a different source (kindly provided by Alan Shaw, Biogen, Geneva) was also used in some experiments. The IL-1 was given as a single i.p. injection in 2% (vol/vol) normal mouse serum in 0.1 ml of pyrogen-free saline. Control mice received heat-inactivated (100°C for 20 min) IL-1. For the experiments with peritoneal macrophages (see below), IL-1 was given i.m.

Infection Models. Mice were rendered granulocytopenic (≤0.5 × 10⁹ granulocytes per liter) by means of i.p. injections of cyclophosphamide (Bristol Myers) at 150 and 100 mg/kg of body weight 4 days and 1 day, respectively, before the inoculation of 1–2 × 10⁷ Pseudomonas aeruginosa (ATCC no. 27853) in the thigh muscle as described elsewhere (9, 10). Unless stated otherwise, doses of gentamicin (Lyomed, Rosemont, IL) at 120 mg/kg were given s.c. 6 and 23 hr postinfection.

A similar infection was established using Streptococcus pneumoniae (10⁹ colony-forming units (cfu) per mouse) obtained from the cerebrospinal fluid of a patient with pneumococcal meningitis. The time schedule for these experiments was the same as with the P. aeruginosa. No antibiotic treatment was given except in a few experiments, in which benzylpenicillin (Pfizer) at 12 mg/kg was administered s.c. 6 and 23 hr after infection.

The mice in each cage were randomized to receive either IL-1 at different time points and in different dosages or heat-inactivated IL-1. Survival was scored over a period of at least 48 hr. In one series of experiments with the pseudomonas model, mice were pretreated with a single s.c. injection of the cyclooxygenase inhibitor ibuprofen (Upjohn) at 10 mg/kg. For the experiments involving peritoneal

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Abbreviations: IL-1, interleukin 1; cfu, colony-forming units.

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Control mice received heat-inactivated (100°C for 20 min) IL-1. (A) Survival with IL-1 administered at different time points is depicted. There were 28–58 mice in each group. The survival with IL-1 given at +6 hr is significantly different from that in the control group (P < 0.001, χ² = 26.5), whereas survival with IL-1 given at −30 min or +6 hr is not different from that in the control group. (B) Each group represents at least 21 mice. The survival rates are not significantly different between groups given 800 ng and 80 ng of IL-1, whereas survival rates in groups given 800 ng, 80 ng, or 8 ng are significantly different (P < 0.05) from the survival rates in the control animals and those given 0.8 ng. (C) The number of mice is at least 18 per group. IL-1 was given at −24 hr at a dose of 80 ng. The difference in survival between IL-1-treated mice not treated with gentamicin and mice that received no treatment is significant at P < 0.05 (χ² = 6.26).

**RESULTS**

**Survival of Mice.** Human recombinant IL-1β, given as a single i.p. injection of 80 ng, did not influence the survival of infected mice when it was given either shortly before or 6 hr after injection of *P. aeruginosa*. However, when IL-1 was given 24 hr before infection, dramatic improvement of survival was observed compared to the survival of controls (χ² = 26.5, P < 0.001) (Fig. 1A). The effect of IL-1 on survival was dose-dependent; dosages of 800, 80, or 8 ng per mouse afforded protection, whereas 0.8 ng was without effect (Fig. 1B). In subsequent experiments we used the relatively low dose of 80 ng per mouse (3.0 µg/kg). The results obtained with recombinant IL-1β from the two sources were similar.

When gentamicin treatment was withheld, the differences in survival between IL-1-treated mice and controls were still apparent (Fig. 1C). Pretreatment with a single injection of ibuprofen, given s.c. 30 min before the injection of IL-1, did not significantly influence the survival of either the IL-1-treated group or the control group.

In mice infected with *S. pneumoniae*, the differences between IL-1-treated and untreated mice were less prominent. Although we observed increased survival with IL-1 in some experiments, the overall reproducibility of the IL-1 effect was less clear with this microorganism than with *P. aeruginosa* (Fig. 2). Treatment with benzylpenicillin did not increase the differences between mice treated with IL-1 and controls.

**Blood Leukocytes and Peritoneal Macrophages.** At the time of infection and during the subsequent days, animals were profoundly granulocytopenic (Table 1). The recovery of granulocyte counts was approximately synchronous in mice treated with IL-1 and controls treated with heat-inactivated IL-1.

The number of macrophages obtained from the unperforated peritoneal cavities (cyclophosphamide given s.c. and IL-1 given i.m. for these experiments) was not different for IL-1-treated mice and controls (Table 1). Fewer than 0.5% of the peritoneal cells were granulocytes in these mice. Super-
In this study, we report the efficacy of a relatively low dose of IL-1/β (3.0 and 0.3 µg/kg) in preventing death in severely leukopenic animals with a P. aeruginosa infection. These results hold promise that such treatment might decrease Gram-negative infections in patients with severe granulocytopenia. Patients whose granulocyte counts fall below 0.1 × 10^9 per liter are at high risk of acquiring Gram-negative bacterial infection (14, 15). In this circumstance, periodic prophylactic administration of IL-1, started when the number of granulocytes falls below 0.5 × 10^9 per liter, is a feasible option that should be explored further. It is likely that an equivalent dose of IL-1/β employed in these studies can be used with acceptable toxicity in humans.

Because IL-1 is a major endogenous pyrogen (7, 8), an important side effect that can be anticipated is fever. Therefore, our finding that pretreatment with the antipyretic drug ibuprofen did not influence the protective effect of IL-1 is relevant. Other studies have shown that indomethacin, another cyclooxygenase inhibitor, in fact, increased nonspecific resistance to infection (16).

The results of our studies demonstrating a protective effect expand upon those obtained by Kampschmidt and Pulliam (17), who used a partially purified rat cytokine preparation in Salmonella typhimurium infection, and those reported recently by Ozaki et al. (18). The latter investigators used human recombinant IL-1α in nonneutropenic mice that were infected with either P. aeruginosa or Klebsiella pneumoniae.

In the present studies, the time of administration of IL-1 in relation to time of infection was critical to the outcome. These findings are reminiscent of those in earlier studies (1-3, 5) in which bacterial endotoxin was given to mice 1 hr prior to infection (i.e., "negative phase"). The lack of any beneficial effect when IL-1 was given shortly before infection with P. aeruginosa may also be explained by the rapidity of the spread of the bacteria in granulocytopenic mice. The observation (18) that IL-1 was effective when given at the time of infection with Klebsiella in nongranulocytopenic mice, in which host defenses may be adequate, is in agreement with this interpretation. A similar observation has been made previously with muramyl dipeptide in a model of intramuscular infection in normal mice (4).

The mechanisms responsible for the IL-1-induced increase in survival remain unclear. A direct antibacterial effect of IL-1 or a potentiation by IL-1 of the antibacterial effect of gentamicin was ruled out in vitro. The rapid clearance of i.p. injected recombinant IL-1/β from the circulation (19) also makes a direct effect of the molecule on bacterial growth unlikely. Because the animals were profoundly granulocytopenic, an effect of IL-1 on the neutrophils was considered unlikely. Administration of IL-1 induces hypoferremia, which has an antimicrobial effect (20); however, this hypo-

**Table 1. Effect of IL-1/β on peripheral blood granulocytes and peritoneal macrophages in cyclophosphamide-treated mice**

| Blood granulocytes*,  
| no. × 10^-9 per liter  | Mice  |  
| IL-1/β treated | Control  |  
| Day 0 | 0.24 ± 0.04 | 0.20 ± 0.09  |  
| Day 1 | 0.13 ± 0.07 | 0.11 ± 0.07  |  
| Day 2 | 0.17 ± 0.10 | 0.24 ± 0.09  |  
| Day 3 | 0.54 ± 0.07 | 0.38 ± 0.04  |  
| Peritoneal macrophages†,  
| no. × 10^-9 per liter  |  
| 1.44 ± 0.19 | 1.45 ± 0.18  |  
| Superoxide production‡,  
| nmoI per 10^6 cells  |  
| 2.79 ± 0.21 | 3.38 ± 0.91  |  

Cyclophosphamide was given i.p. for studies of blood granulocytes and s.c. for studies of peritoneal macrophages at days 4 and 1. Mice were injected with 80 ng of IL-1/β or heat-inactivated IL-1/β (control) at day -1; for studies of blood granulocytes, IL-1/β was given i.p., and for studies of peritoneal macrophages, it was given i.m. Values are the means ± SEM.

*The number of blood granulocytes before administration of cyclophosphamide was (3.2 ± 0.7) × 10^9 per liter (mean ± SEM).

†Determined at day 0.

‡Superoxide production by macrophages from mice of the two groups was measured at day 0 in three experiments using pooled peritoneal macrophages from six mice per group.

**Fig. 2.** Survival of granulocytopenic mice infected with S. pneumoniae. Each group represents 90 mice. IL-1 was given at −24 hr at a dose of 80 ng. The difference in survival between IL-1-treated mice (□) and mice that received heat-inactivated IL-1 (●) is not significant.

**Fig. 3.** Bacterial clearance in granulocytopenic mice following a P. aeruginosa infection. Mice received either 80 ng of human recombinant IL-1/β (stippled bars) or heat-inactivated IL-1/β (hatched bars) 24 hr before infection. Both groups received a single s.c. injection of gentamicin (120 mg/kg) 6 hr postinfection. Twenty-four hours after infection, animals were killed and cultures were taken from thigh muscle, blood, liver, spleen, and kidney. Each bar and error bar represent the mean ± SEM of cfu/g of tissue in six mice.
ferremic effect does not occur in granulocytopenic animals (21), so it is unlikely to have played a role in our experiments. Also, the beneficial effects of IL-1 on hematopoietic recovery (22) were not demonstrated in our short-term experiments.

An effect of IL-1 on macrophages could explain many of our results. The number of peritoneal macrophages and the ability of these cells to produce superoxide were not influenced by treatment with IL-1. In this respect, the effects of IL-1 treatment do not completely mimic those of muramyl dipeptide on macrophages (12). The most convincing argument against an effect on macrophage activation comes from the bacterial clearance data, which failed to demonstrate a difference between IL-1-treated and control mice. Apparently, IL-1-treated mice survive better than control mice despite similar numbers of bacteria in their blood and in organs, including organs that contain the largest populations of mononuclear phagocytes.

The most likely explanation for the beneficial influence of IL-1 in our studies is through a humoral effect [for example, on acute-phase proteins such as an endotoxin-binding protein (23, 24)]. The time required for an optimal effect of IL-1 treatment comes from the synthesis of a Gram-negative infection. The synergism of IL-1 and IL-2 in order to increase natural resistance to infection is potentially feasible in high-risk patients (e.g., those with declining granulocyte counts and at high risk for development of a Gram-negative infection). The synergism of IL-1 (which is identical to hematopoietin 1 (22)) with factors such as granulocyte-macrophage colony-stimulating factor on hematopoietic recovery could further contribute to the potential utility of IL-1 as a therapeutic agent, especially in granulocytopenic patients.

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