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Prophylactic Administration of Interleukin-2 Protects Mice from Lethal Challenge with Gram-Negative Bacteria

KONG-TECK CHONG

Department of Pharmacology, Cetus Corporation, Emeryville, California 94608

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Prophylactic administration of recombinant human interleukin-2 (IL-2) in mice enhanced survival and produced complete recovery from an otherwise lethal acute bacterial infection. IL-2 was administered as a single intraperitoneal or intravenous bolus dose to CDI mice 18 h before challenge with a lethal dose of a clinical isolate of *Escherichia coli* type O2 (minimal 100% lethal dose, 6×10^7 CFU per mouse). At IL-2 dosages of 7×10^6 U/kg, 90% of treated CDI mice survived as compared to 0% for the excipient buffer control animals ($P < 0.001$). This protective effect was also demonstrable in immune-deficient beige mice. The IL-2 effect was dose dependent; protection was consistently observed in mice pretreated with IL-2 at doses ranging from 1.8×10^6 to 7×10^6 U/kg. However, at 3.5×10^5 U/kg the protective effect was more variable. The route of administration of IL-2 was shown to play an important role; when IL-2 and challenge bacteria were given by the same route (either intravenously or intraperitoneally), protection was readily observable, but when IL-2 and challenge bacteria were given by different routes, little or no protective effect was observed. The protective effect was fully inducible as early as 1 h after IL-2 administration and was effective against various strains of gram-negative bacteria, indicating that the probable mode of action represents control of the establishment of infection by increased activity of the nonspecific host defense mechanisms. The IL-2 effect was abrogated by the administration of carrageenan, suggesting a possible role of macrophages. These data demonstrate that IL-2 may be a potentially useful adjunct for the prophylaxis of bacterial infections in both clinical and veterinary medicine.

Interleukin-2 (IL-2) is a lymphocyte-derived soluble factor (lymphokine) with a broad spectrum of immunoregulatory activity. Its effects on specific cell-mediated and humoral responses have been the subject of numerous studies. For example, it has been shown that in vivo administration of IL-2 results in enhanced natural killer (NK) cell activity (7, 14) and the generation of alloreactive cytotoxic T lymphocytes (7, 14, 31). In addition, Waldmann and co-workers (32) have reported the expression of IL-2 receptors on activated human B cells, suggesting a probable role of IL-2 on humoral immune responses. Recently, Holter and co-workers (15) showed that gamma interferon induces IL-2 receptors on human peripheral blood monocytes, suggesting that these cells are a further target for IL-2. However, few studies have been reported that demonstrate the therapeutic efficacy of this immunoenhancing ability of IL-2 in bacterial infection models. Although administration of IL-2 has been shown to increase survival time of mice infected with *Trypanosoma cruzi* (3) and enhanced survival in *Toxoplasma gondii*-infected mice (28), to date there have been few reports of IL-2 treatment of mice during bacterial infection. In this study we investigated the effect of administration of IL-2 in vivo on host responses against gram-negative bacterial infection. The results demonstrated that, when administered prophylactically, IL-2 protected approximately 90% of the outbred CDI mice from lethal challenge with various gram-negative bacteria; the effect was also demonstrable in immune-deficient beige (*bg/bg*) mice. The effect was dose dependent and was fully inducible as early as 1 h and up to 18 h after a single administration of IL-2.

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MATERIALS AND METHODS

Mice. Female CDI mice (virus antibody free) were purchased from Charles River Laboratories, Inc., Wilmington, Mass. Beige (*bg/bg*) mice of C57BL/6 background were purchased from Jackson Laboratories, Bar Harbor, Maine. Animals were maintained on Purina Certified Lab Chow and water ad libitum. All animals used in experiments were 8 to 10 weeks old and were housed in specific-pathogen-free animal facilities at Cetus Corp.

IL-2. Highly purified recombinant human IL-2 (lot 9A; specific activity, 3.9×10^6 U/mg) from *Escherichia coli* (24, 33), provided by Cetus Corp., was reconstituted with sterile water to 10^6 U/ml. One unit of IL-2 is expressed as the reciprocal of a dilution that induces 50% of maximum proliferation of the HT_2 cell line (9) and is equivalent to 2.3 units of the Biological Response Modifers Program. A stock solution of 10^6 U/ml contained less than 0.02 ng of endotoxin per ml as determined by the *Limulus* amoebocyte assay (17). Mice were injected with a single dose of IL-2 at various times before or after infection at the doses specified in Results. The excipient buffer control used in some in vivo experiments consisted of the formulation components without the IL-2.

Bacteria. *E. coli* SM18, a type O2 clinical isolate (from Saint Mary's Hospital, San Francisco, Calif.), was cultured overnight in brain heart infusion broth at 37°C, harvested by centrifuging at 6,000 rpm for 15 min, washed in phosphate-buffered saline, suspended in brain heart infusion broth containing 20% glycerol, aliquoted, and stored at −70°C. The viability of the bacteria was determined by plating 10-fold serial dilutions onto blood agar plates and counting the CFU after a 24-h incubation at 37°C. The titer was

TABLE 1. Effect of IL-2 administration on the survival of CDI mice challenged with *E. coli*

Expt	Treatment[a]	% Survival on day 7	*P*
1	IL-2	80	<0.01
	Saline	0	
2	IL-2	90	<0.001
	Control buffer	0	
3	IL-2	90	<0.001
	Control buffer	0	
4	HSA,[b] 2 mg/kg	0	
	HSA, 6 mg/kg	10	Not significant
	Saline	0	

[a] Groups of 10 to 12 mice were treated with 7×10^6 U of IL-2 per kg or control buffer i.p. 18 h before infection with 6×10^7 CFU of *E. coli* SMI18 per mouse i.p. The test dose of both IL-2 and control buffer contained less than 0.004 ng of endotoxin per mouse.

[b] HSA was included as an irrelevant protein control and was used at the highest dose of IL-2 used (2 mg of protein per kg) or threefold higher (6 mg/kg).

expressed as the mean number of CFU of stock bacteria per milliliter. The bacteria were prepared for injection by diluting a freshly thawed vial in phosphate-buffered saline at room temperature and were administered at 1 minimal 100% lethal dose (LD_{100}) as the challenge dose in vivo. Other strains of gram-negative bacteria used in this study include a clinical isolate of *Pseudomonas aeruginosa* 3632 (kindly provided by L. Young, Kuzell Institute for Arthritis and Infectious Diseases, Pacific Medical Center, San Francisco) and *E. coli* SM1 and *E. coli* SM5 (type O unknown) from Saint Mary's Hospital. All of these strains were grown and titers were determined as for *E. coli* SM18.

Determination of LD_{100}. Groups of mice were injected intraperitoneally (i.p.) with 0.5 ml, or intravenously (i.v.) with 0.2 ml, of a serial 10-fold dilution of virulent *E. coli* in sterile saline, and mortality was recorded daily. The LD_{100} represented the minimum number of bacteria necessary to kill all mice within a group.

Animal model. Test animals were randomly distributed into groups of 10 to 12 animals. Unless otherwise stated below, IL-2 or control buffer was administered i.p. to mice 18 hours before i.p. challenge with viable bacteria (LD_{100}). Mice were observed daily, and mortality was recorded for 7 days. The criterion for protection was significant survival enhancement of IL-2-treated mice as compared with excipient-treated control mice ($P < 0.05$).

Carrageenan. Carrageenan type IV was purchased from Sigma Chemical Co., St. Louis, Mo. This is a nongelling form consisting of essentially pure lambda carrageenan.

Human serum albumin. Albumin (human), USP, 25% solution, 25% buminate, was obtained from Travenol Laboratories, Inc., Glendale, Calif.

Statistical calculations. The proportion of animals surviving on day 7 after bacterial challenge in IL-2-treated mice were compared with that of the control animals treated with saline or excipient buffer by use of the one-tailed Fisher exact test.

RESULTS

Lethal effect of *E. coli* SM18 in mice. Experiments were performed to determine the lethal dose of *E. coli* SM18 in mice and to determine the reproducibility of the infection model, i.e., the viability and virulence (lethal dose) of stock bacteria stored at −70°C. The dose-response curve was steep, with no mortality occurring after i.p. challenge with $\leq 5 \times 10^6$ bacteria per animal, whereas inoculation of $\geq 5 \times 10^7$ bacteria per animals was uniformly lethal. About 80% of deaths occurred between 14 and 30 h after challenge, whereas no further deaths occurred after 72 h. The time course of death was similar with i.v. challenge, but higher doses were required to achieve LD_{100}s (about 2×10^8 CFU per mouse). The steep mortality dose response, the high dose for mortality, and the short time to death raised the possibility that the challenge mice died of the endotoxin content of the inocula. However, heat-killed bacterial inoculation (at the equivalent LD_{100} of 5×10^7 CFU for i.p. injection or at 2×10^8 CFU for i.v. injection) produced no mortality, indicating that in vivo replication of the challenge bacteria was required for lethality. Samples of bacteria stock stored at −70°C retained viability for at least 3 months, as determined by CFU determination, and also virulence, as determined by lethal-dose determination in mice (manuscript in preparation). Thus, these procedures facilitated the development of a reproducible bacteria infection model in mice.

Effect of IL-2 administration on survival of mice challenged with *E. coli* SM18. Groups of 10 to 12 outbread CDI mice were injected i.p. with IL-2 at doses of 7×10^6 U/kg at 18 h before i.p. challenge with LD_{100}s of *E. coli* SM18. Control mice were injected with equal volumes of excipient buffer control. The test dose of both IL-2 and control buffer contained less than 0.004 ng of endotoxin per mouse. Mice treated prophylactically with IL-2 showed marked survival enhancement as compared with the excipient buffer- or saline-treated controls (Table 1). Most of the mortality for both buffer control and IL-2-treated mice occurred within 48 h of bacterial challenge. Typically, between 18 and 30 h, the surviving mice from IL-2-treated groups showed signs of illness (ruffled fur). However, after this time these mice recovered rapidly, showing little signs of illness, and by day 4 of challenge all surviving mice appeared to be completely normal. Separately, further experiments (experiment 4 in Table 1) with human serum albumin (HSA) as an irrelevant protein control were carried out to ascertain that the effect seen with IL-2 administration in this model was not associated with the injection of any foreign protein material. The results showed that HSA was without protective effect even at three times the protein dose of the highest dosage of IL-2 used in this study.

Dose of IL-2 in relation to in vivo protective effect. To determine whether the protective effect induced by IL-2 is dependent upon dose, an experiment was performed in which groups of CDI mice were treated with various doses of IL-2 i.p. 18 h before infection with LD_{100}s of *E. coli* SM18. A noninfected group of mice treated with the highest dose of IL-2 was included to monitor any toxicity that might be induced by IL-2. For non-IL-2 treated controls, mice were similarly injected with the excipient buffer for IL-2. The buffer control animals all died by 48 h after infection, whereas no lethality was observed in IL-2-treated but noninfected animals (Fig. 1). Varied levels of survival ranging from 30% (at 3.5×10^5 U/kg) to 80% were seen in the various IL-2-treated groups, with greatest protection at the high dose of 7×10^6 U/kg (significant at $P < 0.01$), indicating that the IL-2 effect is dose dependent. The protective effect seen with the lower dose of IL-2 (3.5×10^5 U/kg) was more variable, ranging from 30% ($P > 0.05$) to 70% ($P < 0.05$) survival in treated mice in different experiments.

Effect of IL-2 administration time on survival enhancement. Experiments were performed to study the duration of enhanced host resistance against bacterial challenge after a single dose of IL-2. Briefly, mice were injected i.p. with IL-2 at 6×10^7 U/kg and challenged at various times with a standard dose (LD_{100}) of *E. coli* SM18. Treated mice showed enhanced survival when IL-2 was given 1 to 18 h before infection, whereas the same doses given at the time of infection or 1 or 4 h after infection were without effect (Fig. 2). When IL-2 was administered 24 h before infection, some reduced protective effect was observed. The same dose administered at 48 or 72 h before challenge showed no protective effect.

Effect of routes of IL-2 administration on survival of mice challenged with *E. coli* i.p. or i.v. To further explore the nature of this protection induced by IL-2, experiments were designed to determine whether this protective effect was route dependent. When administered i.v., IL-2 is not effective at inducing host resistance against i.p. bacterial challenge, but it is effective when administered i.p. (Table 2).

Separately, experiments were also designed to test the effect of i.v. or i.p. routes of administration of IL-2 on i.v. bacterial challenge. *E. coli* SM18 appeared to be less virulent when administered i.v.; 2×10^8 CFU per mouse was needed as the LD_{100} by this route. Groups of mice were administered various doses of IL-2 i.p. or i.v. at 18 h before i.v. bacterial challenge. IL-2 administered i.v. was effective at protecting mice from lethal i.v. infection, but the same dose given i.p. was without effect (Table 2).

Effect of IL-2 administration on lethal infections with various gram-negative bacteria. Since resistance to infection was induced within a few hours of IL-2 administration, it is postulated that the protective effect is probably mediated via enhanced phagocytosis as a result of activation of macrophages and polymorphonuclear leukocytes. Because it is known that these early host defense mechanisms are immunologically nonspecific, i.e., can be expressed against nonrelated bacteria or other invading foreign materials, experiments were designed to test whether IL-2 was as effective against other bacteria. Accordingly, experiments were performed with various gram-negative bacteria, including two other clinical isolates of *E. coli*, SM5 and SM1, and a strain of *P. aeruginosa*, PA 3632; IL-2 was equally effective at protecting mice against all three isolates (Table 3), suggesting that the host resistance generated is immunologically nonspecific.

Effect of IL-2 in mice with impaired macrophage function. Since the protective effect of IL-2 in this infection model is induced within a short time after IL-2 administration and the enhanced host resistance is immunologically nonspecific, it is likely that the effector mechanism is operative via en-

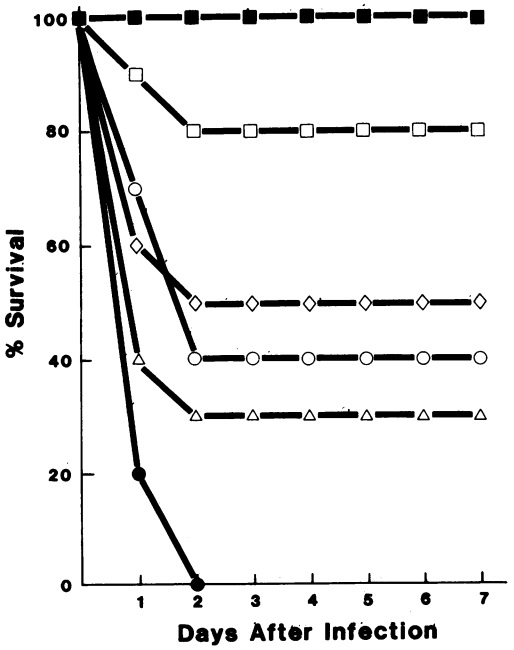

FIG. 1. Effect of IL-2 dose on the survival of mice infected with *E. coli* SM18. Groups of CDI mice were treated with the excipient buffer (●) or various doses of IL-2 (7×10^6 [□], 3.5×10^6 [◇], 1.8×10^6 [○], or 3.5×10^5 [△] U/kg) i.p. 18 h before infection with 6×10^7 CFU of *E. coli* SM18 per mouse. Noninfected control animals (■) were treated with IL-2 i.p. 18 h before an injection of saline i.p. Data are presented as the percentage of mice surviving on day 7 postinfection.

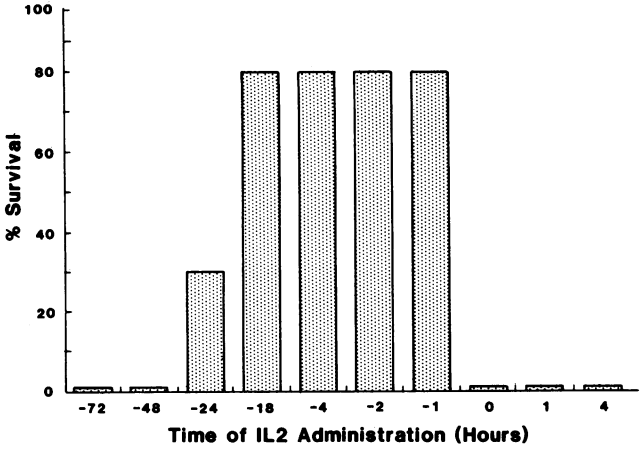

FIG. 2. Effect of administration time of IL-2 on survival of mice challenged with *E. coli* SM18. Groups of CDI mice were treated with IL-2 (7×10^6 U/kg) i.p. at various times before or after an infection with 6×10^7 CFU of *E. coli* SM18 per mouse i.p. Data are presented as the percentage of mice surviving on day 7 postinfection.

TABLE 2. Effect of the route of IL-2 administration in relation to the route of bacteria challenge on survival of mice infected with *E. coli*

Route of infection[a]	Treatment,[b] route	% Survival on day 7	*P*
i.p.	IL-2, i.p.	90	<0.001
	Saline, i.p.	0	
i.v.	IL-2, i.v.	67	0.03
	Saline, i.v.	0	
i.p.	IL-2, i.v.	0	
	Saline, i.v.	0	
i.v.	IL-2, i.p.	0	
	Saline, i.p.	0	

[a] Challenge with 1 LD_{100} of *E. coli* SM18 (6×10^7 CFU per mouse i.p. or 2×10^8 CFU per mouse i.v.).

[b] Groups of CDI mice were treated with IL-2 (7×10^6 U/kg) or saline via the indicated route 18 h before infection.

TABLE 3. Effect of IL-2 administration on the survival of CDI mice challenged with various gram-negative bacteria

Infection (CFU/mouse)	Treatment[a]	% Survival on day 7	P
E. coli SM18 (6.0×10^7)	IL-2	83	<0.01
	Saline	0	
P. aeruginosa (6.5×10^7)	IL-2	83	<0.01
	Saline	0	
E. coli SM5 (5.0×10^7)	IL-2	90	<0.001
	Saline	0	
E. coli SM1 (8.0×10^7)	IL-2	90	<0.001
	Saline	0	

[a] Groups of 10 to 12 mice were treated with IL-2 (7×10^6 U/kg) or saline i.p. 18 h before infection with 1 LD_{100} of bacteria i.p.

hanced phagocytosis. To test the role of peritoneal macrophages in conferring enhanced host resistance, the IL-2 effect was investigated in mice treated with carrageenan, a high-molecular-weight polygalactose. Carrageenan has been shown be toxic to macrophages (2) and in this study was used at 1 mg per mouse, which impaired macrophage function (unpublished data). In our infection model, the LD_{100} of challenge bacteria (*E. coli* SM18) was lowered by 10-fold when mice were given a single dose of carrageenan within 30 min of infection (Table 4). Groups of CDI mice were treated with IL-2 (7×10^6 U/kg) i.p. 2 h before challenge with a lethal dose of *E. coli* SM18, and two groups of mice received further i.p. injection of carrageenan 30 min before or after IL-2 administration. The IL-2 protective effect was abrogated in mice given carrageenan within 30 min of IL-2 administration (Table 4), indicating that macrophages probably plan an important role in generating an enhanced antibacterial state within the peritoneal cavity.

Effect of IL-2 in immune-deficient beige mice. I thought it clinically relevant to determine whether the observed protective effect of IL-2 against bacterial infection was dependent on the immune status of the host. Accordingly, IL-2 effect on host resistance was studied in a naturally occurring immune-deficient strain of mouse. The beige (*bg/bg*) mouse was chosen for study because of its reported deficiency in NK cell activity (22, 29) as well as in T-cell response (25). The lethality of *E. coli* SM18 was determined in beige mice of C57BL/6 background as described above for CDI mice. It was interesting that the LD_{100} as well as the lethal dose curve of *E. coli* SM18 in beige mice was identical to that determined for the immune-competent CDI mice. To study the effect of IL-2, I treated a group of 10 beige mice i.p. with IL-2 (7×10^6 U/kg) or with excipient control buffer at 18 h before challenge with *E. coli* SM18 (i.p., 6×10^7 CFU per mouse). The results showed that none of the buffer-treated animals survived, whereas 100% of the IL-2-treated mice survived and appeared to be normal 7 days after challenge ($P < 0.001$).

DISCUSSION

I have demonstrated that recombinant human IL-2 can induce a potent protective effect in mice challenged with a lethal dose of gram-negative bacteria. This is a novel finding, since IL-2 has not been known to show efficacy against bacterial infection. The protective effect was most marked when high doses of IL-2 were given, especially at 7×10^6 U/kg of body weight, whereas lower doses consistently produced a lesser effect. It is of interest that this dose effect is consistent with an earlier report of IL-2 protective effect in animal tumor models in which protection increases with dose escalation (20). It is likely that lower doses of IL-2 may be effective when more optimal conditions of administration or other formulations (e.g., in liposomes) are employed. It should be noted that in our infection models noninfected mice given the highest dose of IL-2, i.e., 7×10^6 U/kg (i.p. or i.v.), appeared normal with no signs of toxicity throughout the experimental period. Separately, to ascertain that the effect observed with IL-2 administration was not associated with the injection of any foreign protein material into the mouse peritoneal cavity, data were also presented in which HSA was used as an irrelevant protein control. The results indicated that HSA was without protective effect even at three times the protein dose of the highest dosage of IL-2 used in this study. In addition, certain other lymphokine protein materials at equivalent dosage used for IL-2 in this study have been shown to be ineffective (unpublished data).

The IL-2 used throughout this study was clinical-grade material and was assayed to be virtually free of endotoxin. The highest estimated dose of endotoxin (by the *Limulus* amoebocyte lysate assay) that was potentially involved was equivalent to 0.004 ng per animal. Such level of endotoxin has been shown to be ineffective in similar infection models (unpublished data). It is important to dissociate the IL-2 effect from any effect of endotoxin, because of the unique sensitivity of macrophages to endotoxin (19).

The protective effect of IL-2 was inducible within 1 h of administration of a single bolus dose of IL-2, and the effect was still observed after 18 h. Some residual effect was demonstrable 24 h after IL-2 administration (Fig. 2). This finding, coupled with the fact that the protection induced appeared to be immunologically nonspecific (we have extended this observation of IL-2 effect in a viral infection model; unpublished data), suggests that IL-2 may have exerted this action via enhanced activities of the early nonspecific resistance against microbial infections that often involves NK cells and mononuclear and polymorphonuclear phagocytes. The enhanced phagocytic activities are among the most effective antibacterial defenses available before the onset of other immunologically specific responses (1, 4, 5, 10, 29, 30). There is extensive literature relating the activity of various agents that possess macrophage-activating activities and enhanced host resistance against infection (6, 11, 21, 36). This finding of an IL-2 protective effect against bacterial infection is somewhat unexpected, because IL-2, at

TABLE 4. Effect of carrageenan on IL-2 induced protection against gram-negative bacterial infection

Treatment[a]	Carrageenan[b] (mg)	% Survival	P
Saline	1	10	
IL-2	1 (−30 min)	0	
IL-2	1 (+30 min)	10	
IL-2	0 (saline)	100	<0.001

[a] Groups of 10 CDI mice were treated with IL-2 (7×10^6 U/kg) or saline i.p. 2 h before infection with 1 LD_{100} (6×10^7 CFU per mouse or 6×10^6 per mouse when carrageenan was also injected) of *E. coli* SM18.

[b] Mice were injected i.p. with carrageenan at 1 mg per mouse 30 min before (−30 min) or after (+30 min) IL-2 administration. This dose of carrageenan has no toxic effect on CDI mice.

least in in vitro studies, was shown to not directly activate mononuclear leukocytes and polymorphonuclear phagocytes (C. L. Frey, R. C. Allen, and D. J. Drutz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, E16, p. 99).

Any possible effect of IL-2 on macrophage activation may be accounted for by the fact that IL-2 has been shown to directly induce mouse and human lymphocytes in culture to produce gamma interferon (3, 13, 16, 34, 37). Gamma interferon in turn has been shown to be a potent inducer of macrophage activation (26). Thus, these results might add weight to the argument that the study of lymphokine effect at a single-cell population level, although useful at delineating mechanisms at the cellular level, may not predict the final outcome of lymphokine effect in an in vivo situation. Thus, IL-2 may have both direct and indirect effects within the immune network and may thus enhance both immunologically specific and nonspecific host defense mechanisms.

Although this study has not directly examined the mechanism of IL-2-induced host resistance against bacterial infection, there is some indication that macrophages probably play an important role, because the IL-2 effect was abrogated in mice that were treated with carrageenan within a short time (30 min) of IL-2 administration. However, the carrageenan result does not eliminate the potential role of other effector cell mechanisms, because any toxic effect on macrophages in the intact host may indirectly affect other lymphoid cells. For instance, macrophages have been shown to affect the function of a subpopulation of cytotoxic lymphocytes in vitro and to stimulate the formation of suppressor cells in vivo (12).

Another possible mode of action of IL-2-induced host resistance may be via the humoral arm of the host immune response. In this study, the mice used showed no antibody against *E. coli* SM18 (enzyme-linked immunosorbent assay), nor was any antibody detected 2 to 4 h after IL-2 administration. However, further systematic studies on these aspects may be warranted, because a recent report has indicated that IL-2 induced a polyclonal immunoglobulin M response in mice (35). Such responses were induced when nine doses of IL-2 at 5,000 U per dose (per mouse) were injected i.p. over a 3-day period. In our study we have employed different IL-2 administration protocols, i.e., a single dose of IL-2, given 1 to 18 h before infection. It is not known whether the reported polyclonal immunoglobulin M response after IL-2 administration is dependent on a certain dose regimen or on the route of IL-2 administration. Nevertheless, we believe that antibody is less likely to play a role in the findings we observed because (i) the induced effect is dependent on the route of IL-2 administration as well as on the route of challenge bacteria (i.p.-i.p. or i.v.-i.v.), and (ii) the induced effect diminished so rapidly that by 24 h after IL-2 administration only partial protection was observed.

The protective effect of IL-2 against bacterial infection was also demonstrable in a naturally occurring immune-deficient strain of mouse. Beige mice are generally reported to be deficient in NK cell activity (20, 27) as well as in T-cell response (22). However, despite these deficiencies, beige mice are no more susceptible to bacterial challenge when compared with immune-competent CDI mice. This may indicate that NK cell activity and T-cell response perhaps play a minor role in host resistance to primary bacterial infection. In this regard, Morahan and co-workers (18) have recently reported that CDI mice express relatively normal natural resistance to encephalomyocarditis virus, *Listeria monocytogenes*, and herpes simplex virus in spite of profound monocytopenia, granulocytopenia, and depressed NK cell functions caused by ^{89}Sr isotope treatment. In our study, IL-2 is equally effective in enhancing host resistance against bacterial infection in beige mice, suggesting that the effector-cell populations involved were intact in these animals. However, it is not clear whether IL-2 administration would restore NK and T-cell response in these mice.

In addition, we have studied some pharmacological parameters of IL-2 action. It is clear that the route of administration of IL-2 played an important role in its effect on enhancing host resistance to bacterial infection. In this study IL-2 appeared to be effective only when it was administered by the same route as that of the bacteria challenge. It is conceivable that, when administered i.p., the activated phagocytic cells remained largely within the peritoneal cavity, accounting for enhanced resistance at this site. Conversely, when IL-2 was administered i.v., there may have been active recruitment of polymorphonuclear cells into the vascular circulation and perhaps enhanced bacterial clearance by the fixed tissue macrophages of the mononuclear phagocyte system, namely, the Kupffer cells of the liver. Alternatively, this phenomenon may be associated with the in vivo stability and plasma clearance of IL-2. The plasma half-life of IL-2 in mice is generally reported to be biphasic, with an initial distribution phase of about 2 min followed by a slower clearance of about 50 min (7, 23). Similar findings were observed in our study in which CDI mice, as used in the infection model, were administered IL-2 at 10,000 U per mouse (unpublished data). However, until further information is available on the distribution of IL-2 in various body compartments after a given route of administration, it would not be possible to distinguish between the effect of IL-2 stability in vivo versus the efficiency of IL-2 distribution to the target site and its respective role in the protective effect observed. In this regard, a multicompartment pharmacokinetic model for IL-2 distribution in vivo has been proposed (M. Konrad and E. Bradley, Proceedings of the American Society of Clinical Oncology, vol. 5, abstract no. 920, 1986). Thus, it is possible that a bolus dose of IL-2 can only achieve a threshold effective dose at the site or physiologic compartment of administration. The importance of the route of administration has also been shown for recombinant mouse gamma interferon in which the authors reported that gamma interferon was effective in a viral model only when administered i.p. at the site of virus challenge (27).

In the present infection model, bacteria invade the host by way of the lymphatic system or blood vessels or both, and thus the liver and spleen represent some of the initial sites of rapid bacterial growth (manuscript in preparation) and consequential interaction with the fixed phagocytic elements. The activity of tissue macrophages is likely to play a key role in host resistance, since it has been reported that normal as well as immune modulator-enhanced resistance of CDI mice to microbial infection may rely on activated tissue macrophages (18). It should be pointed out that various degrees of spleen enlargement were seen in mice pretreated with IL-2; this is believed to be because of enhanced phagocytic activity (unpublished data). It is probable that the prophylactic effect of IL-2 in this model represents control of the establishment of infection by increased activity of the nonspecific host defense mechanisms and thereby reduced bacterial growth and any associated endotoxin effect. Further studies on the effect of IL-2 in bacterial infection models are in progress with the aims of understanding (i) the relative role of the various cellular effector mechanisms as well as humoral responses and (ii) the mode of delivery of IL-2 in relation to pharmacokinetic parameters. These studies could

facilitate the application of cloned human IL-2 as an adjunct for the prophylaxis of bacterial infection in both clinical and veterinary medicine.

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