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Charles J. Czuprynski, *University of Wisconsin-Madison*
Mary Haak-Frendscho, *University of Wisconsin-Madison*
Nancy Maroushek, *University of Wisconsin-Madison*
James F. Brown, *University of Wisconsin-Madison*

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Effects of Recombinant Human Interleukin-6 Alone and in Combination with Recombinant Interleukin-1α and Tumor Necrosis Factor Alpha on Antibacterial Resistance in Mice

CHARLES J. CZUPRYNSKI,* MARY HAAK-FRENDSCHO, NANCY MAROUSHEK, AND JAMES F. BROWN

Department of Pathological Sciences, School of Veterinary Medicine, University of Wisconsin—Madison, Madison, Wisconsin 53706

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In this study, recombinant human interleukin-6 (rIL-6) was tested for its ability to alter the resistance of mice to experimental *Listeria monocytogenes* infection. Single bolus or repeated injections of rIL-6 by itself did not increase antilisteria resistance. When rIL-6 was injected in combination with suboptimal concentrations of rIL-1α and tumor necrosis factor alpha (rTNF-α), it did not augment their abilities to mediate protection in the spleen and had a marginal effect on the level of protection in the liver. Injection of rIL-6 together with protective doses of rIL-1α did not diminish the protection stimulated by the latter. Unlike rIL-1α and recombinant tumor necrosis factor alpha, rIL-6 appears to have little ability to elevate antibacterial resistance.

Interleukin-6 (IL-6) is a 22-kDa cytokine produced by a variety of cells, most notably mononuclear phagocytes (1, 2). Production and release of IL-6 has been documented to occur during various inflammatory conditions. Like the monokines IL-1 and tumor necrosis factor alpha (TNF-α), IL-6 mediates a wide array of biological activities. These include stimulation of lymphocyte proliferation, alterations in endothelial cell interaction with leukocytes, increased synthesis of acute-phase reactants by hepatocytes, and decreased growth by fibroblasts (1, 10, 12). Levels of IL-6 in serum are elevated during septicemia and endotoxemia, thereby suggesting that it is an important mediator in these clinical syndromes (6, 7, 9, 11, 15). Recent evidence suggests that IL-6 is responsible for some of the biological activities previously attributed to IL-1 and TNF-α during the host response to bacterial endotoxin (7, 11, 15).

Previous studies by several laboratories, including our own, have documented the ability of recombinant IL-1 (rIL-1) and rTNF-α to enhance resistance to a variety of experimental infections (3, 4, 13, 14). We have shown that *Listeria monocytogenes* infection of mice is a useful experimental system for evaluating the effects of IL-1α and TNF-α in host defense (4, 14). If IL-6 indeed mediates many of the effects of IL-1 and TNF-α, one might predict that administration of rIL-6 provides substantial protection against experimental *L. monocytogenes* infection of mice. The purpose of the present study was to determine whether rIL-6 alone had a substantial effect on antilisteria resistance and whether rIL-6 in combination with rIL-1 and rTNF-α might modulate the resistance afforded by these monokines.

MATERIALS AND METHODS

IL-6. The human rIL-6 used in this study (lot 1406-131) was generously provided by Genetics Institute (Cambridge, Mass.). It had a biological activity of approximately 10^6 U/mg in the T1165 assay. The human rIL-α was generously provided by Peter Lomedico of Hoffmann-La Roche, Inc. (Nutley, N.J.). This material (lot 1/87) had a biological activity of 2.5×10^9 U/mg in the D10 cell proliferation assay. The highly purified human rTNF-α was a gift from Cetus Corporation (Emeryville, Calif.). This material had a biological activity of 21.5×10^6 U/mg in the L929 cell cytotoxicity assay.

Mice. The mice used in this study were 6- to 7-week-old male (C57BL/6 × DBA/2)F_1 mice obtained from The Jackson Laboratory (Bar Harbor, Maine). They were certified by the supplier to be free of infection with adventitious viral agents. Mice were given food and water ad libitum and were maintained under sterile microisolator caps at the School of Veterinary Medicine Animal Care Facility.

***L. monocytogenes* infection.** Experimental infection was assessed as we have described previously (14). Log-phase *L. monocytogenes* EGD was stored as aliquots in tryptone phosphate broth with 20% glycerol at −70°C. For each experiment, an aliquot was thawed and diluted to a bacterial concentration of 4×10^4 organisms per 0.2 ml of pyrogen-free saline. This was injected intravenously (i.v.), along with the appropriate concentration of IL-6 or other monokines, into mice via the lateral tail vein. Positive controls included mice that received a dose of IL-1α (0.2 μg) or TNF-α (1.0 μg) known to stimulate significant protection. Mice that received *L. monocytogenes* without cytokine were included as a negative control. Three days after injection, the mice were killed by cervical dislocation and their spleens and portions of their livers were removed to separate sterile glass tissue grinders that contained 5.0 ml of cold phosphate-buffered saline. The tissues were homogenized and then serially diluted in sterile, distilled water. Appropriate dilutions were plated in duplicate on blood agar. The plates were incubated at 37°C for 24 h, and the number of colonies was counted. The results are expressed as the mean ± standard error of the mean (SEM) $\log_{10}$ *L. monocytogenes* per organ (3 or 4 mice per group).

RESULTS

Single bolus or repeated injections of rIL-6 do not enhance antilisteria resistance. In our first experiment, we assessed the effects of a range of concentrations of rIL-6 (0.2 to 2.5 μg per mouse), administered as a single i.v. bolus concomitant with bacterial challenge, on resistance to *L. monocytogenes*

* Corresponding author.

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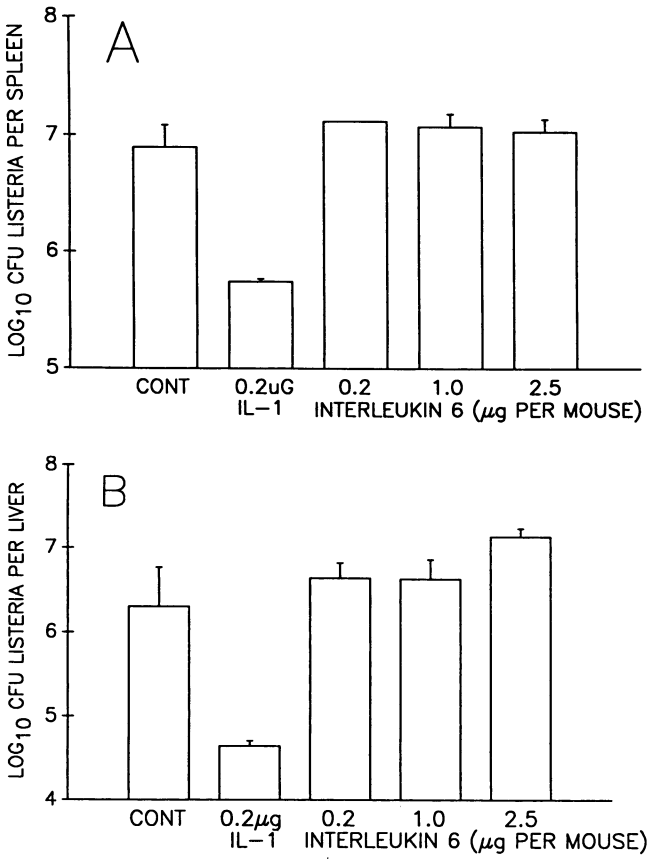

FIG. 1. Human rIL-6 does not increase resistance to *L. monocytogenes* infection. Mice were injected i.v. with 4×10^4 *L. monocytogenes* and the indicated amount of IL-6 in a total volume of 0.2 ml of pyrogen-free saline. Mice were killed 3 days later, and the numbers of viable listeriae in their spleens (A) and livers (B) were determined. Results are the mean ± SEM of three mice per group. Mice that received *L. monocytogenes* alone or *L. monocytogenes* with 0.2 μg of human rIL-1α were included as negative and positive controls, respectively.

infection. Our results demonstrated no protection after injection of the indicated amounts of IL-6, as evaluated by the numbers of listeriae recovered from the spleen and liver (Fig. 1). In contrast, mice that received 0.2 μg of rIL-α exhibited substantial reductions in the numbers of listeriae in their spleens and livers, as reported previously (4). We also examined whether repeated injections of rIL-6 might increase antilisteria resistance. Groups of mice (10 per group) received 0.1 μg of rIL-6 intraperitoneally every 12 h, beginning 24 h before and continuing for 48 h after i.v. challenge with 2×10^4 *L. monocytogenes*. Control mice received pyrogen-free saline intraperitoneally at the same intervals. Repeated injection of rIL-6 did not decrease the numbers of listeriae recovered from the spleens (6.39 ± 0.24 and 6.41 ± 0.10, respectively, for rIL-6 and control mice) and livers (6.20 ± 0.31 and 5.82 ± 0.12, respectively, for rIL-6 and control mice) of *L. monocytogenes*-infected mice (mice were euthanized 72 h after *L. monocytogenes* challenge; $P > 0.4$). Because a previous study indicated that rIL-6 protected mice against lethal *Pseudomonas aeruginosa* infection without decreasing the bacterial burden in treated mice (16), we examined the possibility that rIL-6 might have a similar effect on *L. monocytogenes*-infected mice. Mice received a

TABLE 1. Effects of human rIL-6 on the ability of suboptimal doses of rIL-1α and rTNF-α to stimulate antilisteria resistance

Treatment (μg per mouse)			Mean ± SEM $\log_{10}$ protection[a]	
IL-6	IL-1α	TNF-α	Spleen	Liver
1.0	—[b]	—	0.30 ± 0.12	−0.19 ± 0.16
—	0.025	—	0.68 ± 0.17	0.95 ± 0.13
—	—	0.1	0.32 ± 0.14	0.05 ± 0.25
1.0	—	0.1	0.36 ± 0.05	0.70 ± 0.20[c]
1.0	0.025	—	0.38 ± 0.07	0.59 ± 0.17
1.0	0.025	0.1	1.04 ± 0.05	1.40 ± 0.13[c]
—	0.025	0.1	1.24 ± 0.14	0.90 ± 0.16

[a] $\log_{10}$ decrease in viable listeriae compared with that in control *L. monocytogenes*-infected mice. Results are the mean ± SEM of four mice per group. Positive control mice that received optimal doses of IL-1α (0.2 μg) or TNF-α (1.0 μg) had greater than 1.0 $\log_{10}$ protection in the spleen and liver (data not shown).

[b] —, none given.

[c] $P < 0.05$ compared with that monokine combination without IL-6.

single bolus injection of rIL-6 (1.0 μg) i.v. together with graded doses (2×10^4 to 1×10^5 CFU) of *L. monocytogenes* and deaths were recorded for 7 days following challenge. We observed no difference between the survival of control and rIL-6-treated listeria-infected mice (data not shown).

Effects of rIL-6 on antilisteria resistance mediated by rIL-1α and rTNF-α. In a previous report we demonstrated that suboptimal doses of rIL-α and rTNF-α, which by themselves had a minimal effect on antilisteria resistance, when injected in combination stimulated a substantial level of antilisteria resistance (14). We, therefore, tested whether rIL-6 might have a similar ability to augment the resistance produced by treatment with suboptimal doses of rIL-α and rTNF-α. There was no significant elevation of antilisteria resistance in the spleens of mice that received rIL-6 (1 μg per mouse) i.v. together with suboptimal doses of rIL-α and rTNF-α (Table 1). A small, but statistically significant, protective effect in the livers of mice was observed for mice that received rIL-6 and rTNF-α in combination (compared with rTNF-α alone) or rIL-6, rIL-1α, and rTNF-α in combination (compared with that in mice that received rIL-α and rTNF-α in combination). We went on to determine whether coadministration of rIL-6 would alter the protection afforded by an optimal dose of rIL-1α. Table 2 demonstrates that mice that received 1.0 μg of rIL-6 along with 0.2 μg of rIL-1α did not differ from mice that received 0.2 μg of rIL-1α alone.

TABLE 2. Effects of human rIL-6 on the ability of an optimal dose of IL-1α to stimulate antilisteria resistance

Treatment		Mean ± SEM $\log_{10}$ *L. monocytogenes*[a]	
rIL-6 (0.1 μg)	rIL-1α (0.2 μg)	Spleen	Liver
+	−	5.86 ± 0.32	5.53 ± 0.41
−	+	5.15 ± 0.22[b]	4.83 ± 0.43[b]
+	+	5.48 ± 0.27[b]	5.12 ± 0.36[b]
−	−	6.15 ± 0.32	5.81 ± 0.35

[a] Results are the means ± SEM of four mice per group. Mice received rIL-6 or rIL-1α as indicated i.v. concomitant with 2×10^4 *L. monocytogenes*. Mice were euthanized 3 days after challenge, and the numbers of viable listeriae per spleen and liver were determined as described in Materials and Methods.

[b] $P < 0.05$ compared with control and $P > 0.20$ compared with each other.

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DISCUSSION

The results of this study indicate that rIL-6 does not enhance antilisteria resistance by itself and has a marginal ability to augment the effects of suboptimal concentrations of IL-1α and TNF-α when given in combination with these monokines. Although one might propose that these results were due to our using human IL-6 in mice, other investigators have reported that human IL-6 from the same source (Genetics Institute) stimulates an acute-phase response in mice (12). Our results are generally consistent with the observations of Van der Meer et al. (16), who found that rIL-6 increased survival but did not increase bacterial clearance in granulocytopenic mice infected with *P. aeruginosa*. These authors also reported that rIL-6 did not potentiate the protection offered by IL-1 in their model. Havell and Sehgal (8) recently reported that endogenous levels of IL-6 were elevated in the spleens and bloodstreams of *L. monocytogenes*-infected mice treated with an anti-TNF-α monoclonal antibody. In that study, levels of IL-6 in plasma correlated with the severity of the infection and were largely independent of TNF-α release (8).

Other evidence indicates that endogenous IL-6 levels correlate with active infection with a variety of microbes in both man and rodents (5–9, 11, 15). In particular, it has been suggested that IL-6 is responsible for some of the lethal effects of endotoxemia and septic shock. Both IL-1 and TNF-α have been implicated as mediators responsible for the rise in IL-6 levels observed in these studies (7, 11, 15). To the best of our knowledge, there is no compelling published evidence for the protective effects of rIL-6 in experimental infection models. This is in contrast to the monokines rIL-1 and rTNF-α, which offer substantial protection against a variety of infectious agents when administered to experimental animals (3, 14, 16). Taken together, these findings suggest that, rather than being a major player in the protective host response to infection, IL-6 release represents an attempt by the host to repair tissue damage during microbial infection.

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