Induction of Tumor Necrosis Factor by Legionella pneumophila

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Mice were inoculated with Legionella pneumophila via an intratracheal route to establish an experimental model of infection. Lung lavage fluid obtained from infected mice contained a cytotactic factor identified as tumor necrosis factor (TNF). Peak levels of TNF were produced at about 24 h postinfection and rapidly declined thereafter. Treatment of the mice with dextran sulfate before inoculation with the bacteria resulted in lowered amounts of TNF in the lung lavage fluid, suggesting that macrophages were responsible for production of the cytokine. Furthermore, cultures of adherent lung leukocytes and a macrophage cell line, PU 5-1.8, were stimulated to produce TNF by exposure to Legionella antigens. In addition, adherent lung leukocytes from Legionella-infected mice spontaneously released TNF into the culture supernatant. Inoculation of mice with saline or latex particles failed to induce TNF in vivo, indicating that bacterial antigens or products were the stimulating signals. Since there was no detectable TNF activity in sera at any time after intratracheal inoculation, TNF production appeared to be confined to the site of infection. Pretreatment of PU 5-1.8 cultures with gamma interferon, which was detected in the lung lavage fluid before TNF, resulted in augmented TNF production, suggesting cooperativity may exist between the two cytokines, either in the pathogenicity of the bacterium or in a possible immunomodulatory function of TNF and interferon during infection.

Tumor necrosis factor (TNF) was originally found in the sera of Mycobacterium bovis BCG-infected mice which had been challenged with endotoxin (5). In addition to lipopoly saccharide (LPS), other stimulators can induce TNF, e.g., the streptococcal antigen OK-432 (1), Sendai virus (32), and circulating immune complexes (23). In addition to its antitumor effects in vitro and in vivo (19), TNF has been shown to have immunopotentiating properties. Recently, human TNF was reported to enhance the phagocytic activity in human polymorphonuclear neutrophils (PMN) (16, 24) and to mediate natural cytotoxic cell activity (20). TNF has also been shown to be directly cytolytic for Plasmodium spp. (28) and to stimulate the antiparasitic activity of eosinophils (25). In contrast to its cytostatic and cytolytic effects, TNF has also been shown to stimulate the growth of some cell lines (30) and to mediate the adherence of PMN to endothelial cells (11). TNF has also been identified as cachectin, a protein which is responsible for wasting and weight loss in rodents (2). Some of the activities reported for TNF appear to be synergistically enhanced with gamma interferon (IFN-γ). For example, a cooperative effect between TNF and IFN-γ in their antiproliferative activity on treated cell lines and in enhanced target cell killing of normal and tumor cells in humans has been reported (27). Also, the addition of both IFN-γ and human recombinant TNF to PMN results in higher phagocytic activity and oxidative metabolism than does the addition of either cytokine alone (16, 24). This synergy is possibly due to the induction of TNF receptor expression by IFN-γ (22, 29) and suggests that TNF may participate in the cytokine cascade during an immune response.

Legionella pneumophila is a facultative intracellular bacterium that has been associated with human pulmonary infection (7). Recent studies indicated that cellular immunity plays a major role in resistance to this organism. Whereas the presence of anti-Legionella antibody does not appear to be protective in experimental infections (14), activation of infected macrophages with cytokine-containing fluids enhances intracellular killing of the bacterium (13). We recently reported that Legionella antigens are capable of stimulating the induction of IFNs in mouse spleen cell cultures (4). In the present study, we used an experimental model of infection in which the bacteria were directly inoculated into the lung, and we were able to demonstrate the presence of both IFN-γ and TNF in the lung lavage fluid during the infection. Furthermore, stimulation of adherent lung leukocytes and a macrophagelike cell line with Legionella antigens indicated that macrophages were responsible for the production of TNF.

MATERIALS AND METHODS

Animals and reagents. Inbred BDF1 and C3H/HeJ mice (Jackson Laboratory, Bar Harbor, Maine) were used. The animals were 8 to 10 weeks old at the time of each experiment and were cared for in accordance with standard guidelines. L. pneumophila was a clinical isolate from Tampa General Hospital, Tampa, Fla. L. pneumophila whole-cell vaccine was prepared as described previously (10). Latex particles (diameter, 1.1 μm) and dextran sulfate (DS) were obtained from Sigma Chemical Co., St. Louis, Mo. DS was prepared in pyrogen-free saline at a concentration of 5 mg/ml, and 0.2 ml was injected intraperitoneally 24 h before infection to give a dose of 50 mg/kg of body weight (9).

Intratracheal inoculation. The intratracheal infection protocol was performed as described by Stein-Streilein and Guffee (26). Twenty-five microliters of a bacterial suspension or control fluids was injected directly into the trachea of anesthetized mice.

* Corresponding author.
TABLE 1. Time course of TNF and IFN production in lung lavage fluid of *Legionella*-infected mice

<table>
<thead>
<tr>
<th>Time (h) postinoculation</th>
<th>Concn (U/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF</td>
</tr>
<tr>
<td>0</td>
<td>&lt;4</td>
</tr>
<tr>
<td>3</td>
<td>4/4</td>
</tr>
<tr>
<td>6</td>
<td>16/16 (&lt;4)</td>
</tr>
<tr>
<td>9</td>
<td>16/8</td>
</tr>
<tr>
<td>12</td>
<td>16/8 (&lt;4)</td>
</tr>
<tr>
<td>18</td>
<td>48/56 (&lt;4)</td>
</tr>
<tr>
<td>24</td>
<td>64/66 (&lt;4)</td>
</tr>
<tr>
<td>48</td>
<td>6/4</td>
</tr>
<tr>
<td>72</td>
<td>&lt;4/4 (&lt;4)</td>
</tr>
</tbody>
</table>

a Assays were done in duplicate on specimens from two animals.
b The numbers in parentheses indicate the amount of IFN remaining after neutralization with monoclonal anti-IFN-γ antibodies.
c The numbers in parentheses indicate the amount of TNF activity remaining after neutralization with anti-cachectin/TNF antibodies.

Specimen preparation. Mice were sacrificed by using dry ice-generated CO₂ gas chambers. For TNF and IFN assays of specimens from infected mice, sera and lung lavage fluid were collected. Blood samples were obtained via cardiac puncture and allowed to coagulate. To obtain lung lavage fluid, the animal was tethered as for inoculation and a blunt cannula was inserted through the mouth into the trachea. Complete medium (1 ml) was twice injected into the lung and withdrawn. The fluid was passed through a microfilter (pore size, 0.22 μm; Millipore Corp., Bedford, Mass.) to remove bacteria, cells, and debris. Complete medium consisted of RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) with 10% fetal calf serum (Hyclone, Logan, Utah), penicillin-streptomycin (Sigma), and 5 × 10⁻² M 2-mercaptoethanol (Sigma). Recovery was typically 0.7 to 0.8 ml of fluid per mouse, and the lung lavage fluid was used for assay within 2 days of recovery.

For in vitro induction of TNF, lung leukocytes from uninfected mice were used. Lung tissues were removed from exsanguinated mice and were minced and processed in a Stomacher (Tekmar, Cincinnati, Ohio) to obtain cell suspensions. The suspension was passed through a wire screen to remove large chunks of tissue and then centrifuged over Lympholyte-M (Cedarlane Labs, Westbury, N.Y.) to remove parenchymal cells and debris. The leukocytes were collected from the interface and washed in Hanks balanced salt solution. Cells were used at a concentration of 10⁶ cells per ml of complete medium. Typically, recovery ranged from 0.5 × 10⁶ to 1.5 × 10⁶ cells per mouse.

The murine macrophagelike cell line PU 5-1.8 was cultured in minimal essential medium (GIBCO) with 10% fetal calf serum and antibiotics. For stimulation, cells were diluted to 10⁴/ml of medium, and 1 ml was used per well of a 24-well tissue culture plate (Becton Dickinson Labware, Oxnard, Calif.).

TNF assay. Samples were serially diluted in 96-well flat-bottom microtiter plates. Cells (2.5 × 10⁴) from the cell suspension were added to each well, and the plate was incubated at 37°C in a 5% CO₂ incubator for 24 to 48 h. The monolayer was then microscopically examined for cytolytic activity. The samples were assayed simultaneously on Lm(S) cells, which are TNF sensitive, and Lm(R) cells, which are TNF-resistant. These cells were generously provided by E. A. Carswell (31). Selected samples were neutralized with anti-cachectin antibodies (3), which were a generous gift from B. Beutler, before assay. Titters are expressed as the reciprocal of the dilution of the sample in which 50% of the cells in the monolayer were lysed. A laboratory standard, which was obtained from phorbol myristate acetate-stimulated LuKII cells (31), was included in each assay.

IFN assay. Antiviral activity was determined as described previously (4), using murine L929 cells, which are IFN sensitive but resistant to the cytolytic activity of TNF. The type of IFN that was in the samples was determined by neutralization with monoclonal anti-IFN-γ antibodies generously provided by E. A. Havell, Saranac Lake, N.Y. In these assays, the monoclonal anti-IFN-γ did not interfere with the cytolytic activity of the LuKII laboratory standard on Lm(S) cells, nor did anti-cachectin/TNF antibodies abrogate the antiviral activity of murine recombinant IFN-γ, generously provided by Genentech, Inc., South San Francisco, Calif.).

RESULTS

For the initial experiments, mice were infected with 3 × 10⁶ bacteria per animal, which was equivalent to a 50% lethal dose, by using the intratracheal inoculation route. At the intervals indicated in Table 1, lung lavage fluids were ob-

![FIG. 1. Effect of dose of *L. pneumophila* on induction of TNF in lung lavage fluid of infected mice. Shown is the amount of TNF activity detected in animals infected with live bacteria (●) or inoculated with latex particles or killed *Legionella* cells (○). Lung lavage fluids were obtained 24 h postinoculation. Data are averages for three animals per group.](http://iai.asm.org/)

TABLE 2. Time course of TNF and IFN production in sera of *Legionella*-infected mice

<table>
<thead>
<tr>
<th>Time (h) postinoculation</th>
<th>Concn (U/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF</td>
</tr>
<tr>
<td>0</td>
<td>&lt;4</td>
</tr>
<tr>
<td>3</td>
<td>&lt;4</td>
</tr>
<tr>
<td>6</td>
<td>&lt;4</td>
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<tr>
<td>9</td>
<td>&lt;4</td>
</tr>
<tr>
<td>12</td>
<td>&lt;4</td>
</tr>
<tr>
<td>18</td>
<td>&lt;4</td>
</tr>
<tr>
<td>24</td>
<td>&lt;4</td>
</tr>
<tr>
<td>48</td>
<td>&lt;4</td>
</tr>
<tr>
<td>72</td>
<td>&lt;4</td>
</tr>
</tbody>
</table>

a Assays were done in duplicate with sera from two animals.
b The numbers in parentheses indicate the amount of IFN activity remaining after neutralization with monoclonal anti-IFN-γ antibodies.
TABLE 3. In vitro induction of TNF and IFN in murine lung leukocyte cultures

<table>
<thead>
<tr>
<th>Leukocyte population and treatment</th>
<th>Conc (U/ml) of:</th>
<th></th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>IFN at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPSb</td>
<td>48 (&lt;4)c</td>
<td>64</td>
<td>24</td>
<td>100 (65)c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. pneumophila</td>
<td>16 (&lt;4)</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>65 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adherent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>16 (&lt;4)</td>
<td>16</td>
<td>8</td>
<td>30 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. pneumophila</td>
<td>8 (&lt;4)</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>10 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonadherentc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>65 (10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. pneumophila</td>
<td></td>
<td>&lt;4</td>
<td>30 (&lt;10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Lung leukocytes were cultured at 10⁶ cells per ml. Supernatants were collected at the indicated intervals and assayed for TNF and IFN.

b The LPS concentration was 10 μg/ml.

c The numbers in parentheses indicate remaining activity after neutralization with anti-cachectin/TNF antibodies or monoclonal anti-IFN-γ antibodies.

d Formalin-killed Legionella cells (vaccine) were added at a 10:1 ratio of bacteria to leukocytes.

e Lung leukocytes were adhered for 2 h, and the nonadherent cells were removed and cultured separately.

TABLE 4. Release of TNF into culture supernatants by adherent lung leukocytes from Legionella-infected mice

| Inoculum | L. pneumophila vaccineb | Conc (U/ml) of TNF on day postinoculation:
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>–</td>
<td>&lt;4</td>
</tr>
<tr>
<td>+</td>
<td>8</td>
<td>ND</td>
</tr>
<tr>
<td>Saline</td>
<td>–</td>
<td>&lt;4</td>
</tr>
<tr>
<td>+</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>L. pneumophila</td>
<td>–</td>
<td>12</td>
</tr>
<tr>
<td>+</td>
<td>24</td>
<td>8</td>
</tr>
</tbody>
</table>

* Animals were inoculated with live L. pneumophila (3 x 10⁶ cells) or saline at day 0 and sacrificed at the indicated intervals.

† Adherent lung leukocytes from inoculated mice were cultured with or without Legionella killed-cell vaccine (10:1 ratio of bacteria to cells). Supernatants were collected after 24 h of incubation for TNF assay.

‡ Data represent means for three animals per group.

ND. Not determined.

TNF when much higher numbers of cells (10⁸ per animal) were used.

To determine the cellular source of TNF, lung leukocytes were obtained from normal mice and were used either unseparated or after separation into adherent and nonadherent populations. The cultures were stimulated with Escherichia coli LPS or L. pneumophila killed-cell vaccine, and the supernatants were collected at the indicated times (Table 3). The Legionella vaccine was capable of inducing detectable amounts of TNF in the unseparated leukocytes and in the adherent cell populations. The nonadherent leukocytes did not respond to stimulation by producing TNF, although IFN-γ was produced in these cell cultures. Since E. coli LPS has been reported to induce TNF in macrophage cultures (17), it was included here as a positive control for the induction conditions. As with the Legionella killed-cell vaccine, LPS induced TNF in the unfractonated and adherent cells, although at higher levels, suggesting that macrophages were involved. With both inducers, TNF was present at 24 h after stimulation, remained at about the same level at 48 h, and decreased by 72 h. Furthermore, adherent lung leukocytes obtained from infected mice released TNF into the culture medium (Table 4), and the presence of the bacterial vaccine further stimulated TNF production. Peak spontaneous release of TNF was seen on day 1 after inoculation, correlating with in vivo kinetics (Table 1). No IFN was detected in any of these cultures (data not shown).

To examine the cellular source of TNF in response to stimulation with Legionella antigens, a macrophagelike cell line

TABLE 5. In vitro induction of TNF in macrophagelike cell line PU 5-1.8

| Stimulator (concn) | Conc (U/ml) of TNF at:
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>None</td>
<td>&lt;4</td>
<td>&lt;4</td>
</tr>
<tr>
<td>LPS (10 μg/ml)</td>
<td>64 (&lt;4)b</td>
<td>64</td>
</tr>
<tr>
<td>Legionella vaccinec</td>
<td>12 (&lt;4)</td>
<td>8</td>
</tr>
<tr>
<td>rIFN-γ (100 U/ml)</td>
<td>&lt;4</td>
<td>&lt;4</td>
</tr>
<tr>
<td>IFN + LPS</td>
<td>128 (&lt;4)</td>
<td>96</td>
</tr>
<tr>
<td>IFN + vaccine</td>
<td>32 (&lt;4)</td>
<td>32</td>
</tr>
</tbody>
</table>

* Cells were cultured at 10⁶ cells per ml. Supernatants were collected at the indicated intervals and assayed for TNF.

† The numbers in parentheses indicate remaining activity after neutralization with anti-cachectin/TNF antibodies.

‡ Formalin-killed Legionella cells (vaccine) were added at a 10:1 ratio of bacteria to cells.
line was used. PU 5-1.8 cells have been reported to produce interleukin-1 upon stimulation, are phagocytic, and express Fc receptors (21). These cells were cultured at a concentration of 10^6 cells per ml and were stimulated with LPS or killed Legionella cells. The results (Table 5) were similar to those seen when the lung leukocyte populations were used. This TNF was also neutralized with anti-cachectin antibodies. Pretreatment of the cells with 100 U of recombinant IFN-γ (rIFN-γ) per ml for 1 h before addition of stimulators resulted in augmented levels of TNF.

Since it appeared that macrophages were responsible for TNF production in vitro, the effect of in vivo depletion of macrophage activity was explored. Mice were injected intraperitoneally with 50 mg of DS per kg of body weight 24 h before inoculation. This dose had been reported to specifically suppress macrophage function in a murine listeriosis infection model (8, 9), although the effect of DS treatment on the alveolar macrophages was not explored in this model. The lung lavage fluids were collected at 24 h postinfection and assayed for TNF. The DS-treated mice produced lower levels of TNF than did controls injected with the same number of bacteria (Table 6). This indicated that macrophages were responsible for TNF production in vitro as well as in vivo.

**DISCUSSION**

In this study, live Legionella organisms stimulated the production of TNF and IFN in the lungs of mice during infection. Levels of TNF were maximal at about 24 h after infection and diminished by 48 h. This loss of activity was possibly due to either the proteases present in the normal lung or distribution of the TNF to other parts of the body and indicated that production of TNF occurred early in infection and not continuously during infection. Lethally infected mice succumbed at 3 to 8 days postinoculation, suggesting that TNF was not a direct cause of death since significant TNF activity was not detectable after 48 h. The induction of IFN-γ during the early stages of infection may indicate cooperativity between the two cytokines. Studies using human monocytes have demonstrated that IFN-γ augments the production of TNF (18), and a similar relationship may have occurred in the present study since IFN-γ was detectable 12 to 18 h before TNF. In our study, pretreatment of a macrophagelike cell line with IFN-γ resulted in enhanced TNF production when the cells were stimulated with L. pneumophila or LPS. IFN-γ has also been shown to augment the activities of TNF with regard to cytotoxicity (15), PMN function (24), and antiproliferative effects (27). Additionally, IFN-γ has been shown to induce cellular receptors for TNF on tumor cell lines (22, 29), which may account for the synergism between them.

It is not clear why TNF was not detected in serum, although IFN-γ was present. In the original model of TNF induction, BCG infection of mice is not sufficient to induce TNF and endotoxin is required to detect cytotoxic activity in the serum (5). In the present model, it is possible that TNF was confined to the localized infection in the lung and was not detected in the blood because of low production, local catabolism, or binding to lung cells, endothelial cells, and PMN. It is also possible that there are inhibitors of TNF activity in serum and only under conditions in which overwhelming production of TNF is stimulated is it possible to detect it systemically.

Using lung leukocyte cultures, it was determined that the alveolar cell population was responsible for TNF production in response to Legionella killed-cell vaccine or E. coli LPS. Based on this finding, it is suggested that alveolar macrophages were the responding cell type. The use of the murine macrophagelike cell line PU 5-1.8 further indicated that macrophages were responsible for TNF production when stimulated with Legionella killed-cell vaccine. These cells have been reported to produce TNF in response to LPS (17), and in the present study they responded to killed Legionella cells. It should be noted that the use of killed Legionella bacteria in vitro was done to avoid direct cytotoxicity from live bacteria or their by-products in the assay systems. Although greater numbers of the killed organism were required to induce TNF in vivo, it was apparent that the animals would respond to the vaccine.

Since treatment of mice with DS before infection suppressed the induction of TNF, it is suggested that macrophages were involved in TNF production in vivo. DS was used to specifically interfere with macrophage activity (8, 9, 12). The inability of DS treatment to completely abrogate TNF production may have been due to incomplete neutralization of alveolar macrophages, which are possibly more inaccessible than other macrophage populations, e.g., splenic, liver, and peritoneal cells, which are inactivated by DS treatment (9). It should also be mentioned that increased doses of DS resulted in mortality of the treated animal, indicating that a dose of 50 mg/kg was maximal in our system.

The induction of TNF by E. coli LPS has been well documented (5, 17) and may account for the response to Legionella killed-cell vaccine, which also contains LPS. However, in preliminary studies, infection of C3H/HeJ mice with L. pneumophila resulted in equivalent levels of TNF in the lung lavage fluid (data not shown). This suggests that factors other than LPS (possibly an LPS-associated protein) may be involved in the induction model. In this regard, recent studies indicated that pretreatment of C3H/HeJ macrophages with IFN-γ allows the production of TNF when high concentrations of endotoxin are applied (2). Since killed bacterial cells have been shown to induce IFN-γ in splenocyte cultures from both C3H/HeJ and BDF1 mice (4), IFN-γ may play a role in the induction of TNF.

Based on the present study, it is apparent that TNF is induced in the lungs of Legionella-infected mice early in the infection. It can be speculated that the role of TNF in

**TABLE 6. In vivo induction of TNF and IFN in lung lavage fluid of mice pretreated with DS**

<table>
<thead>
<tr>
<th>Inoculum (amt/animal)</th>
<th>Conc (U/ml) in lavage fluid</th>
<th>Control</th>
<th>DS treated*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF</td>
<td>IFN</td>
<td>TNF</td>
</tr>
<tr>
<td>None</td>
<td>&lt;4</td>
<td>&lt;10</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Saline</td>
<td>&lt;4</td>
<td>&lt;10</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Latex beads (3 × 10⁶)</td>
<td>&lt;4</td>
<td>&lt;10</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Legionella vaccine (3 × 10⁶ cells)</td>
<td>&lt;4</td>
<td>&lt;10</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Legionella vaccine (10⁶ cells)</td>
<td>12</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td>Live Legionella bacteria (3 × 10⁶ cells)</td>
<td>56</td>
<td>65</td>
<td>12</td>
</tr>
</tbody>
</table>

* Lung lavage fluid was obtained 24 h postinoculation and assayed for TNF and IFN activity.
* Data represent means for three animals.
* Mice were injected intraperitoneally with 50 mg of DS per kg of body weight 24 h before inoculation. ND, Not determined.
bacterial infections involves a variety of responses, since this cytokine has been reported to stimulate a diversity of cells. Although the traditional cytostatic and cytolytic effects of TNF may not be relevant in this model, the stimulation of PMN functions (antibody-dependent cell-mediated cytotoxicity and phagocytosis) may help to limit the growth of the bacterium soon after infection. On the other hand, cachectin/TNF has been reported to be toxic to mice (6), and its induction during an infection may contribute to the pathogenicity of the bacterium.

ACKNOWLEDGMENT

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LITERATURE CITED