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Lung Response of Congenitally Athymic (Nude), Heterozygous, and Swiss Webster Mice to Aerogenic and Intranasal Infection by *Nocardia asteroides*

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Congenitally athymic (nude, Nu/Nu), heterozygous (Nu/+), and Swiss Webster mice were exposed to virulent *Nocardia asteroides* GUH-2 inhaled from aerosols or administered intranasally. Clearance of the bacteria from the lungs was determined at 6 h and 1, 2, 3 and 7 days after infection. *N. asteroides* aspirated into the lungs from intranasal administration were killed less rapidly and induced more severe pulmonary infections than did comparable numbers of organisms inhaled from aerosols. Bacterial clearance and histological data indicated that nude mice were significantly more susceptible to nocardial infection than were heterozygous littermates or Swiss Webster mice. From these data we conclude that: (i) pulmonary defenses cope less well with intranasally administered *N. asteroides* than with aerosolized organisms, (ii) alveolar macrophages alone appear not to be an efficient barrier to nocardial infections, and (iii) T cells are important to pulmonary clearance and prevention of dissemination of *N. asteroides* from the lung.

Although the lung is recognized as the target organ for infection by *Nocardia asteroides*, the pathogenesis of these infections is incompletely understood (2). Most nocardial infections result from the inhalation of contaminated dust (12), but on occasion the organism invades the lung after aspiration or bloodstream infections (12, 17, 18). Previous studies with other bacteria (*Staphylococcus aureus* [11, 13, 14], *Listeria monocytogenes* [11, 20], and *Mycobacterium tuberculosis* [11, 20]) have shown that the interaction of bacteria with alveolar macrophages determines the extent of infection. If the entering bacteria are killed by macrophages, serious infection is averted. When bacteria escape eradication by macrophages, they proliferate, and an infiltration with polymorphonuclear or mononuclear cells ensues. Recent studies with alveolar macrophages grown in vitro suggest that the same relationships are important in nocardial infections (3, 5). Virulent strains of *N. asteroides* survived and proliferated within alveolar macrophages, whereas benign strains were killed (3, 5, 7).

The development of murine models permitting the simultaneous determination of bacterial viability in one lung and histological assessment of the interrelationship of infecting bacteria to macrophages and polymorphonuclear leukocytes in the other lung (13) provided a means for further defining the pathogenesis of nocardial infections. Mice were infected with a virulent strain of *N. asteroides* (GUH-2) in aerosols and by intranasal inoculation. The course of infection was studied over the ensuing 168 h. This report documents the results of these studies of the pathogenesis of nocardial infections in intact murine lungs.

MATERIALS AND METHODS

Microorganism. *N. asteroides* GUH-2 was isolated from a fatal human infection and maintained as previously described (4). Its pathogenicity for mice has been described (4).

Animals. Congenitally athymic (nude) mice on an N:NIH(s) background were raised by mating heterozygous (Nu/+) females by Nu/Nu males. The nude and heterozygous littermates were maintained at the Animal Resources Service of the University of California as previously described (9, 10). Specific pathogen-free Swiss Webster (SW) mice (18- to 20-g females) were obtained from Simonson's (Gilroy, Calif.). All infected mice were maintained in a special animal room supplied with filtered air and were fed Purina laboratory chow ad lib and water acidified (pH 2.5 to 2.8) with hydrochloric acid.

Aerosolization procedures. Fresh animal isolates of *N. asteroides* GUH-2 were transferred to brain heart infusion broth (50 ml in 250-ml flasks) and incubated as described previously (4). A 5-ml amount of this starter culture was transferred to each of two flasks containing 500 ml of brain heart infusion broth

(in 2,800-ml Fernbach flasks). The flasks were incubated for 48 h (early stationary phase of growth) at 37°C with rotational agitation (150 rpm). Homogeneous suspensions of the bacteria were collected in sterile centrifuge tubes at ambient temperature at 3,000 × *g* for 15 min. The pellets were aseptically combined and resuspended in sterile saline (0.85%) and washed once by repelleting at 3,000 × *g*. The washed cell pellets were resuspended in sterile saline to give a uniform cell suspension of approximately 10^{11} colony-forming units (CFU) per ml. This thick cell suspension was used directly in the aerosol generating system.

Infection schedules. The aerosol chamber and the experimental procedures used to infect rodents with bacterial aerosols and to determine simultaneously the relationships of bacteria to macrophages and polymorphonuclear leukocytes and rates of bacterial clearance have been described previously (13, 14). Briefly, groups of 20 nude (Nu/Nu), 20 heterozygous (Nu/+), and 20 SW mice were infected with finely dispersed aerosols of *N. asteroides* GUH-2 for 30 min. The nocardial cell suspension (approximately 10^{11} CFU/ml) was aerosolized into a chamber with an air flow of 5 cubic feet (ca. 0.14 m^3) per s. The particle size distribution was determined with the Anderson chamber as previously described (14). The size distribution of the aerosolized bacteria was as follows: 2% were larger than 8.5 μm, 7% were 5 to 8 μm, 83% were from 2 to 5 μm, and 5% of the cells were less than 2 μm. These data imply that most of the bacterial cells in the aerosol occurred as individual organisms. These observations were further substantiated by light microscopy. Mice exposed to this aerosol for 30 min had an average of 1.3×10^6 CFU per left lung. At 0, 6, 24, and 72 h after infection, five mice were sacrificed. Histological relationships of nocardia and phagocytes were determined in the right lungs, and the numbers of viable nocardia were assessed in cultures of the left lungs. Histological examination was carried out on paraffin-embedded sections that were stained by hematoxylin and eosin or the Brown and Brenn tissue Gram stain (16).

Intranasal infection was accomplished by lightly anesthetizing the same numbers of mice as were used in the aerosol experiments with vapors of a mixture of 95% ethanol, chloroform, and diethyl ether (1:2:3, vol/vol/vol) and then introducing 0.05-ml saline suspensions (approximately 10^8 CFU/ml) of nocardia into the nares. At 30 min and at 24, 48, 72 or 168 h after infection, five mice from each experimental group (Nu/Nu, Nu/+, SW) were sacrificed, and measurements of nocardial viability and relationship to macrophages and polymorphonuclear leukocytes were performed.

Animal susceptibility to intranasal infection by *N. asteroides*. Groups of nude (Nu/Nu), heterozygous (Nu/+), and SW mice were infected intranasally as described above. These mice were maintained for several weeks to determine lethality of a given dose of *N. asteroides* GUH-2.

RESULTS

Aerogenous challenge. None of the mice died as a result of infection with aerosolized *N. asteroides*, and Fig. 1 shows that the numbers of CFU of *N. asteroides* declined significantly within the lungs of all three strains of mice in the 72-h period after infection with aerosols. This decline was most marked in the SW mice. At 72 h significantly fewer bacteria were cultured from these lungs than from those of either nude or heterozygous littermate mice. Although fewer bacteria were present in Nu/+ than in the Nu/Nu mice at 72 h, these differences were not significant ($P > 0.1$).

Histological analysis and Gram stains of sections of lung at different time intervals are shown in Fig. 2 and 3. Coccoid cells, many of which were within macrophages, were uniformly distributed throughout alveolar spaces immediately after challenge. The filamentous form of nocardia was not present at this time. At 6 h after infection, more than 99% of the coccoid microorganisms were located within macrophages (Fig. 2B, 3B, and 4B). Many of these intracellular bacteria were elongated or in filamentous form, indicating the onset of growth. Growth as defined by filament elongation was greatest within macrophages of Nu/Nu mice (Fig. 4B, arrows). Small areas of cellular infiltration were present at this time and were most prominent in the lungs of Nu/Nu mice (Fig. 2 and 3).

More extensive infiltration was present within the lungs of all infected mice at 24 h. The response was characterized by the presence of polymorphonuclear leukocytes and aggregates of mononuclear phagocytes (Fig. 2C and 3C). There was extensive elongation of nocardial cells and an increase in the number of such bacteria within macrophages. Large numbers of polymorphonuclear leukocytes and mononuclear phagocytes surrounded these infected macrophages (Fig. 2C). The histological sections suggested a substantial increase in numbers of nocardia within the lungs of all strains of mice at this time. The most extensive lesions with the greatest numbers of nocardia in the filamentous form were present in Nu/Nu mice (Fig. 3C, arrows).

At 72 h after infection, most of the inflammation had subsided, and the filamentous forms of nocardia were no longer present within the lungs. Gram-positive spheres of varying sizes, possibly representing partially digested nocardial filaments, were found within mononuclear phagocytes. It is worth emphasizing that none of the 65 mice infected with aerosols died during this experiment.

Intranasal challenge. Unlike the aerogenous infections which did not cause death, 50% of Nu/+ mice, 5% of the Nu/Nu mice, and 25% of

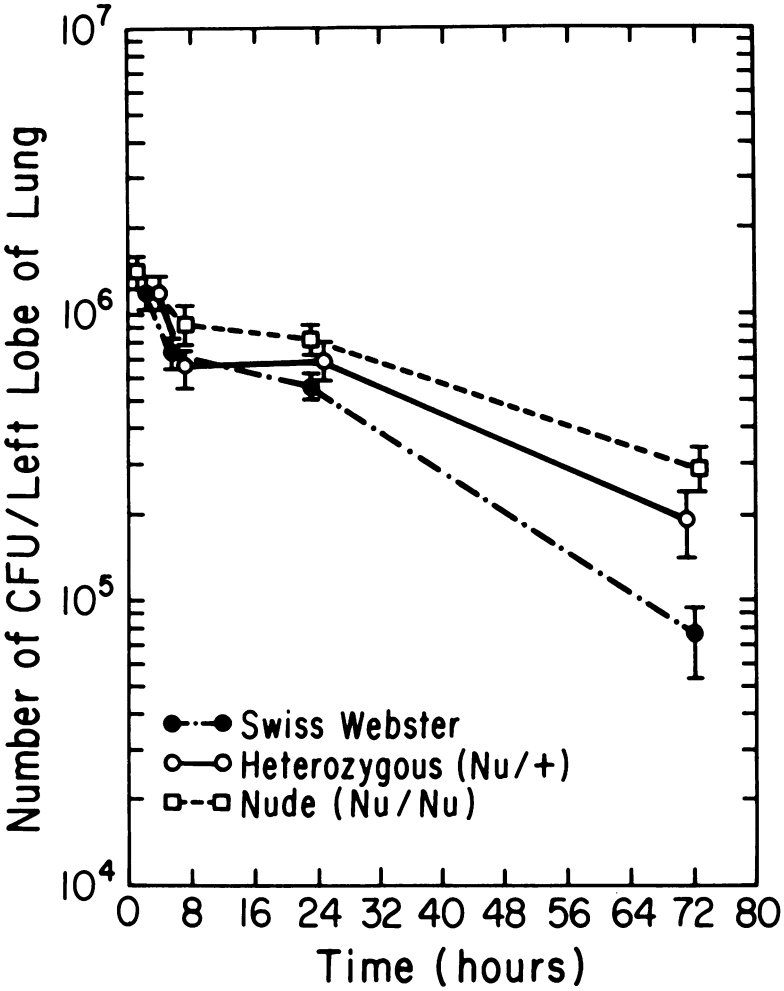


FIG. 1. *Lung clearance of aerosolized cultures of N. asteroides GUH-2. Each point represents an average of five animals, and the bars represent the standard error of the mean.*

the SW mice died after intranasal inoculation at 1 week after infection (Table 1). The Nu/+ and SW mice that survived this period healed their pulmonary infections; deaths were not observed from 1 week onwards (Table 1). Nude mice did not eradicate the nocardia from their lungs (Fig. 4), and at 4 weeks post infection many of them developed disseminated nocardial infection involving primarily the kidneys. Ninety percent of infected mice died from their disseminated infections within 6 weeks (Table 1).

Figure 4 illustrates the clearance data for mice infected intranasally with *N. asteroides* GUH-2. Approximately 10^6 CFU of nocardia, the same number as were present after aerogenous infection, were cultured from the lungs of the three strains of mice immediately after infection. The number of CFU of *Nocardia* remained constant for the first 3 days after infection and then declined to very low levels in Nu/+ and SW mice by day 7. However, in the Nu/Nu mice, the number of CFU persisted unchanged through day 7. Thus, the clearance of *Nocardia* from the lungs of nude mice differed significantly from those observed after aerogenous infection in which nocardial numbers declined during the first 3 days after infection (Fig. 1).

By microscopy (Fig. 2, 3, 5, and 6) it was clear that the nocardia could initiate growth within the lungs of all mice. These observations do not correlate well with the viable plate counts of homogenized lungs (Fig. 1 and 4). This apparent disagreement may be explained by the elongation of nocardial cells to form branching filaments which aggregate within the lung. These clumps of microorganisms are not adequately

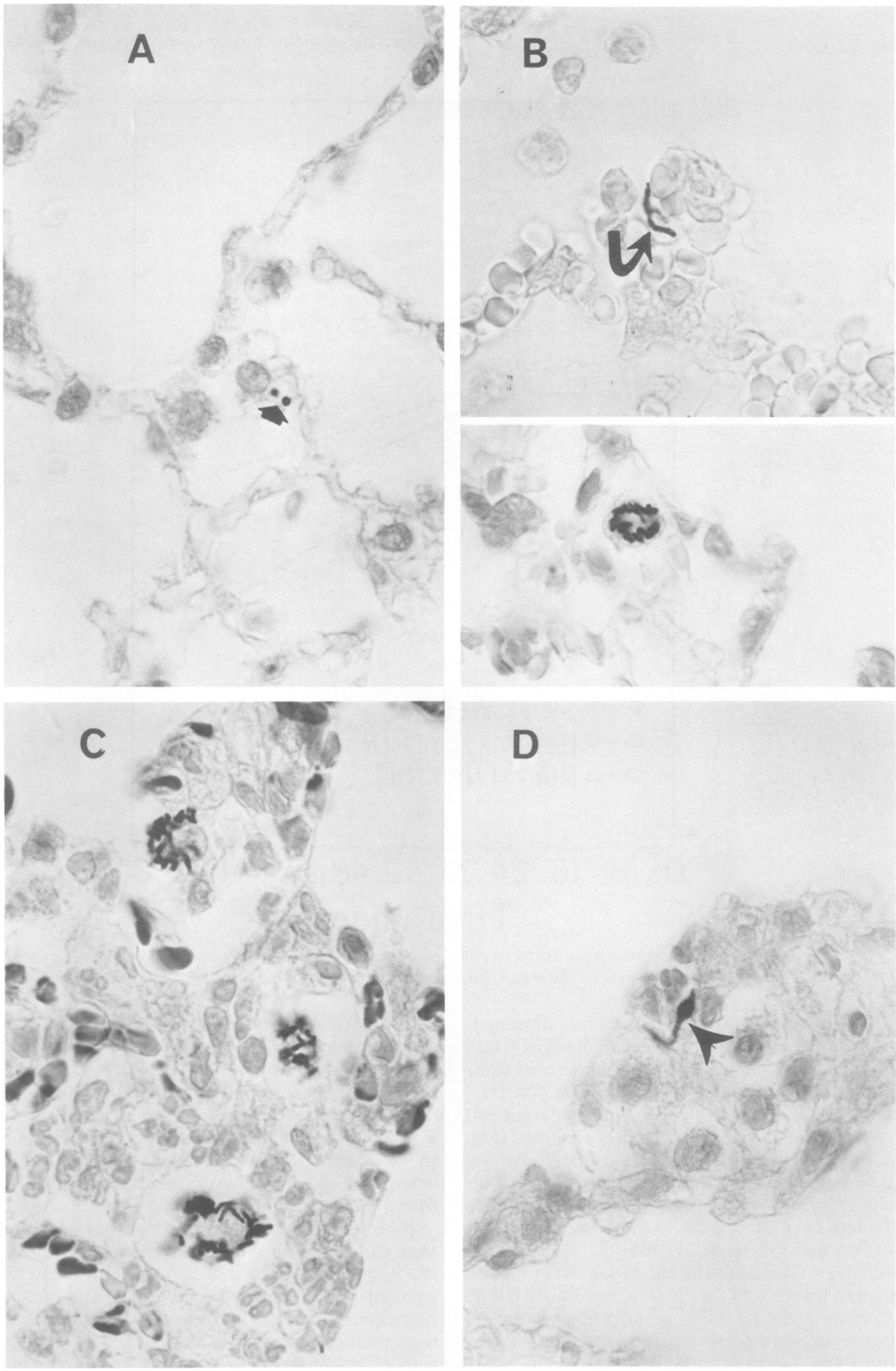


FIG. 2. *Sections of lungs of heterozygous (Nu/+) littermate control mice at time intervals after aerosol exposure to N. asteroides GUH-2. All sections are stained by the Brown and Brenn modification of the Gram stain. (A) Zero time; (B) 6 h postaerosol; (C) 24 h postaerosol; (D) 72 h postaerosol.*

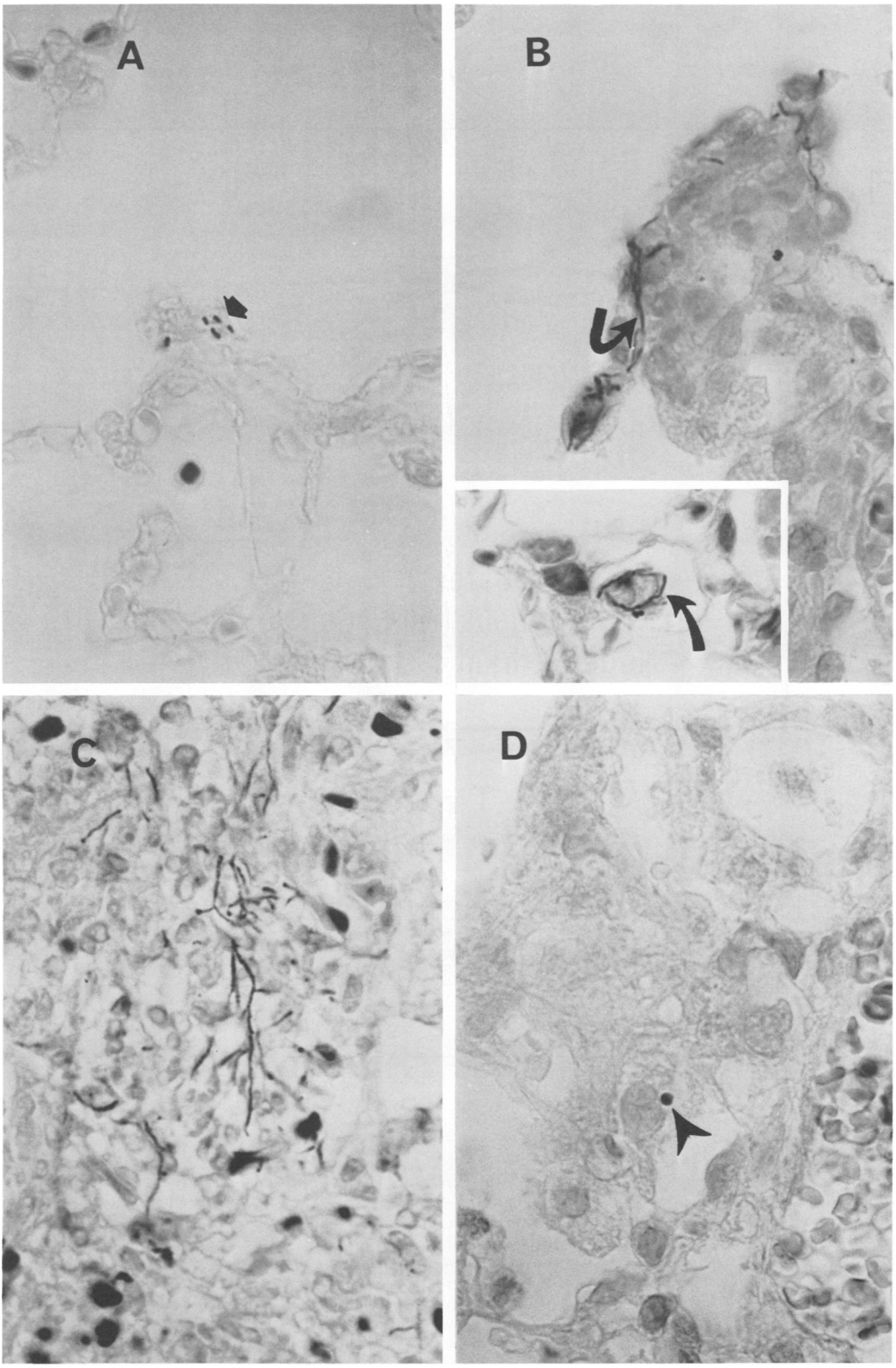


FIG. 3. *Sections of lungs of nude mice (Nu/Nu) at time intervals after aerosol exposure to N. asteroides GUH-2. All sections were stained by the Brown and Brenn modification of the Gram stain. (A) Zero time as in Fig. 2A; (B) 6 h postaerosol. Note increased host cell response with aggregation of macrophages. Arrows indicate long branching filaments of nocardia growing within alveolar macrophages (compare with Fig. 2B). (C) 24 h postaerosol. Note extensive infiltration with many polymorphonuclear leukocytes and macrophages. Large numbers of branching filamentous forms of nocardia are growing throughout the lung infiltrate (compare with Fig. 2C). (D) 72 h postaerosol, as in Fig. 2D.*

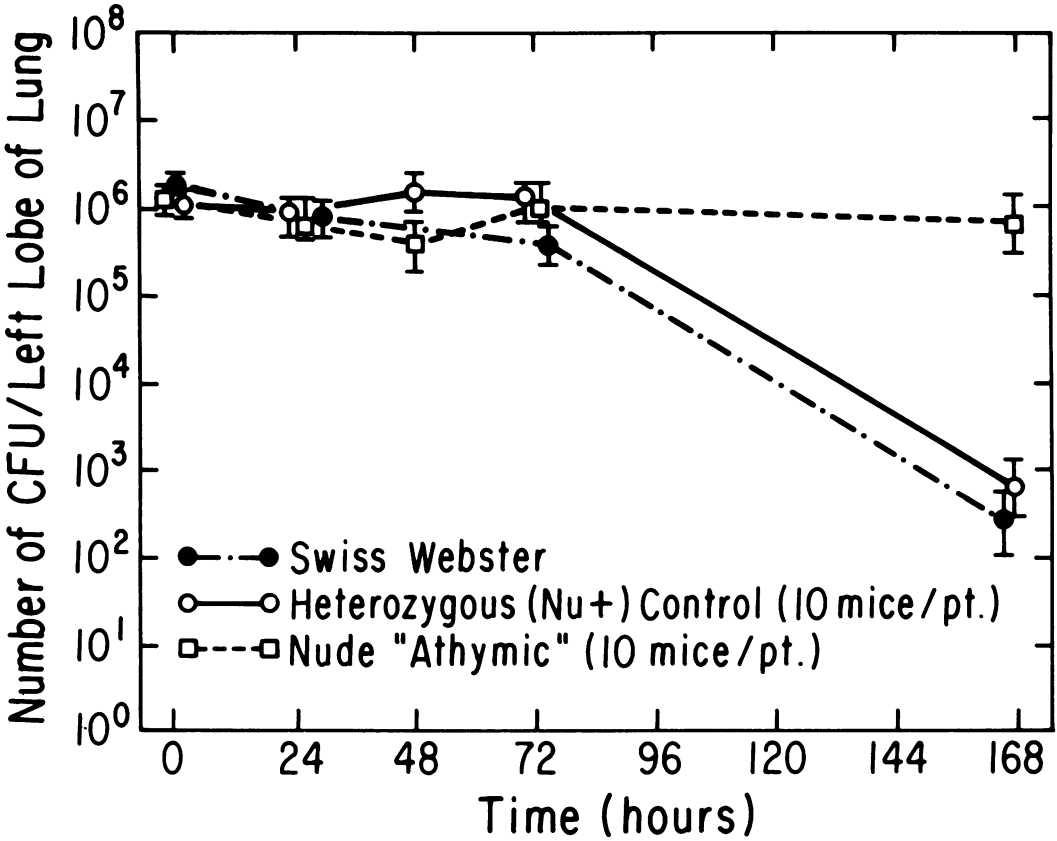


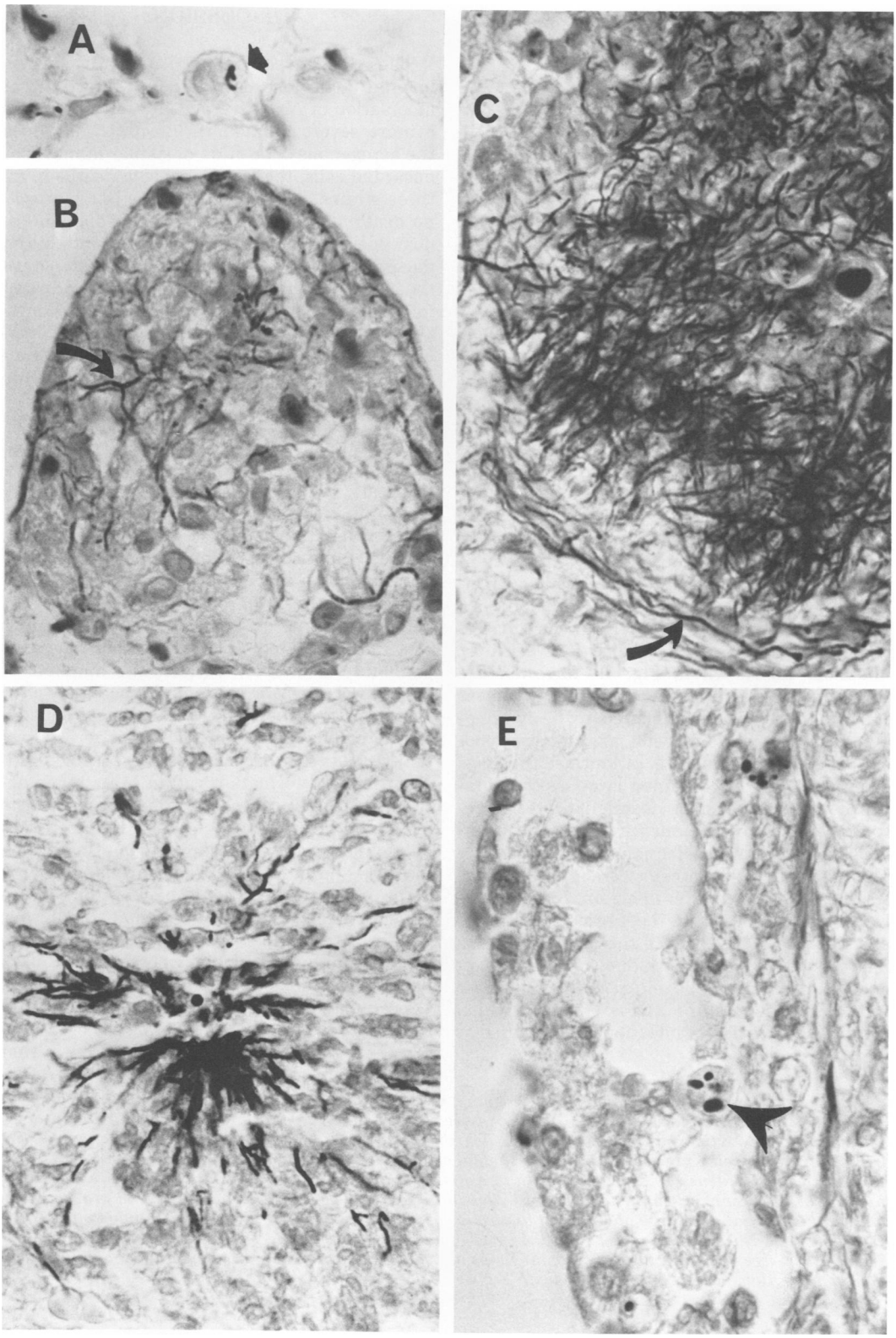
FIG. 4. *Lung clearance of intranasally administered cultures of N. asteroides GUH-2. The bars represent the standard error of the mean.*

TABLE 1. *Comparative mortalities of mice after intranasal administration of N. asteroides GUH-2*[a]

Mouse type	No. of mice	Deaths during week:						Cumulative mortalities	% Mortality
		1	2	3	4	5	6		
Swiss Webster (SPF[b])	20	5	0	0	0	0	0	5/20	25
Heterozygous (Nu/+)	38	19	0	0	0	0	0	19/38	50
Nude (Nu/Nu)	20	1	0	1	1	7	8	18/20	90

[a] Each mouse received approximately 1.5×10^6 CFU per left lobe of lung.
[b] SPF, Specific pathogen free.

FIG. 5. *Sections of lungs of heterozygous (Nu/+) littermate control mice at time intervals after intranasal administration of suspensions of N. asteroides GUH-2. All sections are stained by the Brown and Brenn modification of the Gram stain. (A) Zero time postinfection showing distribution of individual bacterial cells within an alveolar macrophage (arrow). (B) 24 h after infection. Note the extensive host-cell infiltration consisting primarily of macrophages. The arrow indicates extensive growth of nocardial filaments throughout this aggregation of host cells within the alveolar region of the lung. (C) 48 h after infection. Massive nocardial growth has occurred throughout the alveoli within the lung. The arrow indicates long branching filaments permeating the host's cells. (Several mice died at this stage of infection.) (D) 72 h postinfection. Extensive nocardial growth is still evident at this time period; however there is a general decrease in severity (compare with C). (E) 1 week postinfection. Mice studied at this time period represented survivors of the acute infection. Note the absence of branching filamentous cells. The lesions contain gram-positive spheroidal bodies (see Fig. 2D and 3D) and an accumulation of macrophages and lymphocytes.*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dispersed by the homogenization procedures, and an aggregate of nocardial cells would give rise to a single colony.

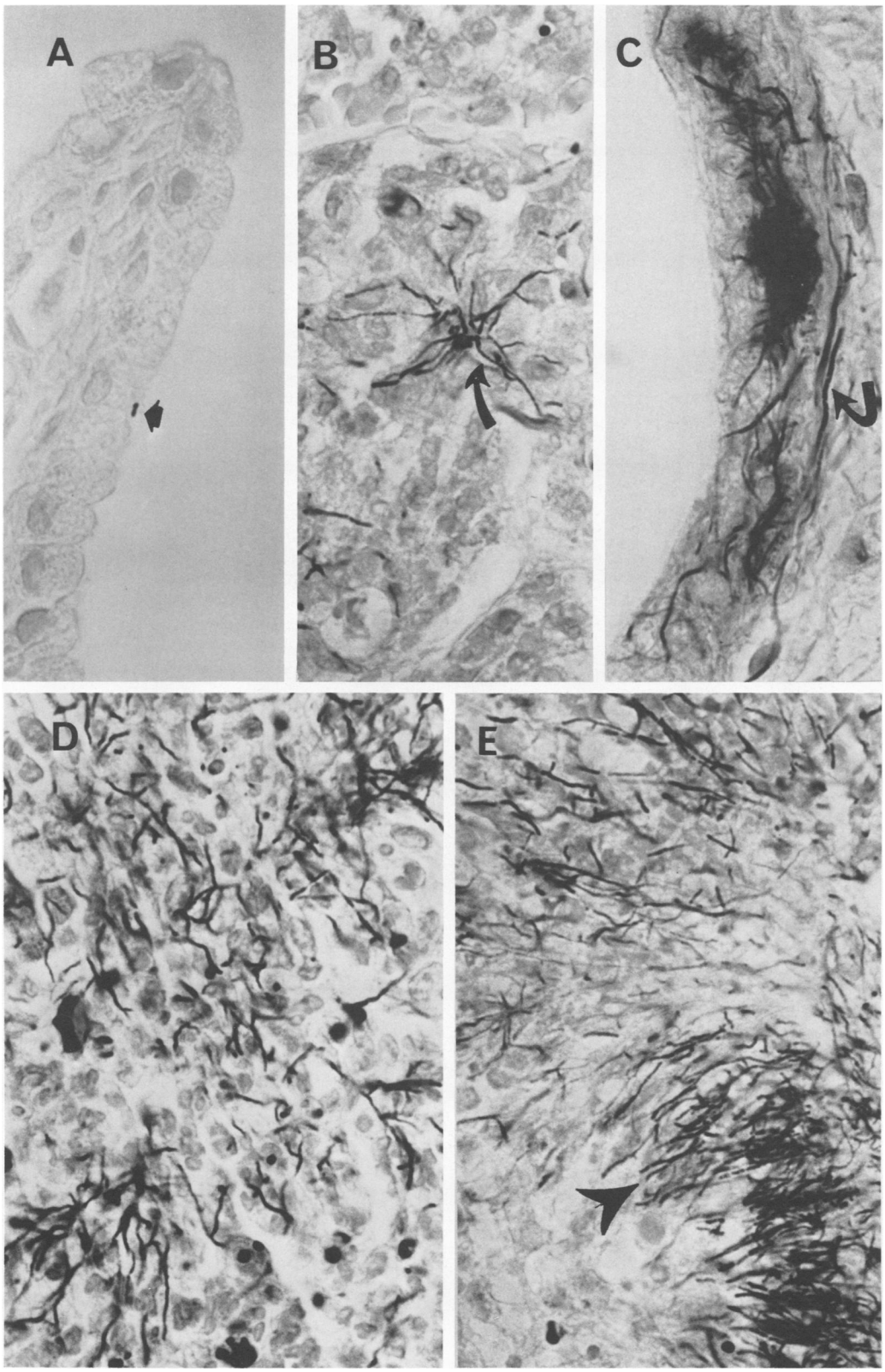
Nocardia in the coccoid or short rod form were observed extracellularly in bronchial epithelium (Fig. 6A) and in alveoli as well as within alveolar macrophages (Fig. 5A) immediately after intranasal infection. At 24 h after infection, mice from all groups were ill. Histological examination showed severe pulmonary infiltration within these mice (Fig. 5B and 6B). The lesions were composed of large numbers of extensively branching filaments of nocardia, polymorphonuclear leukocytes, and macrophages (Fig. 5 and 6). In contrast to the bacteria at zero time which were not acid fast, many of these bacteria were strongly acid fast, suggesting that the bacterial envelope (particularly the lipids) had undergone structural modification during growth within the lungs. The lesions became progressively more severe in all mice in the next 48 h (Fig. 5C and D, and 6C and D), and several mice died during this period. Invasion of bronchial epithelium by nocardial filaments, a finding not observed in mice infected aerogenously, was a prominent feature in the lungs of all mice challenged intranasally. These extensive inflammatory changes were no longer present in the lungs of the surviving Nu/+ and SW mice at 1 week after infection. As shown in Fig. 5E (arrows), the subsiding lesions were composed of mononuclear cells, many of which appeared to be lymphocytes, occasional giant cells, and spherical nocardial cells of varying size. In contrast, the lungs of nude mice still contained large lesions composed of polymorphonuclear leukocytes, macrophages, nocardial filaments, and necrotic material at 1 week after infection (Fig. 6E). Neither lymphocytes nor giant cells were found in these lesions. At 1 month after infection, these lesions were still present within the lungs of nude mice, and in three of seven mice the microorganisms had disseminated to the kidneys. This persistence of infection in nude mice is in sharp contrast to the findings in Nu/+ and SW mice in which complete resolution of infection had occurred at 1 month.

DISCUSSION

These studies show that: (i) under the experimental conditions used herein, the intranasal inoculation of *N. asteroides* into mice provokes a more severe pulmonary infection than does the aerogenous deposition of similar numbers of nocardial cells, the former causing deaths in all three strains of mice whereas the latter caused no deaths; (ii) alveolar macrophages alone appear not to provide a sufficient barrier to initial nocardial infection; (iii) secondary responses are the principal means for suppressing nocardial infections; and (iv) heterozygous but not homozygous nude littermate mice are capable of eradicating intranasally inoculated nocardia, indicating a need for intact T cell function for pulmonary clearance of these organisms.

The data presented herein show that after inhalation cells of *N. asteroides* are destroyed within 72 h, whereas equivalent numbers of intranasally inoculated microorganisms survive and grow in the same strains of mice. One possible explanation for these results is that aerosolization and the resultant dehydration of the nocardial cells injured the organisms and rendered them less vigorous than organisms suspended in saline and inoculated into the lungs in a fully hydrated condition. In our view this possibility is unlikely because the numbers of nocardia grown from the lungs immediately after aerosolization equaled the numbers predicted from previous experiments with this aerosol system (13, 14) and because these organisms initiated filament formation within 6 h after infection. A second possibility is that the difference in virulence might reflect the intranasal introduction of clumps of nocardia in contrast to the dispersed nature of the organisms deposited by aerosol. Such clumps could be more difficult to phagocytize than the individual organisms. These aggregates also would tend to accumulate in a nonuniform fashion, whereas aerosolized bacteria would be more evenly distributed. Gross macroscopic examination of lungs removed from intranasally infected mice at 48 to 72 h is consistent with this explanation in that

FIG. 6. *Sections of lungs of nude (Nu/Nu) mice at time intervals after intranasal administration of suspensions of N. asteroides GUH-2. All sections are stained by the Brown and Brenn modification of the Gram stain. (A) Zero time postinfection showing distribution of individual bacterial cells within the lung. The arrow indicates a cell on the surface of the bronchial epithelium. (B) 24 h after infection (as in Fig. 5B). (C) 48 h after infection. Extensive nocardial growth has occurred. In many of the lung sections there is significant invasion of the bronchial epithelium by nocardial filaments (arrow). Active penetration and growth in the bronchial epithelial cells were observed at all groups of mice, but it was most extensive in nude mice. (D) 72 h postinfection. Nocardial growth within the lungs of nude mice was not decreasing at this time period as in Fig. 5D. (E) 1 week postinfection. Most of the lungs of nude mice at this time period contained large numbers of branching filamentous cells (compare with Fig. 5E).*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the dorsal, cephalad region of the right lung was much more extensively damaged than were the ventral, caudad regions, a finding not observed in the lungs of animals infected with bacterial aerosols. Because microscopic observations were made only from the median lobe and these sections did not show more nocardial clumps in the intranasally infected mice, differences in nocardial deposition could not be confirmed. A third possibility is that the instillation of organisms in liquid suspensions may have affected the interaction of nocardia and macrophages. We could not assess the importance of the liquid milieu from the present study. Finally, the anaesthetic given to the intranasally infected animals could have adversely altered macrophage function; however, this has not been confirmed by previous investigations (1, 6). Although the mechanisms responsible for the increased virulence of intranasally administered nocardia are uncertain, it is worth emphasizing that similar differences in virulence related to the route of administration occur with *S. pneumoniae* (1) and with *Klebsiella pneumoniae* (6), the latter organism being 75 times more virulent for mice when instilled intranasally as compared with aerosol administration.

Once within the lung, the nocardia are rapidly phagocytized by alveolar macrophages, but not killed inasmuch as transformation of nocardia from coccoid to filamentous forms was noted in the surrounding lung. Although there is little information on the interactions of alveolar macrophages and *N. asteroides* within the host lung, Beaman and Smathers (5) and Beaman (3) demonstrated that virulent forms of *N. asteroides* were phagocytized readily by rabbit alveolar macrophages maintained in vitro. These organisms initiated growth within the macrophages, ultimately destroying the cells by intracellular outgrowth, a process that is similar to that observed in vivo. Similar sequences occurred in in vitro studies utilizing murine or guinea pig peritoneal macrophages (7, 19). The proliferating nocardia within the lung elicit an inflammatory response which involved an influx of polymorphonuclear leukocytes and additional mononuclear cells. This reaction successfully eliminated the nocardia from the lungs of SW (specific pathogen free) and heterozygous (Nu/+) mice regardless of the method of administration. Inhaled nocardia were also eradicated by this reaction in nude (Nu/Nu) mice. The nude mice were unable to eradicate intranasally instilled organisms, indicating a marked difference in susceptibility for this route of infection.

Although the mechanisms by which these secondarily attracted phagocytes kill nocardia are not known, it is tempting to speculate that the smoldering infection produces a hypersensitivity response which augments the bactericidal capacity of newly arrived phagocytes. This kind of phagocytic activation occurs in listerial infections (20). Krick and Remington (15) demonstrated that peritoneal macrophages obtained from mice given *N. asteroides* were "activated". Furthermore, these mice were resistant to challenge with *L. monocytogenes*. Conversely, mice that had been infected with *Listeria* appeared to be more resistant to subsequent infection with *N. asteroides* in gastric mucin (15). The similarity in pattern of infection of nocardia and listeria, which is also an intracellular parasite, further supports the concept of hypersensitivity being important in nocardial infections. The sequence of infection for both organisms consists of ingestion by alveolar macrophages, proliferation and outgrowth of nocardia, and subsequent suppression of infection by a second inflammatory response (20).

The finding that macrophages from nude mice are less resistant to nocardial outgrowth and filament formation than are macrophages from heterozygous (Nu/+) littermate or SW specific pathogen-free control mice suggests that T-cell function is important in the interaction of nocardia with alveolar macrophages. One possible mechanism for this relationship may be that T cells activate alveolar macrophages and, therefore, in their absence augmentation of bactericidal capacity does not occur. Perhaps, this inhibitory effect may also apply to the phagocytes involved in the secondary response. Because of the increased difficulty in killing intranasally instilled nocardia, these infections are never eliminated from T cell-deficient mice, and the nocardia eventually disseminate to other organs, causing death. The data presented herein show that the heterozygous (Nu/+) mice are significantly more susceptible to acute pulmonary infection after intranasal administration than are nude (Nu/Nu) mice (Table 1). These observations are similar to previous studies of *L. monocytogenes* in nude mice which showed the Nu/+ mice to be more susceptible to listeria than the Nu/Nu littermates (8). The precise mechanism of this enhanced susceptibility to acute pulmonary infection by heterozygous (Nu/+) mice remains to be elucidated. It has been attributed to the chronic activation of macrophages in nude mice (8) and possibly to an elevated number of clonogenic plaque-forming unit cells and CFU cells of bone marrow and spleen cell populations in nude mice but not in the heterozygous (Nu/+) littermate mice (21). As indicated above, *N. asteroides* grew better

within the alveolar macrophages of nude mice than in the heterozygous littermates. This observation appears to be inconsistent with the explanation that chronically activated macrophages result in nude mice being more resistant to acute infection than their heterozygous littermates. Perhaps these data support the suggestion that elevated CFU and plaque-forming unit bone marrow and spleen cell populations in nude mice play a role in the increased resistance of nude mice to acute nocardial infection of the lungs.

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LITERATURE CITED

1. **Ansfield, M. J., D. E. Woods, and W. G. Johanson, Jr.** 1977. Lung bacterial clearance in murine pneumococcal pneumonia. Infect. Immun. **17**:195-204.
2. **Beaman, B. L.** 1976. Possible mechanisms of nocardial pathogenesis, p. 386-417. *In* G. Brownell, M. Goodfellow, and J. Serrano (ed.), Biology of the Nocardia. Academic Press, London.
3. **Beaman, B. L.** 1977. In vitro response of rabbit alveolar macrophages to infection with *Nocardia asteroides*. Infect. Immun. **15**:925-937.
4. **Beaman, B. L., and S. Maslan.** 1977. Effect of cyclophosphamide on experimental *Nocardia asteroides* infection in mice. Infect. Immun. **16**:995-1004.
5. **Beaman, B. L., and M. Smathers.** 1976. Interaction of *Nocardia asteroides* with cultured rabbit alveolar macrophages. Infect. Immun. **13**:1126-1131.
6. **Berendt, R. J.** 1978. Relationship of method of administration to respiratory virulence of *Klebsiella pneumoniae* for mice and squirrel monkeys. Infect. Immun. **20**:581-583.
7. **Bourgeois, L., and B. L. Beaman.** 1974. Probable L-forms of *Nocardia asteroides* induced in cultured mouse peritoneal macrophages. Infect. Immun. **9**: 576-590.
8. **Emmerling, P., H. Finger, and H. Hof.** 1977. Cell-mediated resistance to infection with *Listeria monocytogenes* in nude mice. Infect. Immun. **15**:382-385.
9. **Gershwin, M. E., R. M. Ikeda, T. G. Kawakami, and R. B. Owens.** 1977. Immunobiology of heterotransplanted human tumors in nude mice. J. Natl. Cancer Inst. **58**:1455-1461.
10. **Gershwin, M. E., B. Merchant, and A. D. Steinberg.** 1977. The effects of synthetic polymeric agents on immune responses of nude mice. J. Immunol. **32**:327-336.
11. **Goldstein, E., W. Lippert, and D. Warshauer.** 1974. Pulmonary alveolar macrophage: defender against bacterial infection of the lung. J. Clin. Invest. **54**:519-528.
12. **Goodman, J. S., and M. G. Moenig.** 1970. Nocardia infections in a general hospital. Ann. N.Y. Acad. Sci. **174**:552-567.
13. **Green, G. M., and E. Goldstein.** 1966. A method for quantitating intrapulmonary bacterial inactivation in individual animals. J. Lab. Clin. Med. **68**:669-677.
14. **Kim, M., E. Goldstein, J. P. Lewis, W. Lippert, and D. Warshauer.** 1976. Murine pulmonary alveolar macrophages: rates of bacterial ingestion, inactivation, and destruction. J. Infect. Dis. **133**:310-320.
15. **Krick, J. A., and J. S. Remington.** 1975. Resistance to infection with *Nocardia asteroides*. J. Infect. Dis. **131**:665-672.
16. **Luna, L. G.** 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology, 3rd ed. McGraw-Hill Book Co., New York.
17. **Poretz, D. M., M. N. Smith, and C. H. Park.** 1975. Intracranial suppuration secondary to trauma. J. Am. Med. Assoc. **232**:730-731.
18. **Roberts, G. D., N. S. Brewer, and P. E. Hermans.** 1974. Diagnosis of nocardiosis by blood culture. Mayo Clin. Proc. **49**:293-296.
19. **Splino, M., V. Merka, and F. Kyntera.** 1976. Phagocytosis and intracellular proliferation of *Nocardia asteroides* (strain Weipheld) in cell structures *in vitro*. 2. Peritoneal macrophages of guinea pigs. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **235**:512-520.
20. **Truitt, G. L., and G. B. Mackaness.** 1971. Cell-mediated resistance to aerogenic infection of the lung. Am. Rev. Respir. Dis. **104**:829-843.
21. **Wilson, F. D., M. E. Gershwin, M. Shifrine, and R. Graham.** 1977. Increased clonogenic (CFU-C, PFU-C) populations from bone marrow and spleen of nude mice. Dev. Comp. Immunol. **1**:373-383.