

Nocardia asteroides and *Nocardia brasiliensis* Infections in Mice

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A model for *Nocardia asteroides* and *Nocardia brasiliensis* infections in Swiss white mice has been established without the addition to the inocula of any form of adjuvant. Serial histopathological studies revealed that these two actinomycetes cause lesions that are quite different in their features. An acute suppurative abscess characterizes the lesions of *N. asteroides*. In the case of *N. brasiliensis* infections a granuloma is produced in which a striking feature is the presence of large numbers of foam-laden macrophages, although occasional exceptions to this pattern were noted. Electron microscopic studies demonstrated that these macrophages contain within their cytoplasm organisms in varying stages of degeneration. Repeated mortality studies in mice failed to demonstrate differences in mortality rates produced by *N. asteroides* and *N. brasiliensis*. Thus, despite relatively trivial biochemical and antigenic differences between these two species of *Nocardia*, the local pathogenic response is quite different. The presence in the "brasiliensis lesion" of foamy macrophages with intracellular organisms is reminiscent of the histopathological features of lepromatous leprosy and of disseminated *Mycobacterium bovis* infection when this occurs in the immune suppressed situation. It is possible that *N. brasiliensis* infection produces a depression of cellular immunity that modifies the local host response to the organism.

Infection with *Nocardia asteroides* and with *Nocardia brasiliensis* in man may occur spontaneously or in the immune-compromised host. There appears to be a particular association between *N. asteroides* infection and the prolonged use of steroids. Nocardiosis is becoming increasingly recognized following renal and cardiac transplantation (2, 7). *N. asteroides* infection of the lung is a relatively common complication of pulmonary alveolar proteinosis (1). Likewise, systemic *N. brasiliensis* infection may be opportunistic (unpublished data, manuscript in preparation). However, both organisms may produce systemic disease in an otherwise healthy person (4, 8).

It has been shown that *N. asteroides* and *N. brasiliensis*, injected with adjuvant into experimental animals, produce two different histopathological lesions. These have been referred to as the "asteroides lesion" and the "brasiliensis lesion," respectively (13). There is disagreement in the literature as to which of these two organisms is more virulent for experimental mice (5, 11).

The objectives of these studies were to establish experimental models in Swiss white mice for both *N. asteroides* and *N. brasiliensis*,

without the addition of adjuvant to the inocula, so that a detailed comparison could be made of the pathology and natural history of the two infections. Electron microscopical examination of the brasiliensis lesion was performed to clarify certain features noted on light microscopy. A comparison was made of the virulence for mice of the two organisms.

MATERIALS AND METHODS

***Nocardia* organisms.** The cultures of *N. asteroides* and *N. brasiliensis* were isolated, respectively, from the sputum and from a subcutaneous abscess of patients suffering from clinical illnesses. The actinomycetes were identified and characterized by established criteria (3, 6). Confirmation of the *N. asteroides* isolate was obtained from Ruth Gordon, Rutgers Univ., N.J. The *N. brasiliensis* culture was confirmed by L. Kaufman, Center for Disease Control, Atlanta, Ga.

Experimental mice. Swiss white mice weighing between 25 and 30 g were used.

Inoculations. The mice were inoculated either intraperitoneally (i.p.) or subcutaneously into the footpad. In the case of the i.p. injections, 5 mg (dry weight) of the organism, previously cultured on tryptose-agar slants and suspended in 0.5 ml of sterile saline, were introduced through a narrow-gauge

needle through the lower left abdominal wall. The footpad lesions were produced by injecting a suspension of 10 mg (dry weight) of the organism suspended in 0.1 ml of sterile saline into the subcutaneous tissues of the footpad.

Pathological studies. In four separate series of experiments *N. asteroides* and *N. brasiliensis* infections were produced i.p. and in the footpad. In each case 24 mice were studied. From each group four mice were examined at intervals of 2, 11, 21, 45 and 60 days, and at 7 months after inoculation. The mice were anaesthetized and killed with ethyl ether. A full postmortem examination was performed, and portions of the organs, including the brain, were taken for histological examination.

Routine staining of the histological sections was with hematoxylin and eosin. Selected stains were used in certain instances: viz. Gram, P.A.S., modified Ziehl Neelsen, Giemsa, Van Kossa, Masson trichrome, and Congo red with microscopic examination by polarized light.

Regular bacteriological examination of the lesions was performed. Impression smears of i.p. lesions and exudates were air-dried and stained with Ziehl Neelsen and Gram stains.

Electron microscopy. Selected lesions (*N. brasiliensis*) were removed for electron microscopical study. Immediate fixation was with 6% glutaraldehyde at pH 7.2. After two washes with sucrose buffer, the tissues were fixed in 1% osmic acid for 2 h. After dehydration in alcohol, the specimens were washed in propylene oxide and embedded in Epon. The grids were cut in an L.K.B. Ultratome, stained with uranyl acetate and lead citrate, and examined under the AEI 6B electron microscope.

Cumulative mortality studies. In another experiment 10 mice were injected i.p. with 10 mg (dry weight) of *N. asteroides* cells. Likewise, 10 mg of *N. brasiliensis* cells were injected i.p. into another group of 10 mice. A control group of 10 mice was injected i.p. with 0.5 ml of sterile saline. (This dose of inoculum was chosen, as higher doses of either organism produced a uniformly high mortality rate). This experiment was repeated three times.

RESULTS

In every instance of both i.p. and footpad inoculation of *N. asteroides* and *N. brasiliensis*, a local inflammatory reaction was noted. There were no fatalities with the doses of organisms that were used. (This does not refer to those experiments in which cumulative mortality was studied.)

The macroscopic appearances of the lesions produced by the two organisms were similar. I.p. lesions could be identified on day 2 after inoculation, and pathological changes were clearly defined by day 11. At that stage characteristic, yellow-white, spherical nodules ranging in size from 1 to 5 mm in diameter were found in the gastric bed, at the hilus of the spleen, and at the porta hepatis, on the surface of the liver and between the liver lobes. In

addition, occasional lesions were noted on the surface of the left leaf of the diaphragm and at times at the root of the mesentery and on the antimesenteric surface of the small bowel. Rarely abscesses were noted extending to the injection site on the anterior abdominal wall. Sectioning of the intra-abdominal lesions 11 to 21 days after inoculation revealed yellow pus in which colonies of acid-fast organisms could be demonstrated and which on culture yielded a pure growth of the *Nocardia* species. On examination 60 days after inoculation i.p. lesions were still visible, but by 7 months there was invariably either resolution or fibrosis of the lesions, associated with local peritoneal and intestinal adhesions. Local footpad swelling was noted 2 days after inoculation into that site, and this persisted until 21 days or longer. On dissection of the footpad, macroscopic lesions were invariably found at 11 and 21 days.

The histopathological lesion produced by *N. asteroides*, both by i.p. and by footpad inoculation of the organism, was characteristic (Fig. 1). By 11 days after inoculation there was a localized lesion with central necrosis surrounded by large numbers of macrophages and sheets of histiocytes which did not show evidence of obvious phagocytic activity. Plasma cells were prominent in the marginal area. Intracellular organisms were not noted within the macrophages. Small clusters of organisms, often surrounded by a minute rim of granulocytes, were scattered throughout the lesion. With a single exception, hyaloid material was not noted in the *asteroides* lesions. At the periphery of the central necrotic area dense accumulations of Ziehl Neelsen-positive material were frequently found (Fig. 2). Surrounding this reaction was a thin fibrous zone. In the later stages (45 and 60 days) compact sheets of histiocytes in the peripheral zone dominated the local histological response. These cells increased in size and in number with time. Fibrosis and capsule formation were scantily developed even at the later stages, and they took the form of fine strands of reticulin. The fibrosis increased to a limited extent with time.

With regards to the *brasiliensis* lesion, it was found that at each stage of its development the histological features differed significantly from those of the *asteroides* lesion. The pattern was that of a granuloma, which by 11 days had a highly distinctive appearance. The lesions had a multilocular pattern; the number of locules tended to increase with the age of the lesion so that at 11 days there were 5 to 10 locules, and at 3 weeks between 10 and 30 locules were noted. Each individual locule had well-defined zones (Fig. 3). At the center was a felt-like mass of

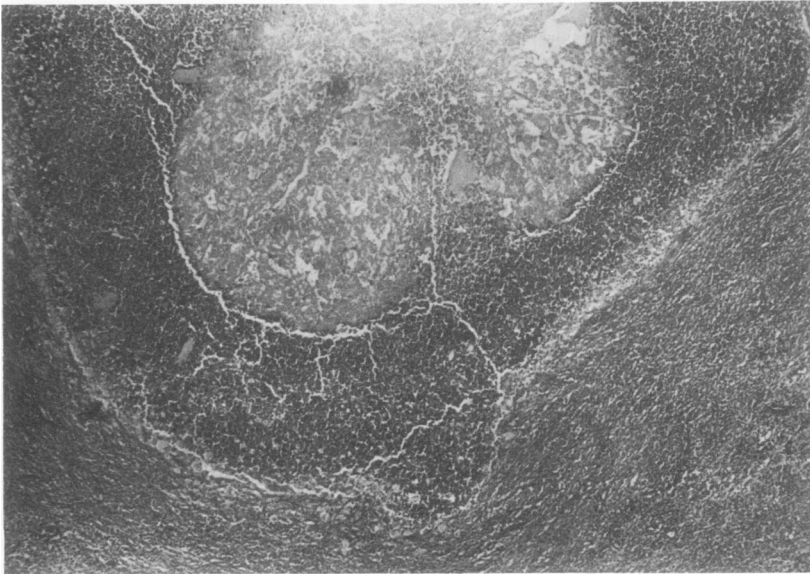


FIG. 1. *N. asteroides*. I.P. lesion (omentum) 11 days after inoculation. Haematoxylin and eosin. $\times 25$.

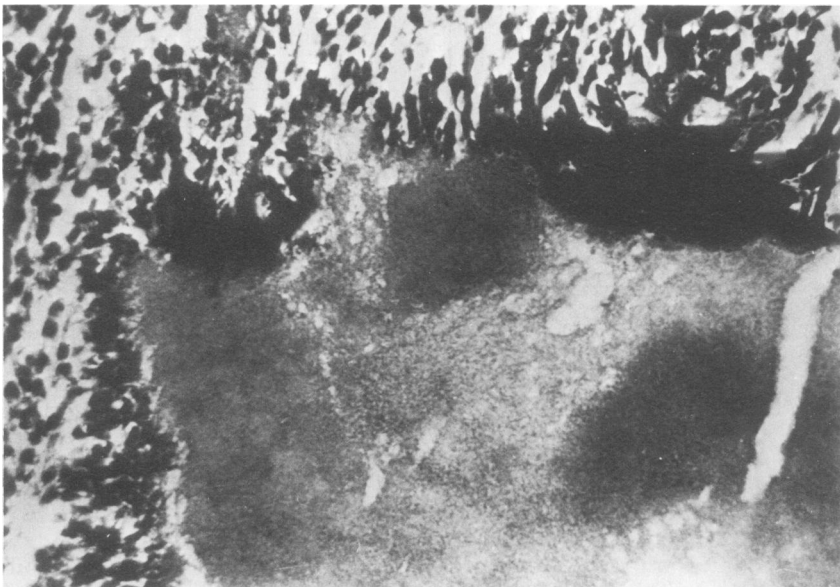


FIG. 2. *N. asteroides*. I.P. lesion (omentum) 21 days after inoculation. Dense accumulations of acid-fast material are seen at the periphery of the necrotic area. Ziehl-Neelsen. $\times 378$.

organisms forming a granule, usually circular. The edge of the granule was frayed, and it appeared eosinophilic. Granulocytes were tightly packed between the projecting strands and around the granule. Hyaloid material could be seen in the granule and at the junctional zone between the host and the parasite. This hyaloid material had definite staining characteristics with various histochemical

stains (Table 1). Surrounding the zone of compact granulocytes was a wider zone with both granulocytes and histiocytes in various proportions. Phagocytosis was seen in this area, and occasionally scattered organisms were found between the cells (Table 1). Even by 10 days foamy macrophages were apparent, especially at the periphery of the locule (Fig. 4). The thickness of this foam cell layer varied and was

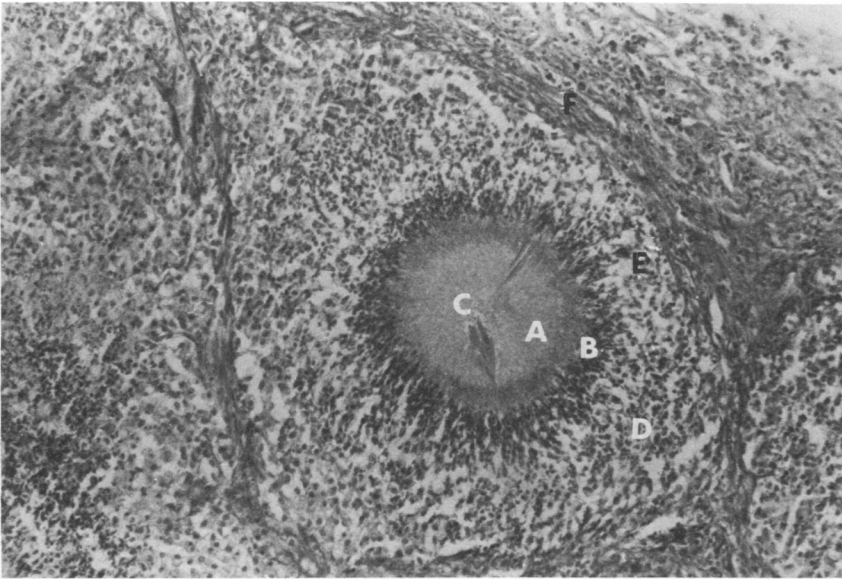


FIG. 3. *N. brasiliensis*. I.P. lesion (omentum) 11 days after inoculation. Symbols: (A) Central granule consisting of masses of organisms; (B) granulocytic layer; (C) hyaloid material; (D) zone of granulocytes and histiocytes; (E) foamy macrophages; (F) rim of fibroblastic cells with concentric orientation. Hematoxylin and eosin. $\times 100$.

TABLE 1. Staining characteristics of *N. asteroides*, *N. brasiliensis*, and the hyaloid material

Stain	Staining reaction	
	<i>N. asteroides</i> and <i>N. brasiliensis</i>	Hyaloid material
Hematoxylin and eosin	Variable	Strongly eosinophilic
Periodic acid-Schiff	Strongly positive	Negative
Ziehl Neelsen	Variably positive; weak	Strongly positive
Giemsa	Purple	Pink
Van Kossa	Negative	
Masson trichrome	Green	Red
Refractibility		Positive; variable
Congo red, polarized light		Negative

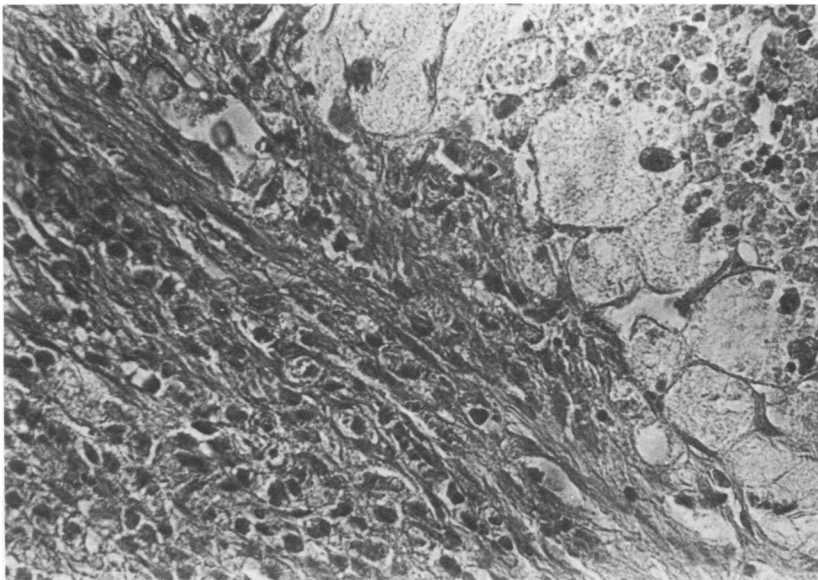


FIG. 4. *N. brasiliensis*. I.P. lesion (omentum) 11 days after inoculation. The illustration demonstrates foamy macrophages at the periphery of the locule. Giemsa stain. $\times 252$.

greatest where necrosis was found in the area of the mixed granulocytic and macrophage response. A feature of the *brasiliensis* lesion, not noted in the case of *N. asteroides* infection, was the presence of organisms within the histiocytic cells. The locule was limited by a rim of fibroblastic cells with concentric orientation, separating one locule from the next. By 21 days the layer of foamy macrophages had become prominent. The cells were swollen and contained vast amounts of clear, bubbly cytoplasm. Mast cells were conspicuous, and the number of lymphocytes increased with time. By 40 days plasma cells, some with globular secretion, appeared in the fibrous zone. Calcification became apparent at the junction of the histiocytic fibrous zone, especially in areas of necrosis.

It is important to qualify the above description of the histological findings by two points. In the first place, some differences were noted between the i.p. and the footpad lesions with respect to the *N. asteroides* infection. Although the features of the lesions in the two sites were essentially similar, the footpad lesions tended to be more extensive and destructive, and the cellular composition more compact than the i.p. equivalent. These differences can probably be ascribed to local and regional tissue factors. No notable differences were noted in the *brasiliensis*

lesion in the two sites. Secondly, although clear-cut differences were noted in the lesions produced by *N. asteroides* and by *N. brasiliensis* in the vast majority of the mice studied, these differences were not invariable. In particular, occasionally *N. brasiliensis* was found to produce an *asteroides*-type lesion. This unusual occurrence was probably due to individual immunological factors operating within a particular mouse.

In the case of both *N. asteroides* and *N. brasiliensis* i.p. and footpad infections, systemic abscess formation in other organs was not noted as a rule. With both organisms an occasional microabscess was noted in the liver. In the case of the footpad lesion, inguinal lymphadenopathy with sinus histiocytosis was sometimes found.

Electron microscopic studies of the *N. brasiliensis* lesion demonstrated conclusively the presence of organisms within the cytoplasm of the foamy macrophages. These organisms were noted in the intact form and in various stages of degeneration (Fig. 5, 6).

DISCUSSION

Our findings are in accordance with those of Uesaka et al. (13). It is possible to distinguish a characteristic *asteroides* lesion and a *brasiliensis*

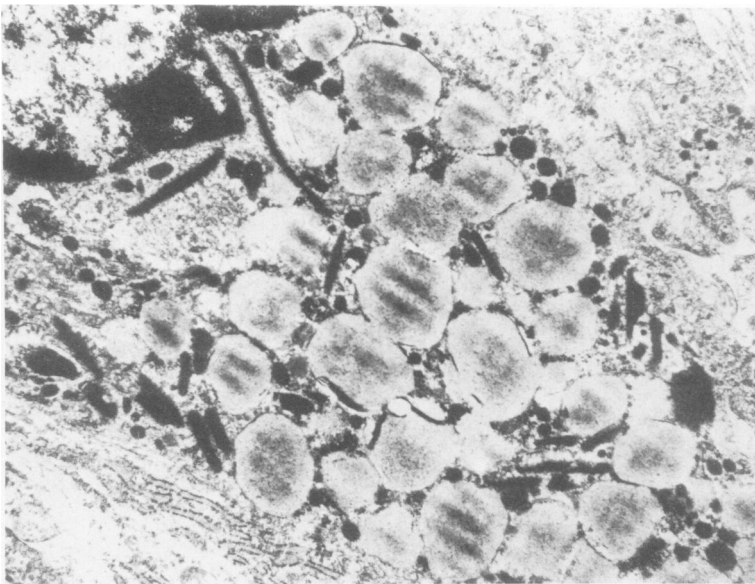


FIG. 5. *N. brasiliensis*. Footpad lesion 21 days after inoculation. Electron micrograph showing portion of a foamy macrophage. At the upper left hand corner the edge of the nucleus is seen with clumps of chromatin condensed along the nuclear membrane. The cytoplasm of the cell contains numerous organisms as well as lipid vacuoles. $\times 12,000$.

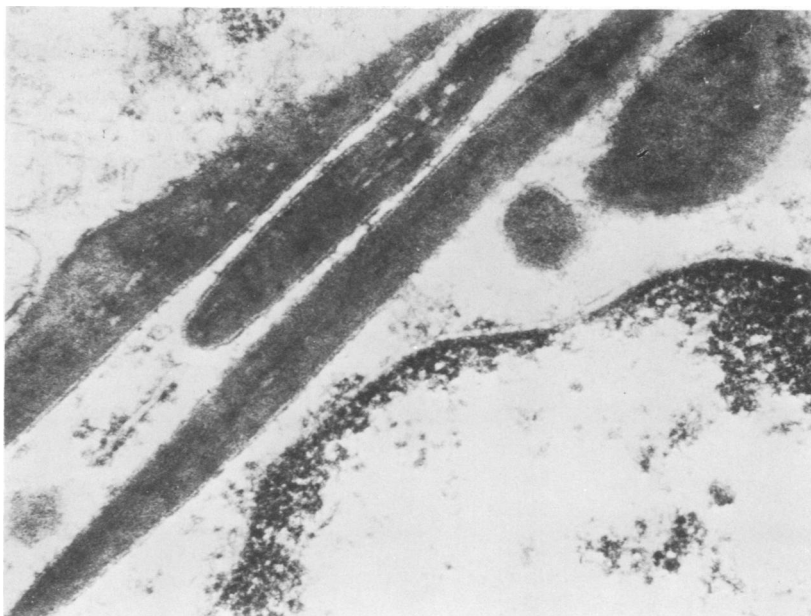


FIG. 6. *N. brasiliensis*. Foodpad lesion 21 days after inoculation. Electron micrograph showing relatively intact organisms lying within the cytoplasm of a macrophage in close proximity to the nuclear membrane. The outer cell membrane of the organisms, and a laminated internal structure can be clearly seen. $\times 60,000$.

sis lesion histopathologically. As adjuvant was not added to the inoculum in our experiments, these differences can only be due to differing responses by the mouse to the two organisms.

The microscopical features of the lesion of *N. brasiliensis* are of special interest. The presence of large numbers of foamy macrophages, and the presence of organisms within the cytoplasm of the macrophages are reminiscent of the features noted in lepromatous leprosy in man, and of systemic *Mycobacterium bovis* (BCG strain) infection in the immune-suppressed mouse (10, 12). There is considerable evidence that in lepromatous leprosy there is an immune defect in the cellular-immune function (9). If, indeed, there is a parallel between the lesions of *M. leprae* (lepromatous form), *M. bovis* infection in the congenitally athymic (nude) mouse, and *N. brasiliensis*, it is possible that the pathological effects of *N. brasiliensis* are related to inactivation of normal T-lymphocyte function by the organism. This property does not appear to be shared by *N. asteroides*. Such a hypothesis is one which can be further tested.

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