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Enhancement of Antibacterial Resistance of Neutropenic, Bone Marrow-Suppressed Mice by Interleukin-1α

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The effect of recombinant human interleukin-1α (IL-1) on the resistance of normal and bone marrow-suppressed mice against bacterial infection was evaluated. IL-1 induced neutrophilia and enhanced the resistance of normal mice against acute, systemic intraperitoneal infection with *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus*. Mice with cyclophosphamide-induced bone marrow suppression were neutropenic and exhibited increased susceptibility to infection. Treatment of neutropenic C57BL/6 and C3H/HeJ mice with IL-1 before infection accelerated recovery of peripheral neutrophil counts and stimulated resistance against infection. Increases in neutrophils and enhancement of resistance induced by IL-1 were both dose and time dependent. Both neutrophilia and augmented resistance to infection were eliminated by a second dose of cyclophosphamide administered during the IL-1 treatments. Bone marrow-suppressed mice treated with IL-1 showed, at 4 h postinfection, greater increases in peripheral blood neutrophils and in numbers of peritoneal exudate neutrophils than suppressed mice treated with vehicle. The data suggest that the IL-1-stimulated recovery of myelopoiesis is an important factor in the enhancement of antibacterial resistance in bone marrow-suppressed, neutropenic mice. These findings indicate that IL-1 may be efficacious in limiting the duration of the neutropenia and of the increased risk for the development of bacterial infection associated with bone marrow suppression.

Interleukin-1 (IL-1) plays a central role in modulating inflammatory reactions and immune responses in vivo. Originally shown to be produced by stimulated macrophages (12), IL-1 is now known to be synthesized by a variety of cell types (for a review, see reference 23). The two major forms of IL-1, IL-1α and IL-1β, exhibit similar biological activities in vitro and in vivo (8, 9, 17). Recently, IL-1 has been shown to enhance the proliferation and differentiation of myelopoietic precursors in the bone marrow in both normal as well as immunosuppressed mice (20, 22). IL-1, also known as hemopoietin 1 (19), synergizes with various hematopoietic colony-stimulating factors (CSF) to increase the growth of progenitor cells (20), although it does not evoke directly the proliferation and maturation of myeloid precursors (32). Additionally, IL-1 induces the production of a variety of CSF (27, 31) which appear to be at least partially responsible for its in vivo stimulation of granulopoiesis.

Suppression of bone marrow function is a crucial factor complicating the use of cytotoxic drug and radiation therapies in treatment of cancer and in bone marrow transplantation (25, 28). Such therapy frequently results in neutropenia, thrombocytopenia, and anemia. As neutrophil suppression becomes more profound and of longer duration, patients are at progressively greater risk for the development of severe or life-threatening infection (4, 5).

We have recently analyzed the activity of IL-1 in promoting the regeneration of bone marrow myelopoiesis and the recovery of cytotoxic drug-induced neutropenia in mice (W. R. Benjamin, N. S. Tare, T. J. Hayes, and T. D. Anderson, J. Immunol., in press). In this report, we evaluate IL-1 for its capacity to enhance the nonspecific antibacterial resistance of mice to infection following bone marrow suppression. The present results demonstrate that IL-1 is a potent stimulator of antibacterial resistance in neutropenic mice. Furthermore, this increased antibacterial resistance correlates with rapid IL-1-induced recovery of bone marrow myelopoiesis. We propose that IL-1 may be of clinical utility as an adjunctive therapy in bone marrow-suppressed patients.

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MATERIALS AND METHODS

Mice. C57BL/6J and endotoxin-resistant (33) C3H/HeJ female mice (Jackson Laboratory, Bar Harbor, Maine) were used between 7 and 10 weeks of age (approximately 18 to 20 g of body weight). Mice were maintained in sterilized cages with filter tops and autoclaved food, water, and bedding. Mice were routinely acclimated for at least 1 week prior to use.

Reagents. Recombinant human IL-1α (lot 1/87; specific activity, 1.5×10^9 units/mg of protein) (referred to hereafter as IL-1) was diluted in a vehicle of sterile Dulbecco Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (GIBCO Laboratories, Grand Island, N.Y.) containing mouse serum albumin (0.2 mg/ml) (Organon Teknika, West Chester, Pa.). Mice were injected subcutaneously (s.c.) with 0.1 ml of IL-1. Control mice were injected with 0.1 ml of vehicle. The IL-1 used in these experiments was shown by the *Limulus* amebocyte lysate assay (Associates of Cape Cod, Woods Hole, Mass.) to contain less than 0.2 ng of endotoxin per mg of IL-1; vehicle contained less than 0.2 ng of endotoxin per 0.1 ml. Cyclophosphamide (CPA; Sigma Chemical Co., St. Louis, Mo.) was dissolved in sterile, pyrogen-free distilled water (GIBCO) and injected intraperitoneally (i.p.) at a dose of 100 or 150 mg/kg of body weight. 5-Fluorouracil (5-FU; Sigma) was dissolved in sterile saline and injected intravenously via the retro-orbital sinus at a dose of 150 mg/kg.

Hematology. Peripheral leukocytes (WBC) were enumerated by hemacytometer counts of EDTA-anticoagulated whole blood diluted in 3% acetic acid. Differential WBC counts were performed on Wright-stained smears. Absolute neutrophil counts were calculated by multiplying the total

WBC by the percentage of neutrophils obtained from differential counts.

PEC. Peritoneal exudate cells (PEC) were collected by injecting 4 ml of serum-free RPMI 1640 medium (GIBCO) into the peritoneal cavity. Following massage of the abdomen, medium was withdrawn and viable (trypan blue dye-excluding) cells were counted. Cytocentrifuge (Shandon Southern Instruments, Sewickey, Pa.) smears of PEC were stained with Wright stain for differential counts. Total PEC neutrophils were calculated from the percentage of neutrophils on differential counts and the total PEC per mouse.

Bacterial infection. Within a given experiment, all mice were infected on the same day. In some instances (e.g., kinetics experiments), the day on which CPA was administered to different groups was staggered such that the day of infection for all groups would coincide. *Klebsiella pneumoniae* A and methicillin-resistant *Staphylococcus aureus* 1191-2, a clinical isolate from the Detroit Receiving Hospital, were grown overnight in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). For infection, serial 10-fold dilutions of *K. pneumoniae* were prepared in Trypticase soy broth and similar dilutions of *S. aureus* were prepared in 10% hog gastric mucin (American Laboratories, Inc., Omaha, Nebr.). Six mice were infected i.p. (0.5 ml) at each dilution of bacteria, and survival of the infected mice was monitored daily. The concentrations of bacterial CFU were determined by spreading 0.1 ml of 10-fold bacterial dilutions on Trypticase soy agar plates and counting colonies after 24 h of incubation at 37°C.

Statistical analysis. Data are presented as the mean ± standard deviation of three to five mice per group. The 50% lethal dose (LD_{50}) was calculated by the method of Reed and Muench (26). The determination of statistical significance between the absolute neutrophil counts of different treatment groups utilized the Student *t* test.

RESULTS

IL-1-enhanced antibacterial resistance of normal mice. Our studies were predicated on the hypothesis that IL-1 administration would increase antibacterial resistance by stimulating myelopoiesis or immune function or both in mice. To be certain that IL-1 did not have direct bacteriostatic or bactericidal properties, we added IL-1 to broth freshly inoculated with cultures of *S. aureus* and *K. pneumoniae*. At concentrations up to 200 μg/ml (the highest concentration tested), IL-1 had no inhibitory activity on bacterial growth (data not shown).

We examined the ability of IL-1 to enhance antibacterial resistance of normal mice, as has been demonstrated recently by others (6, 7, 24). To rule out the potential effects of localized IL-1-induced accumulations of inflammatory cells on antibacterial resistance (6), we used different routes for the injections of IL-1 and bacteria. Mice were treated with s.c. injections of 0.5 μg of IL-1 on four consecutive days, and then they were infected with bacteria by the i.p. route 24 h after the last IL-1 injection. Mice treated with IL-1 exhibited markedly enhanced resistance to subsequent infection by *S. aureus* and *K. pneumoniae* as shown by a 200-fold higher LD_{50} compared with control mice (Table 1). This enhanced resistance was correlated with increased numbers of neutrophils in the peripheral blood of IL-1-treated mice at the time of infection.

Resistance of bone marrow-suppressed, IL-1-treated mice. Transient suppression of bone marrow function by treatment of mice with modest doses (100 to 200 mg/kg) of CPA resulted in significant neutropenia at 3 to 4 days with a gradual return of normal cell numbers in 6 to 7 days posttreatment (35). The recovery of neutrophil numbers to normal (and above-normal) levels was enhanced by IL-1 administration (Benjamin et al., in press). To determine whether IL-1 would increase antibacterial resistance of bone marrow-suppressed mice, we treated mice with CPA and then administered IL-1 for 4 days. Table 2 shows that CPA treatment of mice decreased resistance to bacterial infection compared with control mice. IL-1 treatment of bone marrow-suppressed mice augmented the level of antibacterial resistance above that of mice treated with CPA alone and even beyond that of vehicle-treated controls. This enhanced resistance was correlated with significantly ($P < 0.005$) increased numbers of peripheral blood neutrophils. Thus, bone marrow-suppressed mice receiving IL-1 survived infecting doses of bacteria that were 200- to 2,000-fold higher than those that bone marrow-suppressed control mice survived.

A similar experiment conducted with endotoxin-resistant C3H/HeJ mice also revealed increased antibacterial resistance and elevated peripheral blood neutrophils as a consequence of IL-1 treatment to reverse CPA-induced bone marrow suppression (Table 3). These data demonstrate that the neutrophilia and the enhanced resistance to infection induced by IL-1 treatment were not due to contaminating endotoxin, which is capable of enhancing resistance to infection (29).

Dose-dependent augmentation of resistance by IL-1. Figure 1A demonstrates that the enhanced resistance to *S. aureus* infection induced by IL-1 in bone marrow-suppressed mice was dose dependent. Four daily injections of 0.001 to 1.0 μg of IL-1 per day administered to CPA-suppressed mice resulted in a dose-dependent increase in antibacterial resistance against the *S. aureus* infection. This resistance was

TABLE 1. Antibacterial resistance of normal mice treated with IL-1

Treatment[a]	LD_{50} (CFU/ml)[b]		Neutrophils[c]
	S. aureus	*K. pneumoniae*	
Control	1.0×10^6	3.1×10^4	0.59 ± 0.19
IL-1	$>2.0 \times 10^8$	5.6×10^6	3.05 ± 1.46*

[a] Normal mice were treated with vehicle (control) or IL-1 (0.5 μg/day) s.c. for 4 days.
[b] Mice were infected (i.p.) 24 h after the last treatment. Survival was monitored daily, and LD_{50} was calculated at day 6 postinfection as described in Materials and Methods.
[c] 10^3 per milliliter of blood on the day of infection. Mean ± standard deviation (SD) of five mice. *, $P < 0.01$.

TABLE 2. Effect of IL-1 on resistance of CPA-treated mice to bacterial challenge

Treatment[a]	LD_{50} (CFU/ml)[b]		Neutrophils[c]
	S. aureus	*K. pneumoniae*	
Control	5.6×10^5	3.2×10^6	0.44 ± 0.30
CPA	6.1×10^4	3.2×10^4	0.27 ± 0.09
CPA + IL-1	1.4×10^8	6.1×10^6	12.40 ± 1.89*

[a] Mice were treated with CPA (100 mg/kg) or vehicle. IL-1 (0.5 μg/day, s.c.) or vehicle treatments were begun 24 h later and were continued for 4 days. Mice were infected (i.p.) 24 h after the last treatment.
[b] LD_{50} was calculated at day 6 postinfection.
[c] 10^3 per milliliter of blood on the day of infection. Mean ± SD of five mice. *, $P < 0.001$ versus control or CPA.

TABLE 3. IL-1-enhanced resistance against *S. aureus* infection in endotoxin-resistant C3H/HeJ mice

Treatment[a]	LD_{50} (CFU/ml)[b]	Neutrophils[c]
Control	1.0×10^7	1.96 ± 0.63
CPA	$<1.0 \times 10^1$	0.06 ± 0.04
CPA + IL-1	2.6×10^6	0.56 ± 0.23*

[a] Mice were treated with CPA (150 mg/kg) or vehicle. IL-1 (0.5 μg/day, s.c.) or vehicle treatments were begun 24 h later and were continued for 3 days. Mice were infected with *S. aureus* 24 h after the last treatment.
[b] LD_{50} was calculated at day 6 postinfection.
[c] 10^3 per milliliter of blood on the day of infection. Mean ± SD of five mice.
*, $P < 0.002$ versus CPA.

paralleled by graded increases in peripheral blood neutrophils at the time of infection (Fig. 1B). A treatment regimen of 4 days of 0.001 μg of IL-1 following CPA modestly enhanced bacterial resistance, although this dose was generally ineffective at stimulating significant neutrophilia. Peripheral blood neutrophils were the only cell type whose absolute numbers were significantly increased in a dose-dependent fashion by IL-1 administration; numbers of lymphocytes and monocytes were essentially unchanged (Fig. 2B). Comparable results were obtained in a similar experiment with *K. pneumoniae* A infection as well as in a second experiment with *S. aureus* infection in which mice were treated with IL-1 for 3 days before infection (data not shown).

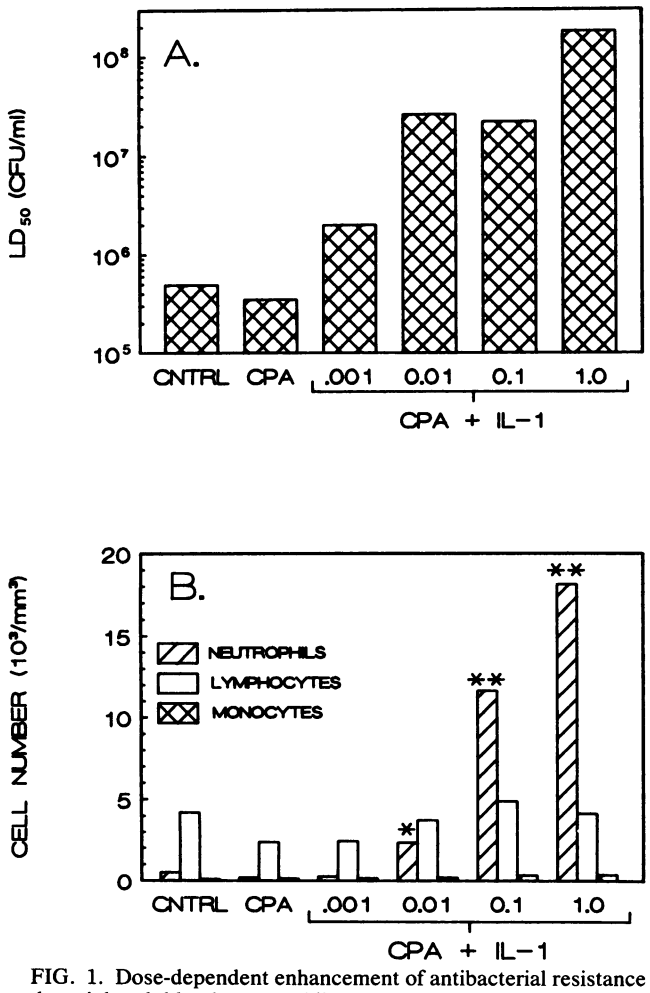

FIG. 1. Dose-dependent enhancement of antibacterial resistance and peripheral blood neutrophils by IL-1. C57BL/6 mice were treated with CPA (100 mg/kg) and then, starting 24 h later, with vehicle or IL-1 for 4 days. Twenty-four hours after the last treatment, mice were bled for WBC and differential counts and then were infected with *S. aureus*. (A) LD_{50}s were determined as described in Materials and Methods. (B) Absolute numbers of different types of peripheral blood WBC determined on the day of infection. *, $P < 0.02$ versus CPA; **, $P < 0.002$ versus CPA.

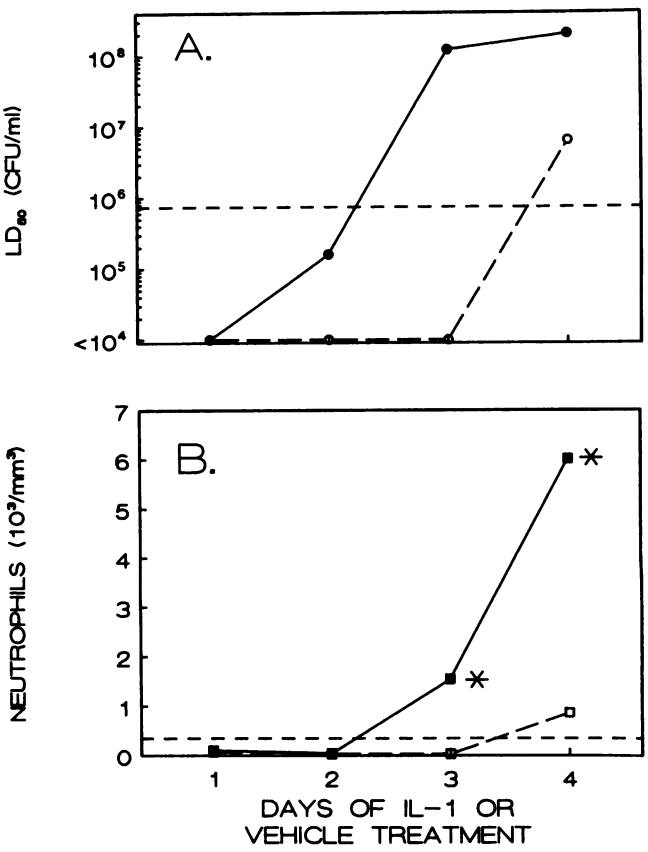

FIG. 2. Kinetics of development of IL-1-enhanced antibacterial resistance and neutrophilia. C57BL/6 mice were treated with CPA (150 mg/kg) and then, starting 24 h later, with vehicle (---) or IL-1 (——) for the indicated number of days. For the different groups of mice, the day on which CPA was administered was staggered so that all mice could be infected on the same day. Mice were infected with *S. aureus* 24 h after the last vehicle or IL-1 treatment. (A) LD_{50}s were calculated at day 6 postinfection. Values for normal control mice are represented by horizontal dashed lines. (B) WBC and differential counts were performed on the day of infection for the determination of total peripheral blood neutrophils. *, $P < 0.001$ versus CPA plus vehicle.

Kinetics of IL-1-stimulated resistance. We next examined the kinetics of the development of IL-1-induced antibacterial resistance in bone marrow-suppressed mice. Mice were treated with CPA and then received 1 to 4 days of treatment with IL-1 or vehicle. Mice were infected 24 h after the last treatment. In mice receiving CPA and vehicle, susceptibility to bacterial infection was maximal during days 1 to 3 of treatment (Fig. 2A). Although 10^4 CFU/ml was the lowest dose utilized in this experiment, other experiments employing a broader range of doses of bacteria indicated that resistance during the first 3 days after CPA administration was often as low as 10^1 to 10^2 CFU/ml (Table 3; and see

TABLE 4. Inhibition of IL-1-enhanced antibacterial resistance against *S. aureus* by two doses of CPA

Treatment[a]	LD_{50} (CFU/ml)[b]	Granulocytes[c]
Control	2.5×10^6	0.67 ± 0.20
CPA (day −5)	2.6×10^6	0.35 ± 0.22
CPA (day −5) + IL-1	$>2.0 \times 10^8$	8.45 ± 1.86*
CPA (days −5, −2)	$<1.0 \times 10^2$	0.01 ± 0.01
CPA (days −5, −2) + IL-1	$<1.0 \times 10^2$	0.01 ± 0.01

[a] Mice were treated with CPA (150 mg/kg, i.p.) on the days indicated. IL-1 (1.0 μg/day, 4 days) or vehicle treatments were begun on day −4.

[b] Mice were infected with *S. aureus* on day 0, 24 h after the last IL-1 dose. LD_{50} was determined at day 6 postinfection.

[c] 10^3 per milliliter of blood on the day of infection. Mean ± SD of three mice. *, $P < 0.002$ versus CPA (day −5) and versus CPA (days −5, −2) with and without IL-1.

TABLE 5. Effect of IL-1 on antibacterial resistance of 5-FU-treated mice against *S. aureus* infection

Treatment[a]	LD_{50} (CFU/ml)[b]	Neutrophils[c]
Control	3.2×10^6	0.72 ± 0.12
5-FU (day −7)	$<1.0 \times 10^1$	<0.01
5-FU (day −7) + IL-1 (6 times)	4.6×10^7	3.37 ± 0.99*
5-FU (day −4)	$<1.0 \times 10^1$	0.28 ± 0.03
5-FU (day −4) + IL-1 (3 times)	$<1.0 \times 10^1$	0.10 ± 0.05
CPA (day −4)	1.0×10^2	0.01 ± 0.01
CPA (day −4) + IL-1 (3 times)	$>2.0 \times 10^8$	3.49 ± 1.27**

[a] Mice were treated with 5-FU (150 mg/kg) or CPA (150 mg/kg) on the indicated days prior to bacterial infection. Daily injection of IL-1 (0.5 μg/day) or vehicle was begun 24 h later.

[b] Mice were infected with *S. aureus* 24 h after the last IL-1 (or vehicle) injection. LD_{50}s were calculated 6 days after injection.

[c] 10^3 per milliliter of blood on the day of infection. Mean ± SD of three mice. *, $P < 0.005$ versus 5-FU (day −7) and versus 5-FU (day −4) plus IL-1. **, $P < 0.01$ versus CPA (day −4).

Table 5). Antibacterial resistance recovered to normal levels following 4 days of vehicle treatment after CPA. This rapid recovery of resistance between 3 and 4 days of vehicle treatment after CPA may also explain the variability in LD_{50}s of mice treated with CPA in other experiments in which the number of days of treatment with vehicle differed (compare Tables 2 and 3 and Table 5).

Mice receiving IL-1 showed progressively enhanced antibacterial resistance with increasing days of treatment, with as few as two doses of IL-1 augmenting resistance (Fig. 2A). Mice receiving IL-1 also showed a more rapid recovery of peripheral blood neutrophils compared with CPA-treated mice receiving vehicle (Fig. 2B). Significant increases in numbers of peripheral blood neutrophils required a minimum of 3 days of IL-1 treatment. These data indicate that recovery of antibacterial resistance and of peripheral blood neutrophils are both dose dependent (Fig. 1) and time dependent (Fig. 2).

Enhanced resistance induced by IL-1 is CPA sensitive. To determine whether a rapidly renewing cell population was responsible for augmented antibacterial resistance, we injected bone marrow-suppressed mice with a second dose of CPA during the course of four IL-1 treatments to inhibit the recovery of myelopoiesis. Table 4 shows that this second CPA treatment abolished the usual increase in peripheral blood granulocytes and completely eliminated the ability of IL-1 to enhance antibacterial resistance. Thus, bone marrow-suppressed mice given four consecutive doses of IL-1 and receiving a second injection of cytotoxic drug were unable to survive infection by as few as 100 *S. aureus* bacteria. In contrast, the IL-1-treated mice receiving only the single dose of CPA before IL-1 treatment survived an infecting dose of bacteria of $>10^8$ CFU. These results suggested that a host component(s) important in mediating antibacterial resistance induced by IL-1 was highly sensitive to the antimitotic effects of CPA.

Recovery of resistance and neutrophils in mice suppressed with 5-FU. Bone marrow suppression induced by 5-FU requires six to eight treatments with IL-1 to promote recovery of peripheral blood neutrophils (20; unpublished observations) compared with suppression by CPA, which requires three to four treatments with IL-1 (Fig. 2B). To determine the effect of IL-1 on antibacterial resistance in this model, we injected mice with 5-FU and then treated them for 3 or 6 days with IL-1. Mice were then infected with *S. aureus* and survival was monitored. Table 5 shows that mice treated with 5-FU and six injections of vehicle were still neutropenic and highly susceptible to infection. This was in contrast to the CPA-induced bone marrow suppression in which resistance against infection recovered after just four treatments with vehicle (Tables 2 and 4; Fig. 1 and 2). Six treatments with IL-1 induced a pronounced neutrophilia and enhanced the antibacterial resistance of 5-FU-suppressed mice, whereas three daily treatments with IL-1 were ineffective at restoring either peripheral blood neutrophils or antibacterial resistance in mice treated with 5-FU. Again, this was in contrast to the results obtained with CPA, in which three daily doses of IL-1 completely restored antibacterial resistance and induced neutrophilia (Table 5). Thus, in this experiment, using two different agents to induce bone marrow suppression, antibacterial resistance was enhanced only in those mice whose numbers of peripheral blood neutrophils were augmented.

IL-1 treatment results in increased neutrophil levels postinfection. In two experiments discussed above, the correlation between enhanced antibacterial resistance and preinfection neutrophilia was not evident. Four treatments with 0.001 μg of IL-1 enhanced the resistance of CPA-suppressed mice without increasing peripheral blood neutrophils (Fig. 1), and two treatments with 0.5 μg of IL-1 had similar effects (Fig. 2). Other studies showed that the bone marrow of CPA-treated mice exhibited significant myelopoietic hyperplasia and increased numbers of granulocytic precursors following as few as two IL-1 injections, but the numbers of peripheral blood neutrophils were not elevated (Benjamin et al., in press). These findings suggested that the enhanced resistance seen in selected groups of mice in those experiments (Fig. 1 and 2) was due to a priming effect of IL-1 that stimulated expansion and maturation of myelopoietic cells in the bone marrow which, upon further stimulation, could be released into the peripheral circulation. We examined this possibility by treating CPA-suppressed mice with two doses of IL-1, infecting them with *S. aureus*, and then examining the peripheral blood and peritoneal cavity for the presence of neutrophils at 4 h postinfection. Figure 3A indicates that by 4 h postinfection bone marrow-suppressed mice treated with two doses of IL-1 had significantly ($P < 0.001$) increased numbers of circulating blood neutrophils despite showing a preinfection level no higher than that of mice receiving CPA alone. Similarly, in CPA-treated mice receiving two doses of IL-1, the numbers of neutrophils in the peritoneal cavity increased 10-fold over control values ($P < 0.05$) at 4 h postinfection, whereas mice receiving only CPA exhibited a 3-fold increase that was not statistically significant (Fig. 3B). Survival of infected mice in these two groups also was

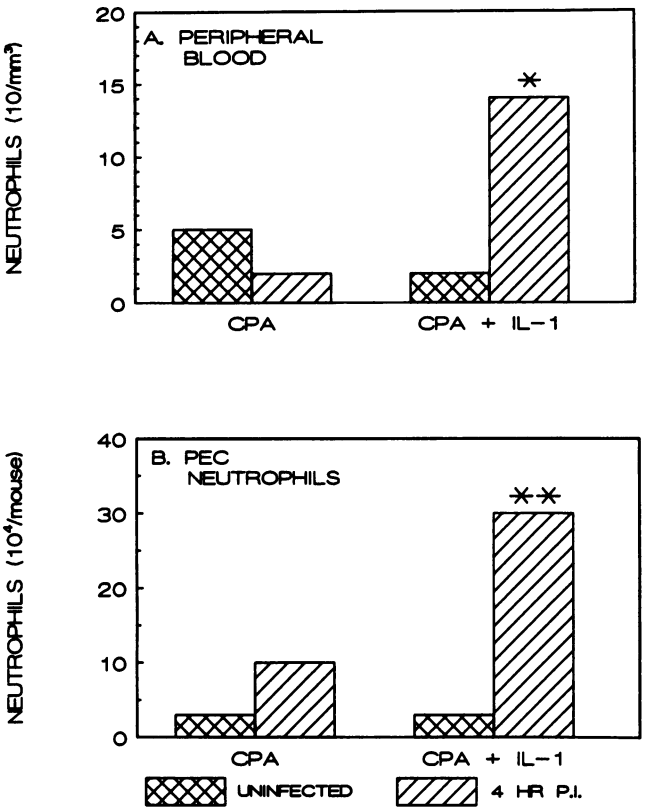

FIG. 3. Peripheral blood and peritoneal cavity neutrophils at 4 h postinfection in mice receiving two IL-1 treatments. C57BL/6 mice were treated with CPA (150 mg/kg) and then received two daily injections of 0.5 μg of IL-1 (or vehicle) beginning 24 h later. Twenty-four hours after the last injection, mice were infected i.p. with *S. aureus*. Blood and PEC were collected from uninfected mice and from infected mice at 4 h postinfection for enumeration of cell numbers and for differential counts. *, $P < 0.001$ versus uninfected mice treated with CPA plus IL-1. **, $P < 0.05$ versus uninfected mice treated with CPA plus IL-1.

markedly different: $LD_{50} = 2.2 \times 10^4$ CFU/ml for CPA plus IL-1 versus $<10^1$ CFU/ml for CPA alone. These data show that the enhanced antibacterial resistance of mice treated with a limited number of doses of IL-1 correlated with an ability to mobilize a rapid neutrophil response early in infection, although no evidence of neutrophilia was present prior to infection.

DISCUSSION

The data presented in these studies demonstrate that IL-1 is a potent inducer of antibacterial resistance against both gram-positive and gram-negative bacteria in bone marrow-suppressed mice. This augmented resistance was paralleled by increases in the number of circulating neutrophils present at the time of infection.

Neutrophils are implicated as important mediators of nonspecific resistance against bacterial infection. Deficiency in their number (25, 28) or functional activity (1) is often manifest in increased susceptibility to potentially lethal infection. Mice made progressively neutropenic by irradiation show a reciprocal increase in numbers of bacteria in tissue following infection (15). Even when antibiotic therapy is begun in these irradiated, infected mice, adequate numbers of circulating granulocytes still constitute an important resistance mechanism, especially when suboptimal doses of antibiotics are used (15). In our experiments, IL-1 elicited a dose-dependent recovery of peripheral blood neutrophils which paralleled enhanced resistance to infection in bone marrow-suppressed mice (Fig. 1). Furthermore, the ability of IL-1 to effectively shorten the period of cytotoxic drug-induced neutropenia was correlated with accelerated recovery of antibacterial resistance (Fig. 2). Multiple linear regression analyses performed to correlate absolute numbers of various leukocyte populations with antibacterial resistance indicated that peripheral blood neutrophils, not lymphocytes or monocytes, correlated most strongly with resistance (data not shown). These findings support the concept that increased granulopoiesis induced by IL-1 is responsible for a significant proportion of the enhanced resistance to bacterial infection in these bone marrow-suppressed mice.

IL-1 has been shown to enhance the resistance of normal rats (16) and mice (6, 7, 24) (Table 1) to bacterial infection. In most cases, maximal enhancement of survival following acute infection was seen when IL-1 treatment preceded infection by 24 to 48 h. This time interval would permit IL-1 to induce a granulocyte response in these immunocompetent mice. Enhanced resistance also has been correlated with a localized IL-1-induced accumulation of phagocytes in the peritoneal cavities of normal mice prior to i.p. infection (6).

An earlier report showed that IL-1 treatment of neutropenic mice led to increased survival following infection with *Pseudomonas aeruginosa* (but not with *Streptococcus pneumoniae*) but that improved survival depended largely on additional antibiotic therapy (30). Our studies presented here demonstrate that IL-1 enhanced resistance of neutropenic mice without additional antibiotics. Thus, with an appropriate treatment regimen, IL-1 alone is capable of rapidly restoring the natural immunity of the bone marrow-suppressed murine host against bacterial infection.

The mechanism(s) by which IL-1 enhances the antibacterial resistance of neutropenic (as well as normal) mice remains to be precisely elucidated, but there appears to be a good correlation between numbers of neutrophils and survival. IL-1 induces the production of granulocyte CSF and granulocyte-macrophage CSF (32, 36) and demonstrates a synergism with CSF in increasing expansion of bone marrow progenitor cells (20, 32). IL-1, or the CSFs that it induces in vivo, also may have direct stimulatory effects on the functional activities of phagocytes. Granulocyte-macrophage CSF activates neutrophils and improves myelopoiesis (2, 10, 11, 14, 34). Thus, it is possible that IL-1 exerts a significant proportion of its stimulatory effects on myelopoiesis (and antibacterial resistance) in vivo by inducing the production of CSF. Indeed, injection of granulocyte CSF alone into CPA-treated mice is capable of reversing neutropenia and of enhancing resistance to infection (18). Exposure of vascular endothelium to IL-1 increases the expression of adhesion molecules for neutrophils (3). This may potentiate leukocyte diapedesis and migration to sites of infection. Together with the increased numbers of neutrophils, these additional elements may contribute to the overall enhancement of IL-1-induced antibacterial resistance.

IL-1 induces a series of physiological changes in vivo mimicking the acute-phase response to bacterial infection (for a review, see reference 8). These changes, including fever, altered synthesis of various hepatic proteins, and reductions in plasma iron, are presumed to be beneficial for the host by increasing opsonization of bacteria and by inhibiting bacterial growth. Although acute-phase proteins

are synthesized within hours after IL-1 administration (21), a single injection of IL-1 failed to enhance resistance against infection 24 h later (Fig. 2). It could be argued that additional injections of IL-1 further augment the production of acute-phase reactants to a protective level. However, administration of CPA to bone marrow-suppressed mice during the course of multiple IL-1 treatments inhibited recovery both of peripheral blood neutrophils and of resistance (Table 3). Additionally, three IL-1 treatments improved neither numbers of peripheral blood neutrophils nor antibacterial resistance in mice suppressed with 5-FU, whereas three treatments with IL-1 induced neutrophilia and concomitantly enhanced resistance in CPA-suppressed mice (Table 5). Recovery of resistance, therefore, was not dependent solely on the myelosuppressed mice having received a given number of IL-1 injections. Thus, only under those conditions of treatment in which granulopoiesis was augmented was antibacterial resistance also restored. Whether a direct causal relationship exists between neutrophilia and enhanced resistance in these experiments remains to be proven formally.

Following the completion of the studies presented here, we became aware of a report by Gladue et al. (13) describing enhanced resistance against *S. aureus* infection induced by human recombinant IL-1β in neutropenic mice. They concluded that in their model there was no role of neutrophils in mediating enhanced resistance. However, differences in experimental protocol, including the species of IL-1 used, the timing of injections, the length of the postinfection observation period, and the routes of administration, preclude a direct comparison of results. Therefore, the discrepancies between the report of Gladue et al. (13) and our current data remain unexplained.

In summary, we showed that IL-1 accelerates recovery of drug-induced neutropenia in mice and effectively shortens the period during which these neutropenic mice exhibit increased susceptibility to bacterial infection. The data indicate that the augmented recovery of myelopoiesis induced by IL-1 is intimately involved in the enhancement of antibacterial resistance associated with IL-1 administration. Furthermore, these findings suggest that IL-1 could prove valuable as an adjunctive therapy in bone marrow-suppressed patients to lessen the duration or severity of neutropenia and to enhance recovery of resistance mechanisms important in the control of life-threatening bacterial sepsis.

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