Role of Gamma Interferon in Induction of Natural Killer Activity by *Legionella pneumophila* In Vitro and in an Experimental Murine Infection Model

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**Legionella pneumophila** has been shown to induce gamma interferon (IFN-γ) both in vitro and in vivo during experimental infections of mice. With complement-mediated serologic depletion of murine splenocytes, the cellular sources of IFN-γ following in vitro stimulation with *L. pneumophila* antigens were Thy-1.2\(^+\), Lyt-2\(^-\), L3T4\(^-\), and asialo-GM1\(^+\), which is consistent with the natural killer (NK) cell phenotype. Additionally, Percoll density discontinuous centrifugation demonstrated that maximal production of IFN coincided with high NK activity in fractions which were enriched for large granular lymphocytes. Furthermore, 18- to 24-h incubation of splenocytes with *L. pneumophila* whole-cell vaccine resulted in augmented NK cytotoxic activity against YAC-1 tumor target cells in a \(^{51}Cr\) release assay. The addition of macrophages to purified large granular lymphocyte populations augmented both IFN-γ production and NK activity, suggesting that antigen is required for optimal responses. In an experimental infection model using an intratracheal inoculation route, NK activity was enhanced in the spleen, peripheral blood, and lung cells of infected mice, with maximal stimulation in the lung leukocytes at the site of infection. The results of the present study indicate that NK cells respond in vivo and in vitro to stimulation by *L. pneumophila* by producing IFN-γ and by increased cytotytic activity.

*Legionella pneumophila* is a facultative gram-negative bacterium that has been identified as the etiologic agent of Legionnaires disease (23). Studies in both humans and guinea pigs have shown that resistance to infections appears to depend to a greater extent on cell-mediated immunity than on humoral immunity. For example, the presence of antilegionella antibodies did not significantly increase the killing of the bacterium by human phagocytes (14, 15), but treatment of monocytes with gamma interferon (IFN-γ) was shown to inhibit the intracellular growth of the bacterium (2). IFNs are known to be antiviral, antitumor, and antiproliferative proteins which also have immunomodulatory properties (30). Recent studies have indicated that IFN-γ is similar, if not identical, to at least one of the macrophage-activating factors (31). The addition of native or recombinant IFN-γ to macrophage cultures resulted in activation of the macrophage, as shown by increased tumor cytosis (8), increased Ia antigen expression (33), and increased Fc receptor numbers (11). IFN has also been shown to upmodulate natural killer (NK) cell activity (34).

NK cells have been identified in normal human and mouse lymphocyte populations as those cells capable of spontaneously lysing various neoplastic cells in vitro, and these cells may be important in tumor surveillance mechanisms and early defense against microbes (26).

IFNs appear to regulate NK activity in at least two ways. First, IFNs can trigger the conversion of a noncytotoxic cell into a cytotoxic cell. This can be monitored by the appearance of Lyt-5 antigen, a differentiation marker, on the surfaces of murine NK cells (26). Second, IFNs can increase the activities of existing NK cells, presumably by accelerating the recycling event, which results in increased numbers of NK-sensitive target cells being lysed. In addition to their responses to IFNs, NK cells are also able to produce both IFN-α and IFN-γ (34). This could result in amplifications of their effects once the NK cells are activated.

NK activity has been shown to be induced in vivo by intraperitoneal injections of live *Listeria monocytogenes* bacteria into mice (13). From day 2 to day 6 postinoculation, high levels of NK activity were detected in the nonadherent populations of peritoneal exudate cells. The ability of nonadherent cells to produce IFN when stimulated in vitro with listerial antigens correlated with the levels of NK activity, although a causal relationship was not established. NK cell activity has also been reported to be modulated by in vivo exposure of mice to *Corynebacterium parvum* (7). Either augmentation or depression of NK activity has been described, depending on the conditions of treatment. NK activity is augmented on day 1 to day 3 in the splenocytes of mice given *C. parvum*, but suppressor cells to NK activity are produced in mice by 5 to 7 days after treatment (24). Human NK activity has also been reported to respond to bacterial stimulation. Incubation of glutaraldehyde-fixed *Salmonella* bacteria with high-density lymphocytes resulted in activation of NK-like cytotoxicity that was independent of IFN (32). However, similar stimulation of low-density lymphocytes (a population enriched for NK cells) with bacteria appeared to involve IFN production (32). The augmentation of NK activity by bacteria and their products could be a direct result of the induction of IFNs or interleukin 2 (IL-2), another potent activator of NK cells (12, 16). Thus, while the mechanisms of NK activation by bacteria have not been clearly determined, it is likely that IFN and IL-2 play important roles in the augmentation of this activity.

In the present study, we identified the source of legionella-stimulated IFN-γ as large granular lymphocytes (LGLs) in murine splenocyte cultures. Furthermore, *L. pneumophila* was found to stimulate NK cytotoxic activity in vitro and in...
the lung, spleen, and peripheral blood leukocytes (PBLs) of intratracheally (i.t.) infected mice.

MATERIALS AND METHODS

Animals and reagents. Inbred female BDF1 mice (Jackson Laboratory, Bar Harbor, Maine) were used for these studies. The animals were 8 to 10 weeks of age at the time of the experiments. The culture medium used for the murine splenocytes was RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.), supplemented with 10% fetal calf serum (Hyclone, Logan, Utah), 2 mM L-glutamine (GIBCO), penicillin, and streptomycin, and 5 \times 10^{-5} M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.), and will be referred to as complete medium. Recombinant human IL-2 was generously provided by Hoffmann-La Roche, Inc., Nutley, N.J. Recombinant murine IFN-γ (specific activity, 1.03 \times 10^6 U/mg) was provided by Genentech Corp., South San Francisco, Calif., and was derived from transfected Escherichia coli cultures.

Preparation of bacteria. L. pneumophila, serogroup 1, was obtained at autopsy from a case of fatal legionellosis at Tampa General Hospital, Tampa, Florida, and was generously provided by Hoffmann-La Roche, Inc., Nutley, N.J. Recombinant murine IFN-γ (specific activity, 1.03 \times 10^6 U/mg) was provided by Genentech Corp., South San Francisco, Calif., and was derived from transfected Escherichia coli cultures.

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Preparation of lung lavage fluid. Lung lavage fluid for the IFN assay was collected as previously described (4). Briefly, 1 ml of warm, complete medium was twice injected into lungs and withdrawn. The fluid was passed through a microfilter ( pore size, 0.22 μm; Millipore Corp., Bedford, Mass.) to remove bacteria, cells, and debris and was either used immediately for the assay or stored at -70°C.

Collection of murine PBLs. Murine PBLs were obtained from both control and infected mice for determination of NK activity. Blood was collected via cardiac puncture by using a heparinized syringe with a 25-gauge needle from mice that were sacrificed in dry-ice chambers. The heparinized syringe was prepared by aspirating small volumes of sterile heparin at 1,000 U/ml into a tuberculin syringe and by then expressing all but the amount which remained in the needle hub. Typically, 0.5 to 0.8 ml of blood could be collected per animal. The blood was then diluted with an equal volume of HBSS and centrifuged over a cushion of Lympholyte-M, as described for the recovery of lung leukocytes. PBLs were collected from the fluid interface, washed twice in HBSS, and suspended in complete medium at 2 \times 10^6 cells per ml for the NK assay.

Serologic depletion studies. To identify the cellular source(s) of legionella-induced IFNs, serologic depletions of splenocyte cultures were performed. T-cell-depleted cultures were obtained by incubating 10^7 whole-spleen cells in 0.1 ml of RPMI 1640 and with 0.05 ml of undiluted anti-Thy-1.2 antibodies for 30 min at 37°C. T cells and LGLs were then lysed by adding complement at a dilution of 1:10 and incubating for an additional 30 min at 37°C. Cells were washed three times in HBSS and suspended in complete medium. T-cell depletion was assessed by measuring mitogenic responses of the residual cells to 5 μg of concanavalin A (Sigma) per ml or 10 μg of E. coli lipopolysaccharide (Sigma) per ml. Cultures were considered to be selectively depleted of T cells if the response to concanavalin A was not above that of cells incubated in medium alone and the response to lipopolysaccharide was not inhibited. Similarly, splenocytes were treated with anti-asialo-GM1 antibodies plus complement to eliminate NK cells (19) or anti-L3T4 antibodies plus complement to eliminate T helper cells (9). The treated populations were assessed for NK activity as described below. Anti-Thy-1.2 antibody was obtained from Cappell Laboratories (West Chester, Pa.), rabbit anti-asialo-GM1 antibodies were obtained from Wako Chemicals (Dallas, Tex.), and anti-L3T4 antibodies were obtained from the culture supernatants of a hybridoma, GK1.5 (9). Rabbit complement, used with the above antibodies to accomplish lysis, was obtained from Cedarlane.

Purification of LGLs. As an alternative method to determine the cellular source(s) of legionella-induced IFNs, separations of the nonadherent splenocytes were performed, in addition to the serologic depletion studies described above. Nonadherent cells recovered from the plastic wells were passed through nylon-wool columns to remove residual macrophages and B cells (17). A 10-ml column containing 1 g of brushed nylon-wool was incubated at 37°C in HBSS plus 1 ml of warm complete medium for 1 h, and 1 ml of warm complete medium were then loaded onto the column. The column was incubated at 37°C for 45 min, and nylon-wool nonadherent (NWNA) cells were then eluted with 25 ml of warm complete medium. The recovery was usually 18 to 23% of the original number of cells applied to the column, and treatment of a sample of the eluted cells with anti-Thy-
1.2 plus complement indicated that 90 to 95% of the eluted cells were positive for Thy-1.2 (i.e., T cells and LGLs).

The NWNA cells either were used at 4 x 10^6 cells per ml for IFN induction or were further purified by Percoll discontinuous density centrifugation. Percoll and complete medium were adjusted to 285 mosM. Each of seven concentrations of Percoll (2 ml), ranging from 38.6 to 70.1%, was layered into a 15-ml conical centrifugation tube (22). NWNA cells (1 ml), at a concentration of 5 x 10^7 cells per ml, were then layered onto the surface of the gradient, which was then centrifuged at 300 x g for 45 min. Cells were removed as separated populations from each of the seven interfaces of the gradient, washed in RPMI 1640, and suspended in complete medium at 2 x 10^6 cells per ml for IFN induction or NK determination. The morphology of the cells in the resulting fractions was determined by staining cyt centrifuged preparations with Wright stain. Typically, 30 to 40% of the cells in fraction 2 (formed between 38.6% Percoll and the next denser concentration) were LGLs, with decreasing numbers of LGLs and a corresponding increase of small lymphocytes throughout the remainder of the fractions.

IFN assay. Antibody activity was measured as described previously (29) by using murine L929 cells, which were challenged with vesicular stomatitis virus. One unit of IFN was calculated as the reciprocal of the dilution of the sample in a well which protected 50% of the cells in the monolayer from the virus-induced cytopathogenic effects. As a positive control, standard mouse IFN-α/β calibrated against a reference reagent, G002-902-026 mouse IFN-α/β, obtained from the National Institute of Allergy and Infectious Disease, was used. The type of IFN that was present in the samples was determined by neutralization with monoclonal anti-mouse IFN-γ antibodies, kindly provided by Edward A. Havell, Trudeau Institute, Saranac Lake, N.Y.

NK cell assay. For NK target cell preparations, 10^9 YAC-1 cells were labeled in 0.1 ml of medium with 0.1 mCi of sodium chromate for 60 min at 37°C. After three washes with medium, 5 x 10^3 51Cr-labeled target cells were incubated with various numbers of effector cells in a final volume of 0.2 ml in round-bottomed, 96-well microtiter plates. Cultures, in triplicate, were incubated at 37°C for 4 h, after which time 0.1 ml of supernatant fluids was harvested from each well. Target cells without effector cells in 0.1 ml of medium were mixed with an equal volume of medium to determine spontaneous 51Cr release, and maximal release was calculated as half of total radioactivity of 0.1 ml of target cells. Percent cytototoxicity was calculated according to the following formula: % specific lysis = ([release (experimental) - release (spontaneous)]/[maximal release - release (spontaneous)]) x 100. NK activity is expressed as lytic units (LU) per 10^6 cells, with LU being the number of effector cells required to lyse 20% of the target cells.

RESULTS

Identification of cellular sources of L. pneumophila-induced IFNs. We previously reported that L. pneumophila induced IFNs both in vitro and in vivo (4, 5). To identify the possible source(s) of IFNs induced by L. pneumophila antigens, both positive and negative cell selection approaches were taken in vitro. First, whole splenocyte suspensions were treated with specific antibody plus complement to eliminate various populations of cells, and the remaining cells were then stimulated with L. pneumophila vaccine in the presence or absence of IL-2. The IL-2 was added to assess whether IFN production might be further enhanced by this cytokine, as previously shown in the induction of IFN-γ by E. coli lipopolysaccharide (3). The supernatant fluids from these treated or untreated cells were assayed for IFN activity. Second, the various populations of cells were physically separated, and the cell preparations enriched for various populations were similarly stimulated with the L. pneumophila antigens.

The results indicated that depletion of whole splenocyte populations with antibodies to Thy-1.2 and asialo-GM1 abrogated the induction of IFN by the vaccine in the presence or absence of IL-2 (Table 1). However, treatment of cultures with antibodies to L3T4, a helper T-cell marker, did not affect IFN production or NK activity. It was also noted that NWNA cells could produce IFN upon stimulation, but for optimal levels to be achieved, the presence of macrophages at concentrations of about 10% of the total lymphoid cells was required. Nylon-wool adherent cells, which are primarily composed of B cells, were not found to produce IFN either in the presence or absence of IL-2 or macrophages (data not shown). The IFN induced appeared to be of the gamma type because monoclonal anti-IFN-γ antibodies completely neutralized all activity in the IFN-containing supernatants.

 Purification of LGLs for IFN induction. Since the previous studies indicated that Thy-1.2-positive, asialo-GM1-positive cells were responsible for the production of IFN-γ upon stimulation with L. pneumophila antigens and since NWNA cells appeared to contain the responsive population of cells, it was thought that NK or NK-like cells were the potential source of IFN-γ. To further purify the LGL subpopulation of lymphocytes, Percoll density gradient separations were performed with the NWNA cells. The purified cell fractions were then stimulated with L. pneumophila vaccine in the presence or absence of IL-2 or macrophages. Figure 1 shows the IFN activity induced by whole-cell vaccine in the various cell populations isolated from the different Percoll fractions. The results demonstrate that IFN production was maximal in cells from fractions 2 to 3, which also correlated with the highest NK activity as measured by the cytolytic activity of freshly isolated cells against YAC-1 tumor target cells. These results indicate that IFN was produced by NK or NK-like cells, since Percoll purification procedures of LGLs substantially increased production and depletion studies.

**TABLE 1. Identification of the cellular source of IFN induced by L. pneumophila antigens**

<table>
<thead>
<tr>
<th>Cell induced and antibody</th>
<th>Amt of IFN (U/ml) induced by*</th>
<th>NK activity (LU/10^6 cells)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine</td>
<td>Vaccine plus IL-2</td>
<td></td>
</tr>
<tr>
<td>Whole spleen</td>
<td>100 ± 10 (&lt;10)</td>
<td>5.0</td>
</tr>
<tr>
<td>Thy-1.2</td>
<td>&lt;10</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Asialo-GM1</td>
<td>&lt;10</td>
<td>&lt;2</td>
</tr>
<tr>
<td>L3T4</td>
<td>100 ± 10 (&lt;10)</td>
<td>4.4</td>
</tr>
<tr>
<td>NWNA</td>
<td>20 ± 5 (&lt;10)</td>
<td>9.0</td>
</tr>
<tr>
<td>Macrophage</td>
<td>&lt;10</td>
<td>ND</td>
</tr>
<tr>
<td>NWNA plus Macrophage</td>
<td>100 ± 10 (&lt;10)</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Cultures were incubated alone or with IL-2 (20 U/ml) and stimulated with legionella whole-cell vaccine (10^6 cells per ml) for 24 h. Under these conditions, IL-2 alone did not induce detectable amounts of IFN. Numbers represent the mean IFN activity ± the standard error of the mean, and numbers in parentheses indicate residual IFN activity after neutralization with anti-murine IFN-γ monoclonal antibodies.

* The NK activity of freshly isolated cells is expressed as LU/10^6 cells required to lyse 20% of target YAC-1 cells. ND, Not done.

* Splenocytes were treated with the indicated antibodies plus complement.
FIG. 1. Effect of macrophages and IL-2 on production of IFN by cells isolated from Percoll density gradient fractions upon stimulation with whole-cell vaccine. Whole splenocyte cultures and purified Percoll fractions were incubated in medium alone (stippled areas) or in the presence of IL-2 (20 U/ml) (represented by total bar height) and stimulated with $10^6$ killed cells per ml. Lymphocytes were also incubated in the absence (-) or presence (+) of 10% macrophages in the culture. The media were harvested after 24 h of incubation and assayed for IFN activity. Bar height represents the mean from three separate experiments, and brackets indicate the standard error of the mean. The NK activities of whole-spleen cells and cells freshly isolated from the fractions were 4.2, 9.0, 29.1, 5.0, and 0.0 LU/10⁶ cells, respectively.

using antibodies to asialo-GM1 totally eliminated the cells responsible for IFN production. The bacterial vaccine consistently stimulated the induction of IFN, and the presence of macrophages and IL-2 augmented the levels produced.

In vitro induction of NK activity by *L. pneumophila*. Since the studies described above showed that NK or NK-like cells were responsible for the production of IFN-γ after stimulation with *L. pneumophila* antigens, the possibility that *L. pneumophila* could activate NK activity was examined. For these experiments, NWNA cells or cells purified from Percoll fractions were stimulated in vitro with *L. pneumophila* vaccine for 24 h. The splenocytes were harvested, washed in fresh medium, and assessed for NK activity in a 4-h $^{51}$Cr release assay using YAC-1 tumor cells as targets. As with the experiments on the induction of IFN by *L. pneumophila* components, the effects of exogenous IL-2 and macrophages on the generation of NK activity were explored. The results are shown in Fig. 2. The basal level of cytolytic activity of murine spleen cells which were incubated for 24 h in medium alone was always less than 2 LU/10⁶ cells. As with the production of IFN, the maximal *L. pneumophila*-induced NK activity was found in fraction 3, although the addition of macrophages to the mixture did not appear to be as important as it was for IFN production. IL-2 could activate NK activity alone, and the levels of NK activity seen with the combination of vaccine and IL-2 appeared to be additive rather than synergistic. However, there was a correlation between the cellular source of IFN-γ and *L. pneumophila*-activated NK activity, suggesting that similar cells responded to the bacterial signals. Additionally, treatment of NWNA cells with anti-asialo-GM1 antibodies abrogated both IFN induction by *L. pneumophila* components (Table 1) and activation of NK activity by either the bacterial antigens or IL-2 (data not shown).

In vivo induction of IFN-γ and NK activity by *L. pneumophila*. We previously reported that IFN-γ was found in the circulation and at the site of local inoculation during *L. pneumophila* infection of mice (4). Since NK cells were activated by *L. pneumophila* vaccine in vitro, the induction of NK activity during the course of in vivo infection was also examined. The results in Table 2 show the in vivo kinetics of IFN production and NK activity in the circulation and at the local site of infection during i.t. inoculation of mice. The kinetics of IFN production in both the lung lavage fluid and serum was similar to that reported previously (4), and increased levels of IFN appeared to correlate with augmented NK activity of PBLs and lung leukocytes.

<table>
<thead>
<tr>
<th>Time (h) postinoculation</th>
<th>NK activity (LU/10⁶ cells)</th>
<th>IFN production (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung²</td>
<td>Blood³</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>0</td>
<td>65</td>
<td>&lt;10</td>
</tr>
<tr>
<td>24</td>
<td>117</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>48</td>
<td>358</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>72</td>
<td>164</td>
<td>&lt;10</td>
</tr>
<tr>
<td>120</td>
<td>130</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* Mice were inoculated i.t. with $3 \times 10^6$ bacteria per animal (sublethal dose).

² At the indicated times postinoculation, lung lavage fluid was obtained for the IFN assay and lung leukocytes were obtained from separate animals for the assay for NK activity. Each result of NK activity is from a representative of four experiments that were performed. IFN activity is expressed as the mean ± the standard error of the mean.

³ Sera were obtained for the IFN assay at the indicated times, and PBLs were collected from heparinized blood from separate animals for the assay for NK activity.
To further assess the effect of i.t. infection by *L. pneumophila* on NK activity, three populations of lymphoid cells were assayed: spleen cells, PBLs, and lung leukocytes. The effect of an approximate lethal (10^7 bacteria per animal) versus sublethal (3 x 10^6 bacteria per animal) infection on NK activity was also investigated. Figure 3 illustrates the effect of *L. pneumophila* infection on NK activity in the spleen cells of lethally and sublethally infected animals. The injection of pyrogen-free saline had no significant effect on NK activity, while lethally infected mice were suppressed on days 2 and 3. In contrast, sublethally infected animals were found to have augmented NK activity on days 1 and 2, with levels returning to normal by day 3. The NK activity in PBLs shows a slightly different pattern (Fig. 4). Lethally infected animals demonstrated augmented activity at days 1 and 2, with levels then falling. In sublethally infected animals, NK activity reached 300% of the level in uninfected controls and was maximal at 2 days postinoculation. The augmentation in the lung leukocytes of sublethally infected mice at day 2 postinoculation was even more pronounced than in PBLs of the same mice (Fig. 5), with maximal levels reaching 550% of the levels in control mice. The lung leukocytes of lethally infected mice also demonstrated augmented NK activity until day 3, with levels then falling. It should be noted that mice begin to succumb to lethal infection by legionella by day 3 postinoculation and were therefore unavailable for the NK assay beyond that point.

**DISCUSSION**

We previously reported that legionella could induce the production of IFN-γ both in vitro (5) and in vivo (4). In this study, we extended our investigation to show that the asialo-GM1+ or NK-like cells are the source of IFN-γ in mouse splenocyte cultures. Additionally, *L. pneumophila* antigens stimulated NK activity in the same Percoll-fractionated cells that produced IFN. It was noted that the presence of macrophages or IL-2 resulted in maximal production of both IFN-γ and NK activity. While NK cell activation is not macrophage dependent, the enhanced NK activity in the presence of macrophages here may be mediated by IFN-γ in an autostimulatory fashion.

During in vivo infection of mice by *L. pneumophila*, there also appeared to be augmentation of NK activity, with the largest changes found in the lung, which is the site of inoculation. There also appeared to be a mobilization of NK cells in the lungs of infected mice. For example, in addition to the augmented cytolytic activity present, the total number of leukocytes recovered from infected lungs was 20- to 25-fold higher than that recovered from uninfected mice (data not shown). The accumulation of LGLs has also been reported in the lungs of virally infected mice in response to a chemotactic stimulus (25). LGLs are also chemotactically attracted to f-met-leu-phe (25), a bacterial product which is likely present during infection by legionella.

The results of these experiments indicate that *L. pneumophila* antigens stimulate NK cell function in at least two ways. These cells could respond to bacterial stimulation by producing IFN-γ both in vivo and in vitro. In addition, the
tumorcidal activity of NK cells was augmented after an 18-h coculture of splenocytes in vitro or during in vivo infection of i.t. inoculated mice.

The role of NK cells during bacterial infections has not been fully determined. While their ability to lyse tumor or virally-infected cells is well documented (26, 34), their effect on bacteria is less clear. In this regard, a recent report has demonstrated that LGLs can directly phagocytize and kill gram-positive microorganisms (1). More characteristic of NK function is the lysis of Shigella-infected HeLa tissue culture cells by NK-like cells, described by Klimpel et al. (21). Similarly, we have recently demonstrated the lysis of legionella-infected human monocytes by NK or activated killer cells (6), which may serve to limit the course of human infection.

In the present model, however, BDF1 macrophages are unable to support the intracellular growth of L. pneumophila (Y. Yamamoto, T. W. Klein, C. A. Newton, and H. Friedman, in T. K. Eisenstein, W. Bullock, and N. Hanna, ed., Host Responses and Immunomodulation to Intracellular Pathogens, in press), indicating that NK-cell-mediated lysis of infected murine macrophages is not applicable in the present model. Thus, the effect of activation and apparent mobilization of NK activity in the lungs of infected animals must lie elsewhere. One possible role of LGLs in the present model is to serve as a source of cytokines, e.g., IL-1, IL-2, colony-stimulating factor (18), tumor necrosis factor (10), as well as IFN-α and IFN-γ (34). These cytokines may then serve a variety of functions. For example, IFN and tumor necrosis factor both activate the phagocytic capacity and oxidative burst of polymorphonuclear leukocytes (20, 27), and IFN-γ has been shown to have macrophage-activating functions (31).

The results of this study suggest a myriad of responses which may result after stimulation of NK cells by L. pneumophila bacteria. Whether augmented cytotoxic activity which occurs as a consequence of bacterial infections has a direct effect on the pathogenesis of the disease is yet to be determined. However, the production of IFN-γ could lead to augmented bactericidal capacity of both macrophages and polymorphonuclear leukocytes, thereby accelerating the clearance of the bacteria from the host. Thus, the responses of NK cells to bacterial stimulation may vary from direct lysis of bacterially infected cells (6, 21) to augmentation of macrophage and polymorphonuclear leukocyte bactericidal properties via the production of soluble factors such as IFNs and tumor necrosis factor.

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LITERATURE CITED


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