INFECTION AND IMMUNITY, Nov. 1980, p. 578-587
0019-9567/80/11-0578/10$02.00/0

Vol. 30, No. 2

Interaction of Alveolar Macrophages with *Nocardia asteroides*: Immunological Enhancement of Phagocytosis, Phagosome-Lysosome Fusion, and Microbicidal Activity

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Normal and specifically activated rabbit alveolar macrophages were infected in vitro with *Nocardia asteroides* GUH-2. In the presence of serum from normal rabbits, no significant differences were noted between normal and activated alveolar macrophages with respect to phagocytosis, incidence of phagosome-lysosome fusion, or nocardicidal activity. However, all of these macrophage functions were enhanced by various immunological components. Serum from immunized rabbits enhanced phagocytosis of nocardial cells by activated macrophages, and there was an additional increase in phagocytosis observed when alveolar lining material was present. Complement had no effect on the ability of the macrophages to phagocytize nocardial cells. The greatest percentage of organisms phagocytized was observed when specifically primed lymph node cells, alveolar lining material, and serum from immunized rabbits were present in the incubation medium. *N. asteroides* GUH-2 inhibited phagosome-lysosome fusion in normal macrophages in the presence of serum from normal rabbits. However, addition of serum from immunized rabbits or the addition of specifically primed lymphocytes increased the amount of phagosome-lysosome fusion, whereas complement had no effect on this fusion process. Nocardial viability was not reduced when either normal or activated macrophages were infected with bacteria in the presence of normal serum, immune serum, or alveolar lining material. However, specifically activated macrophages incubated with primed lymph node cells obtained from immunized rabbits were able to both decrease the number of viable organisms recovered and to increase the incidence and extent of bacterial cell damage. The greatest number of organisms were killed by specifically activated macrophages when the bacterial cells were incubated with primed lymph node cells suspended in immune serum and alveolar lining material. These results indicate that activated macrophages alone are not sufficient to kill ingested *N. asteroides* GUH-2 and that specifically primed lymphocytes are important in host resistance to nocardial infections.

Although alveolar macrophages constitute one of the major components involved in antimicrobial defense of the lung, additional humoral and cellular factors present in the lung can modulate the antimicrobial activities of these cells. For example, alveolar lining material has been shown to enhance the bactericidal activity of alveolar macrophages against *Staphylococcus* (28, 32) and *Pseudomonas* (46). Additionally, both locally synthesized and serum-derived immunoglobulins are present within the lung (49, 50). These antibodies have been shown to enhance phagocytosis of organisms such as *Pseudomonas* (44) and *Mycoplasma* by alveolar macrophages (16, 42). In addition to these humoral factors, cell-mediated immunity has been demonstrated in the lung (11, 25, 38), and it has been observed that factors derived from T cells may be important in the antimicrobial activity of alveolar macrophages against such intracellular parasites as *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella typhimurium*, and *Brucella* (35, 51). Thus, in evaluating host defenses against infectious agents encountered by the pulmonary route, both humoral and cellular factors must be considered.

Previous work in this laboratory has been directed toward understanding the host response to *Nocardia*. Earlier studies have established that the majority of pulmonary infections in humans due to *Nocardia* are caused by the common soil organism *Nocardia asteroides* (7). This organism is often responsible for fatal infection in immunocompromised patients, but severe infection in noncompromised individuals also occurs (7, 30, 41). Both humoral antibodies and delayed-type hypersensitivity to nocardial antigens have been demonstrated in individuals

systemically infected with *Nocardia* (4). Although it has been suggested that antibodies may be important in host resistance to some strains of *Nocardia* (6, 9), attempts to transfer humoral immunity in animals have been unsuccessful (29). On the other hand, since it has been shown that *N. asteroides* can grow as a facultative intracellular parasite within alveolar macrophages (5, 6, 9), it seems likely that cell-mediated immunity may be of major importance in host resistance to the more virulent strains of *Nocardia* (8). Studies indicating an enhanced susceptibility to *Nocardia* in athymic mice, relative to their heterozygous littermates and normal mice, have suggested the importance of T cells in resistance to systemic nocardial infection (8, 18). Other studies with both mice (17, 29) and rabbits (6) have suggested the importance of activated macrophages. However, the relative importance of cellular and humoral immunity to resistance to systemic and pulmonary nocardial infections is still not clearly defined. In the present study we have undertaken to assess the effect of both cellular and noncellular immunological components on the interaction of rabbit alveolar macrophages with *N. asteroides*. Our results suggest that both may in fact play a role in host resistance to *N. asteroides*.

MATERIALS AND METHODS

Preparation of bacteria. *N. asteroides* GUH-2, originally isolated from a fatal human infection, was maintained as previously described (10). Single-cell suspensions were prepared from early stationary-phase cultures grown in brain heart infusion broth (Difco) and quantitated as described elsewhere (9).

Specific immunological priming of rabbits. Female New Zealand White rabbits (Herbert's Rabbitry, Plymouth, Calif.) weighing 2 to 3 kg were injected subcutaneously in the dorsal neck region with a saline suspension of 4% formaldehyde-killed *N. asteroides* GUH-2 as previously described (10). Six days before sacrifice, the rabbits again were injected in the dorsal neck region and also in both hind footpads specifically to prime the popliteal lymph nodes. The cold agglutinating antibody titer of the serum at the time of sacrifice was 1:256. The specificity of the antibody was assessed by indirect immunofluorescence labeling. Complement activity was assessed by incubation of non-heat-inactivated serum with hemolysin-sensitized sheep erythrocytes. The use of either autologous or pooled immune serum did not alter experimental results.

Preparation, characterization, and infection of alveolar macrophages. Alveolar macrophages were obtained by lavage from either normal or primed rabbits by a modification of the technique of Myrvik et al. (39) and maintained as previously described (10). The cell population was assessed microscopically after an aliquot of the lavage fluid had been cytocentrifuged and stained with Wright stain. Macrophages were allowed to adhere to sterile cover slips for 3 h and were then washed and subsequently infected as previously described (6, 10). The viability of the infecting organisms after incubation with alveolar macrophages was assessed by bacterial plate counts as previously described (6, 10). Both the percentage of organisms recovered and the percentage of organisms phagocytized were calculated based on a comparison of experimental values with control cultures incubated without macrophages.

For each experiment, comparable numbers of organisms were used to infect the alveolar macrophages for the 3-h time period. In vitro infection of alveolar macrophages from a single rabbit per experiment permitted us to concurrently assess the relative effect of the various immunological parameters examined. These experiments were repeated three times with similar results.

Macrophage and lymphocyte activation. The state of activation of macrophages from specifically primed animals was assessed on the basis of their ability to kill *L. monocytogenes* (34). Macrophages were considered activated if they were able to kill greater than 50% of the listeriae after a 3-h incubation.

The production of migration inhibition factor by sensitized lymphocytes present in the initial lung lavage fluid was demonstrated by a modification of the agarose gel technique (12) in which antigen was incubated with both the lymphocytes and macrophages present in the lavage fluid. Macrophages present in the lung lavage fluid incubated with nocardial antigen did not migrate from the well. However, macrophages present in the control wells without antigen migrated under the surrounding gel by 10 h.

Preparation of alveolar lining material. After the alveolar macrophages were collected and pelleted, the supernatant from all but the initial lavage fluid was centrifuged at 47,000 × *g* for 20 min at 4°C (31). The recovered pellet was then resuspended in 12 ml of supernatant fluid from the initial lavage. This suspension was then assayed for protein (33) and carbohydrate (26) concentration, the latter being a more satisfactory method of quantitation for this glycolipid-rich component. For the various experiments, the protein was found to vary between 1 and 6 mg/ml, whereas the carbohydrate concentration was relatively constant at about 1.4 mg/ml. The final concentration used in the inoculation medium was 0.1 mg/ml with respect to the carbohydrate.

Preparation of spleen and lymph node cells. The spleen and the primed popliteal lymph nodes were removed, and single-cell suspensions were prepared by aseptically mincing the tissues and washing the released cells through a fine mesh screen with Medium 199 (GIBCO Laboratories, Grand Island, N.Y.). Phagocytic cells were removed with a magnet after incubation with iron carbonyl for 30 min at 37°C, and the suspensions subsequently were enriched for lymphocytes by centrifugation over lymphocyte separation medium (19). The suspension was then washed and counted. The concentration of lymphocytes was adjusted to provide a 1:1 ratio of lymphocytes to macrophages in the culture.

Dark-field fluorescence microscopy. After attachment to sterile cover slips, macrophages were rinsed and incubated for 10 min at 37°C with the

lysosomotropic fluorochrome acridine orange at a final concentration of 5 μg/ml in Hanks balanced salt solution (24). The macrophages were then rinsed thoroughly, infected for 3 h, and processed as previously described (14). Macrophage phagosomes containing nocardial cells that did not fuse with lysosomes were not labeled and thus appeared as dark filaments. In contrast, phagosomes with which fusion of lysosomes had occurred were bright orange and thus were easily identifiable. Random fields were selected, and both the total number of infected macrophages and the number of macrophages with acridine orange-labeled phagosomes were counted and tabulated. The total number of macrophages evaluated by this procedure for each immunological parameter was between 400 and 500. The results presented are based on a total evaluation of 3,800 macrophages.

Electron microscopy. For acid phosphatase after 3 h of infection the macrophages were rinsed and the lysosomes were labeled with lead phosphate by an adaptation of the technique of Gomori and co-workers (23). The cells were then processed as previously described (14).

For horseradish peroxidase, after attachment the macrophages were rinsed and incubated with 5 mg of horseradish peroxidase per ml (type II, Sigma) for 2.5 h at 37°C in Medium 199 containing 40% fetal calf serum (47). The cells were then thoroughly rinsed and incubated in medium without peroxidase for 30 min. Subsequently, the labeled macrophages were infected for 3 h, rinsed, and processed by the technique of Graham and Karnovsky (21) except that a pH 6.0 buffer was used (3). The macrophages were then postfixed overnight in 1.0% osmium tetroxide and processed as previously described (14).

For the assessment of nocardial damage, the organisms were considered to be damaged if there was any electron microscopic appearance of abnormality such as breaks in cell wall or membrane, disorganization of cytoplasm, or disorganization of the nuclear region.

For the assessment of fusion, since both of the electron microscopic techniques used labeled the macrophage lysosomes with an electron-dense substance, lysosomal fusion was identified by the presence of the dark electron-dense label within the phagolysosome. To statistically assess phagosome-lysosome fusion and morphological damage to the organisms, we examined 50 profiles of infected macrophages for each immunological parameter examined in each experiment. All experiments were repeated on three separate rabbits. The structural integrity of the ingested organisms was evaluated, and the occurrence or absence of fusion was recorded. The total number of profiles of phagocytized nocardial cells evaluated by electron microscopy was between 400 and 600 for each immunological parameter. Thus, a total of 1,200 profiles of infected macrophages and a total of almost 4,000 profiles of phagocytized nocardial cells were evaluated during this study. In all experiments, care was taken to avoid serial sections.

RESULTS

Enhancement of phagocytosis. The percent phagocytosis for both normal and specifically activated alveolar macrophages from primed rabbits was calculated from the percentage of organisms recovered from macrophage lysates and infecting medium supernatants after a 3-h infection. Activation of macrophages was demonstrated by listericidal activity (34). Specificity of activation was indicated by inhibition of macrophage migration when nocardial antigen was incubated with the lavaged cells (12). The results presented in Fig. 1 indicate that normal rabbit alveolar macrophages incubated with *N. asteroides* strain GUH-2 in 20% pooled normal rabbit serum phagocytized 41 ± 5% of the organisms present in the inoculum. Activated alveolar macrophages incubated with nocardial cells in 20% normal serum characteristically exhibited a slight increase in phagocytosis. There was an additional enhancement of phagocytosis when activated macrophages were incubated with cells in 20% pooled heat-inactivated immune rabbit serum with an agglutinating antibody titer of 1:256. The use of immune serum with demonstrated complement activity did not further enhance phagocytosis. However, when activated macrophages were incubated with nocardial cells in 20% immune serum containing alveolar lining material at a final carbohydrate concentration of 0.1 mg/ml, there was a marked increase in phagocytosis. This enhancement of

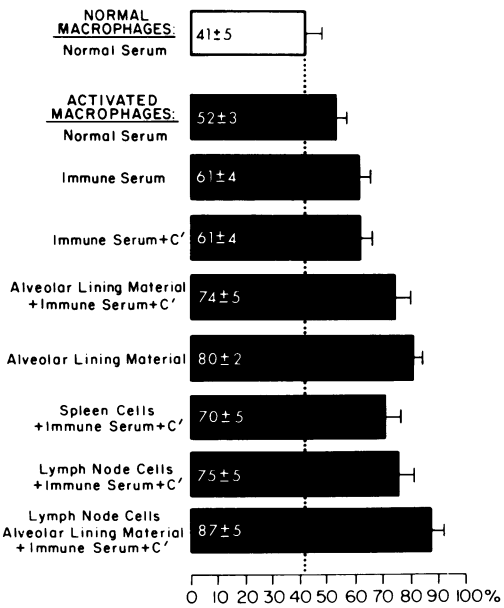


FIG. 1. *Effect of cellular and noncellular immunological components on the enhancement of phagocytosis. Percent phagocytosis was assessed by bacterial plate counts of infected macrophage lysates and infecting medium supernatants. Error bars indicate the standard deviation. C′, Complement activity.*

phagocytosis was independent of the protein concentration within the alveolar lining material. Incubation of macrophages with organisms and alveolar lining material in the absence of immune serum still had an effect on enhancement of phagocytosis. When the incubation medium included autologous spleen cells in addition to immune serum with complement activity, the percent phagocytosis was 70 ± 5%, a 10% increase compared with the incubation medium with immune serum but without spleen cells. The addition of specifically primed autologous lymph node cells to the incubation medium resulted in an increased uptake of nocardial cells. Quite dramatically, phagocytosis by activated macrophages was enhanced to 87 ± 5% when the incubation medium included immune serum with complement, primed lymph node cells, and alveolar lining material (Fig. 1).

Enhancement of phagosome-lysosome fusion. Our previous studies have established that the virulent *N. asteroides* GUH-2 inhibits phagosome-lysosome fusion in alveolar macrophages (14). Therefore, we examined the ability of various immunological components to prevent this inhibition of fusion. Results obtained from dark-field fluorescent microscopy of acridine orange-labeled alveolar macrophages are presented in Fig. 2. When the incubation medium contained 20% normal serum, about 20% of both the normal and the activated macrophages infected with nocardial cells had evidence of phagosome-lysosome fusion. The addition of either heat-inactivated or non-heat-inactivated immune serum, alveolar lining material, or spleen cells to the incubation medium increased the number of infected macrophages with evidence of fusion (Fig. 2). A greater enhancement of phagosome fusion was brought about by the addition of specifically primed lymph node cells to the incubation medium in the presence of immune serum containing complement activity. The inclusion of alveolar lining material with the lymph node cells and immune serum increased the level of fusion to greater than 60% (Fig. 2).

Evaluation of infected macrophages either prelabeled with horseradish peroxidase or labeled for lysosomal acid phosphatase activity permitted electron microscopic assessment of nocardia-containing phagosomes with evidence of lysosomal fusion. The results presented in Fig. 3 indicate that about 30% of the phagosomes in both normal and activated macrophages infected with nocardial cells and incubated with 20% normal serum had evidence of lysosomal fusion. Heat-inactivated immune serum increased the number of phagosomes with evidence of fusion; however, no further enhancement was observed when either non-heat-inactivated serum, alveolar lining material, or spleen cells were included in the incubation medium. Furthermore, incubation of macrophages with nocardiae and alveolar lining material at the same concentration without immune serum did not enhance phagosome-lysosome fusion above the level observed with normal serum. The addition of primed lymph node cells to the incubation medium in the presence of non-heat-inactivated immune serum increased the incidence of phagosome-lysosome fusion to 70%, whereas the addition of alveolar lining material to the incubation medium with lymph node cells and immune serum further increased the incidence of fusion to more than 80% (Fig. 3).

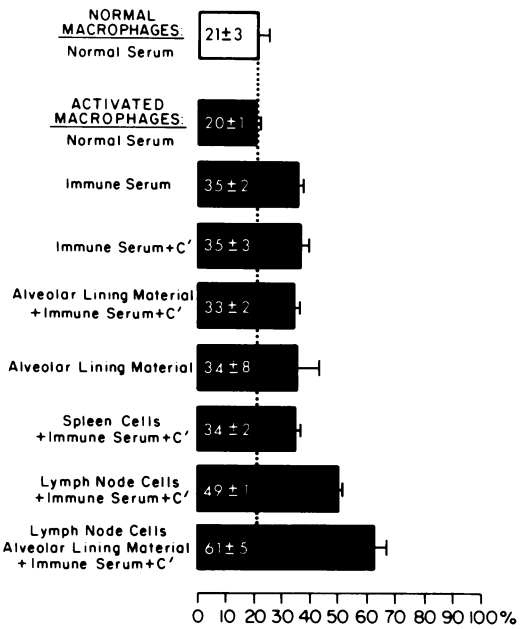


FIG. 2. *Effect of cellular and noncellular immunological components on the percentage of infected macrophages with evidence of phagosome-lysosome fusion. Fusion was assessed by acridine orange fluorescent microscopy of prelabeled infected macrophages. The total number of macrophages evaluated for each immunological parameter was between 400 and 500. Thus, the results presented are based on the evaluation of 3,950 macrophages. Error bars indicate the standard deviation of three experimental determinations. C', Complement activity.*

Representative electron micrographs are presented in Fig. 4. Figure 4A illustrates what was usually observed when either normal or activated alveolar macrophages were infected with this strain of *N. asteroides* in the presence of 20% normal serum. The lack of the electron-dense lysosomal label within the phagocytic vacuole indicates the lack of phagosome-lysosome

fusion. In addition, a prominant, granular zone was frequently observed surrounding the phagosomes containing organisms which had no evidence of lysosomal fusion. Figure 4B illustrates the occurrence of phagosome-lysosome fusion that was observed when activated alveolar macrophages were infected and incubated in the presence of 20% immune serum with complement, alveolar lining material, and specifically primed lymph node lymphocytes (compare Fig. 4A with 4B).

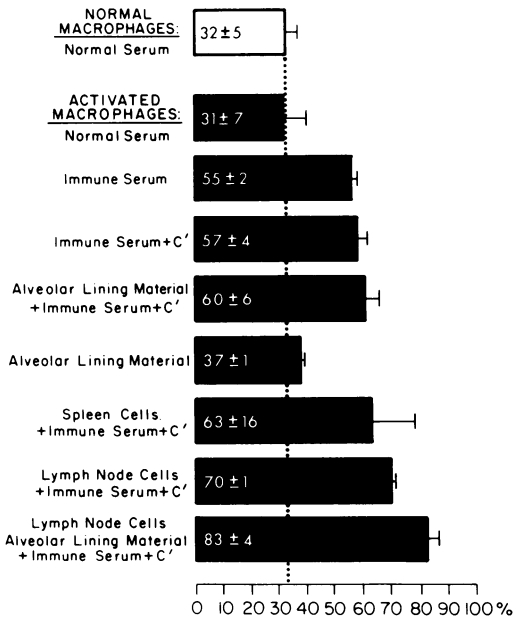


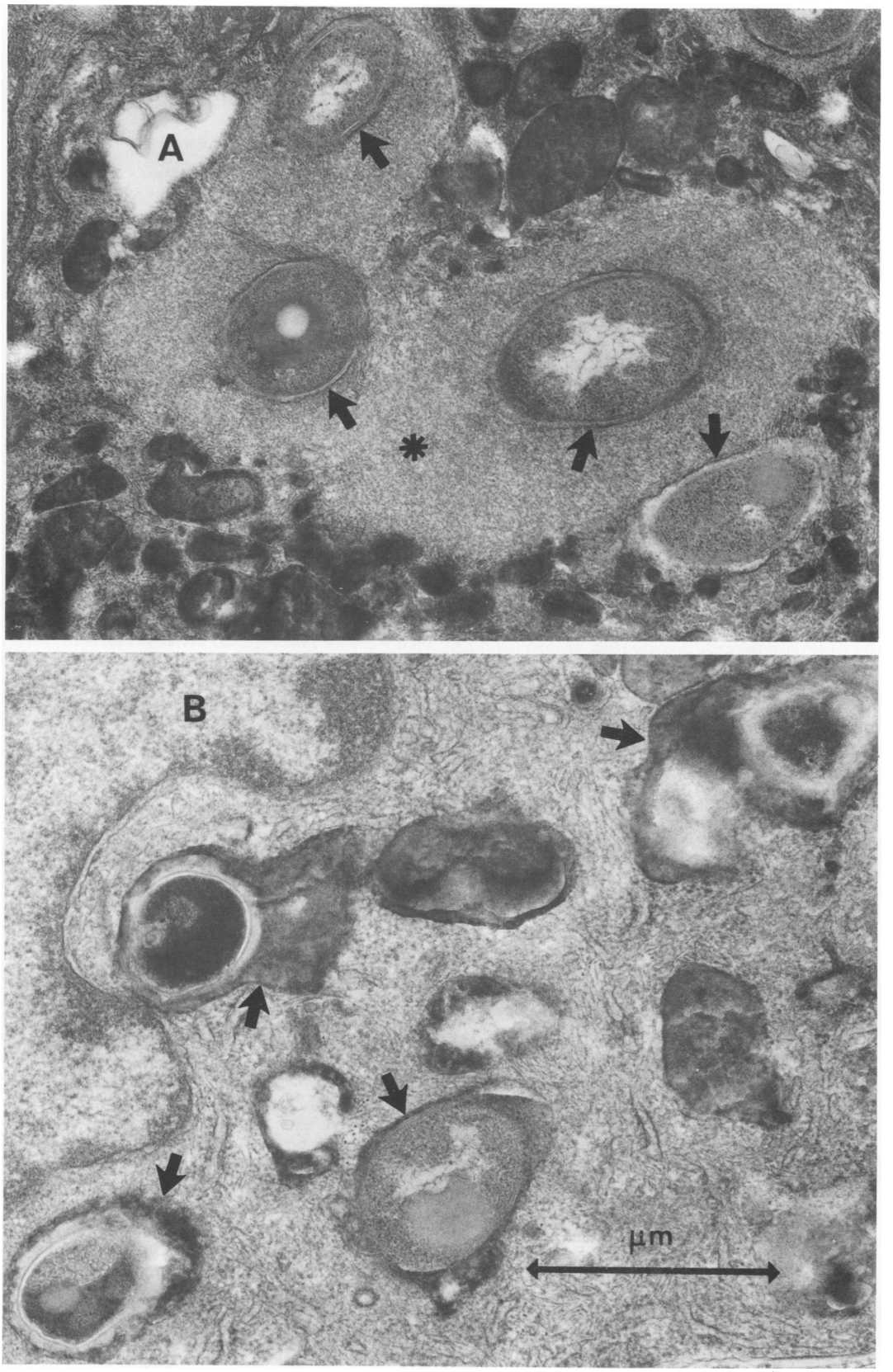
FIG. 3. *Effect of cellular and noncellular immunological components on the percentage of lysosomal fusion with nocardia-containing phagosomes. Fusion was determined by electron microscopic evaluation of infected macrophages either prelabeled with horseradish peroxidase or labeled for lysosomal acid phosphatase activity. Fifty profiles of infected macrophages were examined for each immunological parameter assessed in each experiment. A total of 1,350 profiles of infected macrophages and almost 4,000 profiles of phagocytized nocardia were evaluated. Error bars indicate the standard deviation of three experimental determinations. C', Complement activity.*

Enhancement of nocardial cell damage and nocardicidal activity. Electron microscopic determination of ultrastructurally damaged intraphagosomal nocardial cells indicated that the percentage of damaged cells observed in normal alveolar macrophages was not increased when the organisms were incubated with activated macrophages in the presence of normal serum, heat-inactivated or non-heat-inactivated immune serum, or alveolar lining material. Although the results suggesting an increase with spleen cells were equivocal, the inclusion of specifically primed lymph node cells with non-heat-inactivated immune serum significantly increased the number of obviously damaged nocardial cells (Fig. 5). Furthermore, the addition of alveolar lining material to the lymph node cell plus immune serum incubation medium further enhanced the incidence of damaged cells to approximately 50% (Fig. 5).

The electron micrographs in Fig. 4 illustrate the relative extent of nocardial cell damage observed. Figure 4A shows the lack of nocardial cell damage in activated macrophages incubated in 20% normal serum. In contrast, the micrograph in Fig. 4B demonstrates the range of nocardial cell damage observed in activated macrophages incubated in the presence of immune serum with complement plus alveolar lining material and specifically primed lymph node lymphocytes. Some nocardial cells have only minor indications of cellular damage, whereas others are completely destroyed (Fig. 4B).

Nocardial viability was determined by plate counts of the number of organisms recovered from macrophage lysates and from the incubation medium supernatants after a 3-h incubation. The data presented in Fig. 6 indicate that all of the organisms present in the incubation medium with 20% normal serum were recovered after incubation with either normal or activated alveolar macrophages. No significant decrease in nocardial viability was observed when heat-inactivated or non-heat-inactivated immune serum or alveolar lining material was included in the incubation medium. The results indicating a decrease in the percentage of organisms recovered when spleen cells were included in the

FIG. 4. *Electron micrographs of activated alveolar macrophages infected with N. asteroides GUH-2. Macrophage lysosomes were prelabeled with horseradish peroxidase. (A) Absence of phagosome-lysosome fusion and absence of nocardial cell damage in activated macrophage infected in the presence of 20% normal serum. Arrows indicate the macrophage phagosomal membrane. Note the absence of label within phagosome. (*) Granular zone within the cytoplasm of the macrophage frequently observed when phagosome-lysosome fusion is inhibited by this organism. (B) Occurrence of both phagosome-lysosome fusion and nocardial cell damage in activated macrophage infected in the presence of 20% immune serum with complement, alveolar lining material, and specifically primed lymph node lymphocytes. Arrows indicate the phagosomal membrane surrounding the ingested organisms. Note the presence of the electron-dense lysosomal label within the phagosomes. Nocardial ultrastructural changes range from minor damage to complete destruction.*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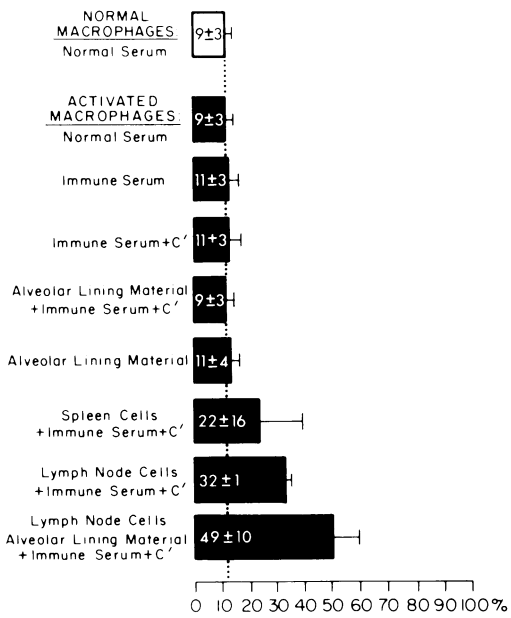

FIG. 5. *Effect of cellular and noncellular immunological components on the percentage of damaged intraphagosomal nocardia. Damage was determined by electron microscopic evaluation of infected macrophages. Fifty profiles of infected macrophages were examined for each immunological parameter assessed in each experiment. A total of 1,350 profiles of infected macrophages and almost 4,000 profiles of phagocytized nocardia were evaluated. Error bars indicate the standard deviation of three experimental determinations. C', Complement activity.*

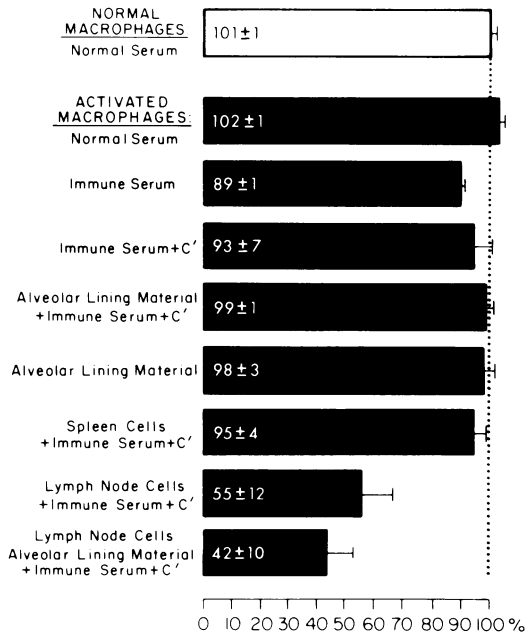

FIG. 6. *Effect of cellular and noncellular immunological components on the enhancement of alveolar macrophage nocardicidal activity. Percent recovery of viable nocardia after a 3-h infection was assessed by bacterial plate counts of infected macrophage lysates and infecting medium supernatants as compared with the same incubation conditions without macrophages. Error bars indicate the standard deviation. C', Complement activity.*

incubation medium were somewhat equivocal. In contrast, the number of viable organisms was reduced by almost 50% when specifically primed lymph node cells were included in the incubation medium, and the addition of alveolar lining material to the incubation medium with lymph node cells further enhanced the ability of specifically activated macrophages to kill *N. asteroides* GUH-2 (Fig. 6).

DISCUSSION

Inhaled nocardial cells which escape mechanical removal in the upper respiratory tract become deposited in alveoli, where they encounter both humoral and cellular immunological components. In the present study we have assessed the effect of alveolar lining material, normal serum, heat-inactivated and non-heat-inactivated immune serum, autologous spleen cells, and specifically primed autologous lymph node cells on the interaction of rabbit alveolar macrophages and *N. asteroides*.

Previous reports have suggested that both non-specifically activated (17, 29) and specifically activated (6) macrophages may be important in host defense against *N. asteroides*. The latter report indicated that although activated macrophages were able to decrease the number of viable bacteria, the bacteria were able to eventually overcome this effect and grow intracellularly. Thus, it was suggested that additional host factors were necessary for an effective host response to *N. asteroides* (6). The results presented in this report indicate that after a 3-h incubation there was no significant difference between normal and specifically activated alveolar macrophages infected in the presence of normal serum with respect to phagocytosis, incidence of phagosome-lysosome fusion, or nocardicidal activity. However, all of these macrophage functions could be enhanced by various immunological components.

The ability of alveolar macrophages to ingest *Pseudomonas* (44) and *Mycoplasma* (42) can be enhanced by specific antibody. In addition, both human and rabbit alveolar macrophages have receptors for the Fc component of immunoglobulin G (13, 20, 43) and receptors for complement

component C3b (13, 20, 45). These receptors have been shown to increase in number and affinity after macrophage activation (37) and have been further shown to be important for particle attachment and ingestion (13, 36, 43). The results presented in this report indicate that immune serum enhanced phagocytosis of *Nocardia* by activated alveolar macrophages to the same extent whether complement was present or not (Fig. 1). However, an additional increase in phagocytosis was observed when alveolar lining material was present in the incubation medium. Alveolar lining material at the same concentration in medium without serum enhanced phagocytosis equally well (Fig. 1). In fact, the same concentration of alveolar lining material in medium without serum enhanced phagocytosis by normal alveolar macrophages to the same extent as activated alveolar macrophages (data not presented). One of several possible explanations is that this highly surface-active material facilitated adherence between the macrophage and the lipid-rich hydrophobic nocardial cell surface.

Phagocytosis of nocardial cells appeared to be enhanced by the addition of autologous spleen cells to the immune serum incubation medium. When specifically primed autologous lymph node cells instead of spleen cells were present, an even greater enhancement was observed. However, the maximal amount of phagocytosis occurred when both primed lymph node cells and alveolar lining material were present in the incubation medium.

Since *N. asteroides* can function as a facultative intracellular parasite in alveolar macrophages (5, 6, 9), the postphagocytic intracellular events were examined. Previous studies have shown that some intracellular parasites, including *N. asteroides*, are able to remain in the phagocytic vacuole of the macrophage and avoid a hydrolytic environment by inhibiting fusion of lysosomes with the phagosome (1, 14, 22, 27, 52). Since our previous observations have established that both viable and formaldehyde-killed nocardial cells inhibit phagosome-lysosome fusion to the same extent (15), the occurrence of fusion most likely indicates neutralization of the nocardial component(s) responsible for inhibition of fusion. These nocardial component(s) may also be responsible for the formation of the granular zone frequently observed surrounding phagosomes which have no evidence of lysosomal fusion (Fig. 4A). However, formation of the granular zone does not appear to be a prerequisite for inhibition of fusion, since fusion may not occur even when the granular zone is absent and lysosomes are adjacent to the phagosome. This lack of fusion in the absence of the granular zone is illustrated by the ingested cell in the lower right-hand corner of Fig. 4A. Both the nature of this granular zone and the nature of the nocardial components involved in these phenomena are currently being examined.

Previous studies have shown that the ability of *Toxoplasma gondii* to inhibit phagosome-lysosome fusion after ingestion by peritoneal macrophages can be abrogated by the previous incubation of the parasite with antibody (27). It has also been reported that incubation of *Mycobacterium tuberculosis* with immune serum prevents the inhibition of phagosome-lysosome fusion normally observed with this organism (2). The present results indicate that a similar phenomenon occurs with *N. asteroides* GUH-2. Thus, the addition of either heat-inactivated or non-heat-inactivated immune serum to the incubation medium increased the level of phagosome-lysosome fusion (Fig. 2 and 3). The addition of alveolar lining material did not further increase the amount of fusion except when present in the incubation medium with specifically primed lymph node cells.

The addition of spleen cells did not appear to increase the number of infected macrophages with evidence of fusion, and spleen cells did not unequivocally increase the number of phagosomes with evidence of fusion. However, the addition of primed lymph node cells increased both the number of macrophages and the number of phagosomes with evidence of phagosome-lysosome fusion. Furthermore, this increase in incidence of fusion was not entirely independent of immune serum, since incubation with lymph node cells and normal serum did not increase fusion to the same extent (data not presented).

A comparison of the data presented in Fig. 3 and 6 indicates that ingested organisms can remain viable even though lysosomal fusion has occurred. No decrease in the number of viable nocardial cells recovered from activated macrophages was observed when the inoculum included immune serum with or without complement activity, even though the immune serum enhanced phagosome-lysosome fusion. This is in contrast to previous reports indicating that specific antibody may enhance post-phagocytic killing of *Pseudomonas* (44) and *Toxoplasma* (27). However, results similar to the present ones have been reported for antibody enhancement of fusion with lack of killing of *M. tuberculosis* (2). Although several reports have indicated that alveolar lining material enhances the bactericidal activity of alveolar macrophages against *Staphylococcus aureus* (28, 32) and *Pseudomonas aeruginosa* (46), no significant enhancement of nocardicidal activity by alveolar lining material was observed in the present studies.

The results of electron microscopic evaluation of ultrastructurally damaged nocardial cells indicated that about 10% of the intraphagosomal organisms had obvious evidence of cellular damage (Fig. 5) even though essentially all of the initial viable organisms were recovered (Fig. 6). A comparison of the data in Fig. 5 and 6 suggests that the damaged nocardial cells in macrophages incubated with the noncellular immunological components were most likely nonviable cells present in the initial incubation medium and not a result of macrophage-induced damage.

Although the humoral components examined did not effect nocardicidal activity or ultrastructural damage to the nocardial cells, specifically primed lymphocytes were able to both decrease the number of viable nocardial cells recovered (Fig. 6) and to increase the incidence of cell damage (Fig. 5; also compare Fig. 4A and 4B). Although the spleen cell data is less conclusive, lymphoid cells from specifically primed lymph nodes yielded consistent enhancement. These observations are thus consistent with results indicating the importance of specifically primed lymphocytes in in vitro enhancement of microbicidal activity reported for activated peritoneal macrophages infected with *Trypanosoma cruzi* (40).

Studies indicating an enhanced susceptibility of athymic mice to *Nocardia* have suggested the importance of T lymphocytes in resistance to nocardial infection (7, 18). Indeed, several studies have suggested that T-cell-derived factors may be important in the antimicrobial activity of alveolar macrophages against intracellular parasites (25, 48). Furthermore, it has been suggested that sensitized lung lymphocytes may play a role in protection against tuberculosis (53). The results presented in this study are in agreement with this concept in that they indicate that nocardicidal activity can be induced in activated alveolar macrophages by concurrent interaction with sensitized lymphocytes.

Since both localized humoral and cellular immunity exist in the lung, the role of both must be considered in evaluating pulmonary defense against infectious agents. Our data indicate that alveolar lining material enhances phagocytosis of *N. asteroides* by alveolar macrophages to a greater extent than does immune serum or primed lymphocytes. In contrast, specifically primed lymphocytes play a much greater role in enhancing phagosome-lysosome fusion and nocardicidal activity of alveolar macrophages than either immune serum or alveolar lining material. These data suggest that all of these components interact within the host to result in effective protection against *Nocardia*. Therefore, a deficiency in any one of these factors may compromise the host towards progressive pulmonary nocardiosis.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-13167 from the National Institute of Allergy and Infectious Diseases.

We thank Steven Scates, Paul Rossitto, Cynthia Vistica, Joanne Rosen, Donald Misumi, and Stephen Moring for their assistance during portions of these experiments. We also thank Marilyn Wheeler for her expert typing of this manuscript.

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