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NOTES

Exogenous Tumor Necrosis Factor Alpha and Interleukin-1α Increase Resistance to *Salmonella typhimurium*: Efficacy Is Influenced by the *Ity* and *Lps* Loci

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Interleukin-1α (IL-1α) or tumor necrosis factor alpha (TNF-α) administered prior to infection with *Salmonella typhimurium* increases survival in mice that are *Ity^r*, not in susceptible *Lps^d* or *Ity^s* mice. Combined IL-1α and TNF-α pretreatment results in greater survival than that seen with either cytokine alone in *Ity^r* mice. Treatment after infection with TNF-α and/or IL-1α increases the mean time to death but not the survival fraction of *Lps^d* mice and was ineffective in either *Ity^r* or *Ity^s* mice.

The cytokines interleukin-1α (IL-1α) and tumor necrosis factor alpha (TNF-α) are intimately involved in the mechanism of inflammation and the response to infectious agents. For example, it is well established that infectious agents, such as bacteria or bacteria-derived products, stimulate the production of IL-1α and TNF-α by many cell types (reviewed in reference 15). The precise role these cytokines play in host defense is not known, but they have complex effects on immune and hematopoietic functions, as well as on metabolism and thermal regulation (15). Recently, it has been shown that administration of these cytokines can prime the host protective response to radiation, chemotherapeutic agents, and infectious diseases (4, 6, 14). In addition, it appears that these cytokines can synergize their activities to produce a more profound effect in combination than is produced by either one alone (20).

The ability of mice to resist a *Salmonella typhimurium* infection is greatly influenced by defined genetic loci (17). Thus, mice which are homozygous for susceptibility at the *Ity* locus (*Ity^s*) cannot efficiently kill or control the growth of *S. typhimurium* and consequently succumb to small numbers of bacteria (2, 10, 18). Similarly, mice which are hyporesponsive to bacterial lipopolysaccharide (LPS; *Lps^d*) cannot resist infection by small numbers of bacteria (16).

We have recently shown that the resistance of mice to a lethal dose of *S. typhimurium* can be enhanced if the mice are pretreated with IL-1α (13). This effect was dependent on the genetic backgrounds of the mice and was observed only in *Ity^r Lps^n* mice. IL-1α pretreatment had no effect on either *Ity^s Lps^n* mice or *Ity^r Lps^d* mice (12, 13). It is possible that the inability of IL-1α to enhance the resistance of these strains of mice was due to a lack of production of a secondary cytokine. As it has been shown that TNF-α can modulate the resistance of mice to bacterial infections (5), we tested the efficacy of TNF-α administration in this system both alone and in combination with IL-1α. We show that the injection of TNF-α in combination with IL-1α prior to infection results in an increase in host resistance greater than that seen with either cytokine alone, but only in *Ity^r Lps^n* mice. In *Ity^r Lps^d* mice, host resistance was increased only when either IL-1α or TNF-α was administered after infection.

A/J, C57BL/6J, and C3H/HeJ mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). The mice used in these experiments were females between 8 and 12 weeks of age. Mice were maintained in a specific-pathogen-free facility (free of mouse viral pathogens). Purified recombinant human TNF-α (produced in *Escherichia coli*) was the generous gift of Abla Creasey (Cetus Corp., Emeryville, Calif.) (21). Recombinant human IL-1α was produced and purified at Immunex Corp. (Seattle, Wash.) as previously described (9, 11). Cytokines were diluted in a 5-μg/ml solution of mouse serum albumin (MSA) (Sigma Chemical Co., St. Louis, Mo.). Endotoxin levels were routinely monitored in all solutions administered to mice and were less than 10 pg/ml. The following three treatment modalities were assessed: (i) cytokines were administered approximately 16 h prior to infection (day −1), (ii) cytokines were administered shortly after infection (within 1 h) and twice the day following infection approximately 10 h apart (days 0 and 1), and (iii) cytokines were administered the day prior to infection and after infection (combination of modalities 1 and 2; days −1, 0, and 1). Mice were challenged with one 100% lethal dose (LD_{100}) of *S. typhimurium* (ATCC 14028) (approximately 2,000 bacteria for A/J mice and 10 bacteria for C57BL/6J and C3H/HeJ mice). Research was conducted according to the principles enunciated in *Guide for the Care and Use of Laboratory Animals* (7a).

The effects of treatment of A/J and C57BL/6J mice with IL-1α and TNF-α prior to infection are shown in Table 1. It can be seen that neither cytokine alone nor both cytokines in combination increased the survival fraction or mean time to death of *Ity^s* C57BL/6J mice. In contrast, pretreatment of *Ity^r* A/J mice with either cytokine alone resulted in a significant increase in mean time to death and, except for the 1.25-μg dose of TNF-α, in survival fraction. Pretreatment with a combination of IL-1α and TNF-α resulted in a survival rate which was greater than that with each cytokine alone.

We have previously shown that the treatment of LPS-hypo-

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TABLE 1. Effects of IL-1α and TNF-α administration on the survival of A/J (Ity^r) and C57BL/6J (Ity^s) mice challenged with one LD_{100} of *S. typhimurium*

Treatment[a]	C57BL/6J mice			A/J mice		
	n[b]	MTD[c] ± SE (days)	% Survival	n[b]	MTD[c] ± SE (days)	% Survival
MSA	16	9.2 ± 2.5	6.25	16	8.8 ± 0.9	0
IL-1α	16	7.6 ± 2.1	6.25	14	18.2 ± 1.1[d]	50[e]
TNF-α (1.25 μg)	16	6.9 ± 2.4	0	14	14.1 ± 1.1[d]	20
TNF-α (5.0 μg)	16	5.3 ± 1.3	0	15	14.9 ± 0.8[d]	46.7[e]
IL-1α–TNF-α (1.25 μg)	16	7.4 ± 1.6	0	16	19.9 ± 1.1[d]	81.3[e]
IL-1α–TNF-α (5.0 μg)	16	5.3 ± 1.3	0	15	26.0[d,f]	93.3[e,g]

[a] Mice were injected intraperitoneally with MSA (1 μg) or IL-1α (200 ng) with or without TNF-α at the indicated dose approximately 18 h prior to intraperitoneal injection with *S. typhimurium*. C57BL/6J mice were administered approximately 10 bacteria, and A/J mice were administered an average of 2,200 bacteria.
[b] Pool of three experiments.
[c] MTD, mean time to death.
[d] Statistically significant from the results for the MSA control group by the log rank test ($P < 0.05$).
[e] Statistically significant from the results for the MSA control group by Fisher's exact test ($P < 0.05$).
[f] Statistically significant from the results for the IL-1α only group and the TNF-α (5 μg) only group by the log rank test ($P < 0.05$).
[g] Statistically significant from the results for the IL-1α only group and the TNF-α (5 μg) only group by Fisher's exact test ($P < 0.05$).

responsive C3H/HeJ mice with IL-1α prior to infection with *S. typhimurium* did not increase the survival rate or mean time to death (12). This was somewhat unexpected since C3H/HeJ mice are homozygous for the resistance allele at the *Ity* locus (16). Thus, it was of interest to assess the efficacy of TNF-α administration to C3H/HeJ mice either alone or in combination with IL-1α prior to infection with *S. typhimurium*. As shown in Table 2, the administration of TNF-α to C3H/HeJ mice prior to infection did not increase either the mean time to death or the survival fraction (compare the results for group A with those for group E). We have previously shown that IL-1α pretreatment of Lps^n C3H/HeN mice resulted in a significant increase in survival fraction (13). TNF-α pretreatment of C3H/HeJ mice was no more efficacious than IL-1α pretreatment (Table 2; compare the results for group B with those for group E), and pretreatment with the combination of IL-1α and TNF-α was no better than pretreatment with either cytokine alone (compare the results for group H with those for groups E and B). We have previously shown that IL-1α treatment of C3H/HeJ mice after infection increased the mean time to death (12), and similar results are shown in Table 2 for group C. TNF-α administration after infection also resulted in a significant increase in mean time to death (Table 2; group F). However, TNF-α alone was no more effective than IL-1α alone (Table 2; compare the results for groups C and F) or the combination of these cytokines (group I). The combination of pre- and posttreatment was no more effective than posttreatment alone (compare the results for groups D and G with those for groups C and F in Table 2). Finally, administering both cytokines before and after infection (Table 2; group J) resulted only in an increased mean time to death that was not significantly different from the mean time to death resulting from treatment after infection only (group I), and combined treatments (group J) did not result in an increase in the survival fraction.

TABLE 2. Effects of IL-1α and TNF-α treatment on the survival of C3H/HeJ mice challenged with one LD_{100} of *S. typhimurium*

Group (n)[a]	Treatment[b]	Day(s) of administration[c]	MTD[d] ± SE (days)	% Survival
A (28)	MSA	−1, 0, 1	5.4 ± 1.5	0
B (27)	IL-1α	−1	6.3 ± 1.7	0
C (26)	IL-1α	0, 1	13.0 ± 1.9[e]	3.8
D (20)	IL-1α	−1, 0, 1	12.1 ± 2.1[e]	0
E (27)	TNF-α	−1	6.9 ± 1.8	3.7
F (24)	TNF-α	0, 1	13.8 ± 2.2[e]	10.7
G (17)	TNF-α	−1, 0, 1	12.2 ± 3.0[e]	5.8
H (27)	IL-1α–TNF-α	−1	8.3 ± 2.5	0
I (25)	IL-1α–TNF-α	0, 1	14.1 ± 2.4[e]	0
J (21)	IL-1α–TNF-α	−1, 0, 1	12.4 ± 3.1[e]	12.0

[a] Pool of three experiments.
[b] Mice were injected intraperitoneally with control MSA (1 μg), human IL-1α (200 ng), human TNF-α (5 μg), or a combination of IL-1α (200 ng) and TNF-α (5 μg).
[c] Mice were injected intraperitoneally with cytokines approximately 16 h before infection (day −1), they were injected on days 0 and 1, or the schedules were combined (days −1, 0, and 1).
[d] MTD, mean time to death.
[e] Statistically significant from the results for the MSA control (group A) by the log rank test ($P < 0.05$).

In this report, we have demonstrated that in Lps^n Ity^r A/J mice, but not in Lps^d or Ity^s mice, both IL-1α and TNF-α can augment antibacterial resistance when they are administered prior to infection. In addition, this combination of cytokines is more effective than either cytokine alone. This pretreatment regimen did not influence the survival fraction of either Ity^s Lps^n (C57BL/6J) or Ity^r Lps^d (C3H/HeJ) mice. We have previously demonstrated a strong correlation between the efficacy of IL-1α pretreatment and the resistance allele at the *Ity* locus, which included the use of *Ity* congenic pairs of mice (13). The enhanced survival fraction seen with combined IL-1α and TNF-α pretreatment is also observed for the CD.2 Ity^r congenic mouse strain (Ity^r allele from DBA/2 mice bred onto a BALB/c background) but not for the Ity^s BALB/c strain (data not shown). Interestingly, the efficacy of cytokine treatment after infection in increasing the mean time to death is observed only in Lps^d C3H/HeJ mice, not in Lps^n mice regardless of *Ity* phenotype (13). The inability of IL-1α and TNF-α to significantly increase survival to a challenge with small numbers of bacteria (LD_{100} = 10 bacteria) indicates that exogenous administration of these cytokines is unable to correct the defect in macrophage activation and function conferred by the Lps^d gene product. It would be of interest to assess the efficacy of IL-1α and TNF-α treatment in combination with gamma interferon treatment since gamma interferon has been shown to partially correct the defect in macrophage function as a consequence of the Lps^d gene (3, 7, 8). Similarly, neither cytokine

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alone nor both cytokines in combination were able to overcome the defect in host resistance conferred by homozygosity of the *Ity*^{s} allele. It would also be of interest to determine the effect of IL-1 and TNF administration on the expression of Nramp, the gene product of the *Bcg*/*Ity*/*Lsh* locus (1, 19). Thus, it is possible that cytokine pretreatment of *Ity*^{r} mice enhances the early resistance response of macrophages (both cytocidal and cytostatic), which allows the survival of these animals until sterilizing immunity occurs. Any compromise of macrophage antimicrobicidal function, as seen in *Ity*^{s} and *Lps*^{d} strains of mice, abrogates the protective function of cytokine pretreatment.

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