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Protection of Mice against *Listeria monocytogenes* Infection by Recombinant Human Tumor Necrosis Factor Alpha

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Recombinant human tumor necrosis factor alpha (rHuTNF-α) administered intravenously to mice resulted in enhanced resistance to a lethal challenge infection of *Listeria monocytogenes* given 24 h later. The observed protection was lost following treatment of the rHuTNF-α preparations with rabbit polyclonal antibody rHuTNF-α but not with normal rabbit immunoglobulin G.

It has been well established that bacterial lipopolysaccharides (LPSs) exert a multiplicity of biological influences in the host, including stimulation of defense systems to infectious diseases and tumors. Numerous investigators have shown that relatively small amounts of LPS, injected 24 h prior to challenge infection, can protect mice from succumbing to lethal infection (for a review, see reference 14). The mechanisms by which LPS exerts its beneficial effects in the host are complex and incompletely understood, but they are believed to be mediated by the action of cytokines liberated by host cells upon interaction with LPS (18). One of the earliest cytokines produced in response to LPS is tumor necrosis factor (TNF), or cachectin. Studies in vivo have shown that the maximum concentration of TNF in serum is attained within 2 h following a single intravenous injection of LPS into LPS-responsive mice (9) and human volunteers (11). The monocyte or macrophage appears to be the principal cell type responsible for the production and release of TNF (10, 15).

There is ample evidence implicating TNF as a key mediator in the deleterious effects of LPS, i.e., endotoxin shock (for a review, see reference 3). However, there are an increasing number of studies indicating that this monokine is capable of influencing numerous other biological pathways that may ultimately be important in enhancing resistance to infectious disease. Included among the immunomodulatory effects described for TNF are induced alterations in leukocyte recruitment and trafficking in vivo (12, 20), up-regulation of interleukin-2 receptors and cytolytic activity of lymphoid cells (17) and complement receptors on neutrophils (1), priming of neutrophils for oxidative burst activity (2), activation of macrophages in vitro and in vivo (4, 16, 19), priming of macrophages and neutrophils for enhanced killing of intracellular parasites (5), and stimulation of proliferation and differentiation in B cells (8), T cells (23), and hematopoietic progenitor cells (22). There are several recent studies which more closely link TNF with increased resistance to infections. Injection of TNF 1 day prior to experimental peritonitis in mice caused by cecal ligation and puncture provided significant protection from mortality. There was no observed protection when TNF was administered immediately prior to surgery (21).

Since it is known that the production of TNF is an early host response to LPS challenge, it was of interest to determine whether TNF could replace LPS in providing protection against *Listeria* infection. Recombinant human TNF alpha (rHuTNF-α) was produced in *Escherichia coli* and purified by high-pressure liquid chromatography (HPLC)–ion-exchange chromatography, HPLC-hydrophobic interaction chromatography, and HPLC-gel filtration. The purified rHuTNF-α gave a single protein band on a sodium dodecyl sulfate-polyacrylamide gel. The activity of the purified molecule (1.0×10^8 U/mg) was assayed and standardized as described previously (9). Neutralizing polyclonal antibody to purified rHuTNF-α was raised in rabbits by standard procedures. The activity of the antiserum was 2.4×10^7 neutralizing units per ml (9). A total of five separate experiments were conducted in which various concentrations of rHuTNF-α were administered intravenously to mice 24 h prior to lethal infection with *Listeria monocytogenes* A25616 (culture collection of Bristol-Myers Co., Wallingford, Conn.). Outbred, male, Swiss-Webster mice [Crl: CFW(SW)] were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Mice were used when they were 4 to 5 weeks of age (weight, 16 to 18 g). Two different lots of TNF were examined; experiments 1 and 2 were carried out with the first lot, and experiments 3 to 5 were carried out with the second lot. The amount of TNF administered to mice was determined by bioassay at the time of treatment. Control groups of mice were treated with various concentrations of *Salmonella typhosa* LPS (Sigma Chemical Co., St. Louis, Mo.) for comparison. On the day of infection, pretreated mice received approximately 10^5 CFU of *L. monocytogenes* intravenously; the dose corresponded to 4 to 7 50% lethal doses. The effectiveness of each pretreatment was evaluated on the basis of cumulative mortality at 7 days following infection. The protective effect of LPS administered 24 h prior to infection is shown in Table 1. Fifty percent of mice were protected from lethal infection by as little as 33 μg of LPS per kg. The results of pretreatment with rHuTNF-α are summarized in Table 2. In each experiment we observed a dose-dependent protective effect of rHuTNF-α on the survival of mice that were lethally infected with *L. monocytogenes*. The 50% effective dose was calculated to be 4.50 to 4.94 ($\log_{10}$) units per mouse. Incubation of rHuTNF-α with neutralizing polyclonal antibody for 30 min at 37°C immediately prior to its administration to mice abolished the protective effect (Table 3). It is important that all TNF preparations, sera, and reagents were free of endotoxin, as measured by the chromogenic enzyme assay (sensitivity, 0.1 ng/ml), and thus, the observed protection could not have been caused by contaminating LPS.

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TABLE 1. Protective effect of *S. typhosa* LPS against lethal *L. monocytogenes* infection in mice

LPS dose (mg/kg)[a]	No. of survivors/ total no.[b]
0.5	15/15
0.125	11/15
0.031	9/15
0.008	2/15
0.002	0/15
Pyrogen-free saline	0/15

[a] A total of 0.5 ml was administered intraperitoneally prior to intravenous infection.
[b] The 50% effective dose was 0.033 mg/kg, and the 95% confidence interval was 0.019 to 0.57.

Additionally, since protection could be blocked by anti-TNF antibody, the effect of LPS acting through other possible pathways is unlikely.

In our studies, the amount of TNF required to achieve protection against *Listeria* infection was higher than that measured in sera of mice that were injected with an amount of LPS necessary to attain a similar degree of protection. It is possible that endogenous TNF produced in response to LPS may, in actuality, reach substantially higher concentrations in discrete microenvironments within the host. Alternatively, given the short half-life of TNF in vivo (6), relatively high levels of exogenous TNF may have to be injected in order to achieve an effective dose at the appropriate target. As is the case with LPS, there appears to be a window whereby TNF-induced protection is optimal. There was little or no protection observed when LPS or TNF was administered immediately prior to a *Listeria* challenge infection (data not shown). During the course of this study, Havell (7) and Nakane et al. (13) reported that treatment of *L. monocytogenes*-infected mice with antibody to recombinant murine TNF resulted in the enhanced growth of *L. monocytogenes* in vivo, with the subsequent conversion of a sublethal infection into a lethal one. While TNF could not be detected during the course of sublethal infection, those studies suggested a role for TNF in the host defense to infection. Our results provide direct evidence that TNF plays an important role in host defense to listeriosis in mice and suggest that TNF may be a key mediator of some of the beneficial effects associated with LPS.

TABLE 2. Protective effect of rHuTNF-α against lethal *L. monocytogenes* infection in mice

Group no.	Mean TNF administered (U/mouse)	No. of survivors/ total no.
1[a]	1.2×10^5	10/10
	2.6×10^4	1/10
	6.5×10^3	1/10
	1.5×10^3	1/10
	3.7×10^2	1/10
	Control	0/20
2[b]	1.2×10^6	15/15
	3.1×10^5	10/15
	7.6×10^4	7/15
	1.9×10^4	2/15
	4.8×10^3	2/15
	Control	0/30

[a] Two separate experiments. The $\log_{10}$ 50% effective dose was 4.50 ± 0.12.
[b] Three separate experiments. The $\log_{10}$ 50% effective dose was 4.94 ± 0.13.

TABLE 3. In vitro antibody neutralization of the immunostimulatory effect of rHuTNF-α[a]

Antibody source	Active TNF recovered (U/ml)[b]	TNF administered (U/mouse)	No. of survivors/ total no.
Rabbit anti-rHuTNF-α[c]	<2.5	<0.25	1/15
Normal rabbit IgG	1.0×10^7	1.0×10^6	13/15

[a] The starting TNF concentration was 1.2×10^7 U/ml.
[b] Following incubation in vitro for 30 min at 37°C.
[c] A total of 2.4×10^7 neutralizing units per ml (immunoglobulin G [IgG] fraction).

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