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Interleukin-1 Administration to C3H/HeJ Mice after but Not prior to Infection Increases Resistance to *Salmonella typhimurium*

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Interleukin-1 (IL-1) treatment of C3H/HeN and C3H/HeJ mice prior to infection with *Salmonella typhimurium* increased the survival fraction only in C3H/HeN mice. IL-1 administration after infection resulted in a significant increase in mean survival time in C3H/HeJ but not C3H/HeN mice. Bacterial growth in IL-1-treated C3H/HeJ mice was less than that in control mice.

In mice, resistance to *Salmonella typhimurium* is controlled by a number of genetic loci, such as *ity*, *xid*, and *Lps* (13–16). C3H/HeJ mice are susceptible to infection with *S. typhimurium* because of homozygosity for the Lps^d allele (18, 22), although they carry the resistance allele at the *ity* locus (13, 15).

Recently, we have demonstrated that administration of exogenous cytokines can increase resistance to *S. typhimurium*, but only if ity^r mice. For instance, the resistance of ity^r mice, but not that of ity^s mice, can be increased by treatment with interleukin 1 (IL-1) prior to infection or with granulocyte-macrophage colony-stimulating factor (GM-CSF) shortly after infection (11, 13).

In this study, we assessed the effect of IL-1 treatment on the survival of ity^r, endotoxin-hyporesponsive C3H/HeJ mice infected with *S. typhimurium*.

Female C3H/HeJ and C3H/HeN mice were purchased from The Jackson Laboratory (Bar Harbor, Maine) and Charles River (Wilmington, Mass.), respectively. The mice were between 10 and 14 weeks old and maintained in a colony free of mouse hepatitis virus, an agent which has been shown to alter resistance to *S. typhimurium* (5).

Production and purification of recombinant human IL-1α and murine GM-CSF have already been described (7–9, 17, 20). The IL-1α and GM-CSF preparations had between 0.5 and 2 pg of endotoxin per μg of protein. Control mice were injected with mouse serum albumin (MSA).

The *S. typhimurium* strain (ATCC 14028, serogroup B) used in these studies was obtained from the American Type Culture Collection (Rockville, Md.).

To test the effect of IL-1 administration on survival after a lethal infection with *S. typhimurium*, C3H/HeJ (ity^r Lps^d) and C3H/HeN (ity^r Lps^n) mice were injected intraperitoneally (i.p.) with 200 ng of IL-1α either prior to or after infection with a 100% lethal dose of *S. typhimurium* (10 bacteria for C3H/HeJ mice and 2,200 bacteria for C3H/HeN mice). Pretreatment of the mice with IL-1α (one injection approximately 16 h prior to infection) significantly increased the survival of C3H/HeN mice but not that of C3H/HeJ mice (Fig. 1, upper panels). Various pretreatment dosages (0.01 to 10 μg) and lengths of pretreatment (1 to 5 days) were assessed for the ability to increase the resistance of C3H/HeJ mice and were without effect (data not shown). It should be noted that neutralization of IL-1 by combining it with its soluble receptor prior to injection negated the protective effects (data not shown) (4).

Treatment of mice with IL-1α after infection (a single injection administered approximately 1 h after infection on day 0 and twice daily thereafter on days 1 through 5) did not increase the survival fraction or mean survival time (MST) of C3H/HeN mice but significantly increased the MST of C3H/HeJ mice (Fig. 1, lower panels).

The effect of varying the length of the posttreatment regimens on the survival of C3H/HeJ mice was assessed (Table 1). Treatment with one injection of IL-1α on only day 0 (within 1 h after infection) was as effective in prolonging the MST of the mice as continuous treatment for 5 or 10 days. Also, delaying treatment for 1 or 2 days following infection resulted in decreased efficacy.

The possibility that a combination of pre- and postinfection treatments with IL-1 or GM-CSF would be effective in increasing the survival fraction of C3H/HeJ mice was investigated (11). The effects of these treatments are shown in

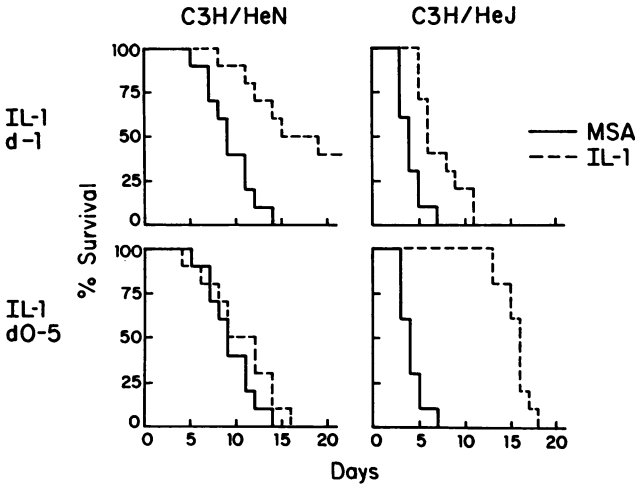

FIG. 1. Effect of IL-1α pretreatment or posttreatment on the survival of C3H/HeN and C3H/HeJ mice after a lethal challenge with *S. typhimurium*. Mice were injected with IL-1α (200 ng i.p.) either 16 h before receiving *S. typhimurium* or on days 0 through 5 after receiving *S. typhimurium*. There were 10 mice per group. The survival fraction of the IL-1α-pretreated C3H/HeN mice is significantly different from that of the controls by the Fisher exact test ($P < 0.01$). The MST of C3H/HeJ mice which received IL-1α postinfection is significantly different by the Student *t* test ($P <$

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TABLE 1. Effect of varying the length of IL-1α treatment on the MST of C3H/HeJ mice after *S. typhimurium* infection

IL-1α treatment[a]	Mean MST ± SEM
None	4.6 ± 1.2
Day 0	12.0 ± 3.9[b]
Days 0, 1	12.1 ± 1.4[c]
Days 0–5	13.4 ± 1.6[c]
Days 0–10	12.4 ± 0.4[c]
Days 1–5	8.7 ± 1.3[b]
Days 2–5	4.8 ± 0.4

[a] IL-1α (200 ng) was administered i.p. once on day 0 within 1 h postinfection and twice daily on the subsequent days indicated. There were 10 mice per group. The mice each received approximately 10 *S. typhimurium* organisms i.p.
[b] $P < 0.05$ in comparison with untreated controls (Student *t* test).
[c] $P < 0.01$ in comparison with untreated controls.

Table 2. In all of the experimental groups, none of the mice survived the infection. Combination of IL-1α pretreatment with IL-1α posttreatment (group D) did not increase the MST of the mice over that of the IL-1α posttreatment alone group. GM-CSF treatment either prior to or after infection did not increase the MST of the mice, either alone or in combination with IL-1α pretreatment (groups E through F).

The possibility that concomitant administration of both IL-1α and GM-CSF were required to increase the survival fraction of C3H/HeJ mice was examined (Table 3). Again, none of the mice in any of the treatment groups survived the infection and the MST was significantly increased only in those groups in which IL-1α was administered after infection.

The effect of IL-1α treatment postinfection on bacterial growth was examined. At various times postinfection, spleens were homogenized and plated to determine the number of bacteria as described previously (11). In Fig. 2, it can be seen that on day 2, the numbers of bacteria in the spleens of IL-1α-treated mice were approximately 10-fold lower than those in the spleens of control mice. Also, in contrast to control mice, bacterial numbers in IL-1α-treated mice increased minimally between days 2 and 5. However, significant increases in the numbers of *S. typhimurium* were seen in IL-1α-treated mice at later time points. Continuation of IL-1α treatment through day 10 did not prevent the increase in the numbers of bacteria from day 7 onward (data not shown). Why continued IL-1 administration did not result in continued bacteriostatic activity is not clear.

In this study, we attempted to overcome the susceptibility of C3H/HeJ mice to *S. typhimurium* by administering IL-1 and/or GM-CSF to the mice. Of all of the treatment combinations studied, only IL-1 administered postinfection significantly increased the MST of C3H/HeJ mice. We have previously shown that pretreatment of *ity*^{r} *Lps*^{n} mice with IL-1 prior to infection significantly increased the survival fraction (12). It is known that the defect due to the *Lps*^{d} allele does not hinder expression of the *ity*^{r} allele (3). Why no beneficial effect of IL-1 pretreatment was observed, especially when combined with cytokine posttreatment, is an enigma.

It is known that macrophages from C3H/HeJ mice are hyporesponsive to endotoxin (10, 19) and this results in a decreased ability to produce IL-1 and tumor necrosis factor alpha (2, 21). Thus, administration of IL-1 postinfection may partially overcome this deficit, resulting in an increased

TABLE 2. Effect of IL-1α or GM-CSF treatment on MST of C3H/HeJ mice after *S. typhimurium* infection

Group	IL-1α treatment		Mean[a] MST ± SEM
	Day −1[b]	Days 0–5[c]	
A	MSA	MSA	4.0 ± 0.4
B	IL-1	MSA	5.1 ± 0.6
C	MSA	IL-1	15.3 ± 1.5[d]
D	IL-1	IL-1	14.2 ± 1.7[d]
E	MSA	GM-CSF	4.0 ± 0.3
F	IL-1	GM-CSF	4.7 ± 0.6
G	GM-CSF	MSA	4.3 ± 0.5
H	GM-CSF	GM-CSF	5.0 ± 0.4

[a] There were nine mice per group.
[b] IL-1α (200 ng) or GM-CSF (500 ng) was administered i.p. 16 h preinjection with approximately 10 *S. typhimurium* organisms.
[c] IL-1α (200 ng) or GM-CSF (500 ng) was administered i.p. within 1 h postinfection on day 0 and twice daily through days 1 and 5.
[d] $P < 0.01$ compared with MSA-treated controls (group A) (Student *t* test).

TABLE 3. Effect of combined IL-1α and GM-CSF treatments on MST of C3H/HeJ mice after *S. typhimurium* infection

Group	IL-1α treatment		Mean[a] MST ± SEM
	Day −1[b]	Days 0–5[c]	
A	MSA	MSA	3.8 ± 0.4
B	MSA	IL-1	15.2 ± 1.4[d]
C	MSA	IL-1–GM-CSF	14.2 ± 0.8[d]
D	IL-1–GM-CSF	MSA	5.9 ± 1.2
E	IL-1–GM-CSF	IL-1	13.9 ± 1.1[d]
F	IL-1–GM-CSF	GM-CSF	4.3 ± 0.5
G	IL-1–GM-CSF	IL-1–GM-CSF	14.9 ± 1.2[d]

[a] There were nine mice per group.
[b] IL-1α (200 ng) and GM-CSF (500 ng) were administered i.p. approximately 16 h prior to i.p. injection with approximately 10 *S. typhimurium* organisms.
[c] IL-1α (200 ng) and/or GM-CSF (500 ng) were administered i.p. within 1 h postinfection on day 0 and twice daily through days 1 and 5.
[d] $P < 0.01$ compared with the MSA-treated control group (Student *t* test).

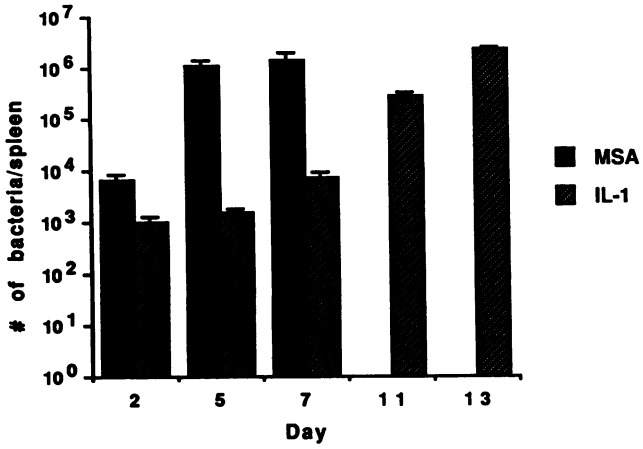

FIG. 2. Effect of IL-1α treatment on the numbers of *S. typhimurium* organisms in the spleens of C3H/HeJ mice. C3H/HeJ mice were injected with approximately 10 bacteria and administered IL-1α (200 ng) or MSA on days 0 through 5. The data are arithmetic averages (± the standard error) of the splenic bacterial numbers of six mice per group per time point. The differences between IL-1-treated and control mice are significant ($P < 0.05$ on day 2; $P < 0.01$ on days 5 and 7).

MST. The inability of IL-1 treatment to increase the survival fraction postinfection with only 10 bacteria suggests that antibacterial mechanisms are not activated optimally and it may be that other cytokines are required. For instance, it has been shown that macrophages from C3H/HeJ mice are deficient in production of tumor necrosis factor alpha in response to stimulation with lipopolysaccharide but that gamma interferon treatment of the macrophages restores tumor necrosis factor alpha production in response to lipopolysaccharide (1, 2, 6). These approaches offer insight into how antibacterial defenses are both activated and regulated and may lead to the development of effective adjunct therapy for bacterial diseases.

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