JOURNAL OF BACTERIOLOGY, Sept. 1969, p. 851-855
Vol. 99, No. 3

Comparison of the Pathogenicity for Mice of *Mycobacterium fortuitum* and *Mycobacterium abscessus*

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Received for publication 10 February 1969

The pathogenicity for mice of 12 strains of *Mycobacterium abscessus* was compared with that for 8 strains of *M. fortuitum*. Both species caused lesions in kidneys and produced "spinning disease" resulting from inner ear infections. No major differences in pathogenicity of these two species were demonstrated. Strain to strain variation was marked, especially with *M. abscessus*. For example, 1.6×10^6 organisms of strain 11188 of *M. abscessus* produced death in four of five animals within 42 days, whereas strain 380 of *M. abscessus* failed to produce any deaths within 42 days. In the case of *M. fortuitum*, the greatest mortality observed was one of five animals, yet the incidence of spinning disease and kidney disease occurred earlier postinfection than in mice infected with *M. abscessus*. Histologically, abscess formation by a strain of *M. abscessus* was greater than by a strain of *M. fortuitum*, but this difference cannot be interpreted as a species difference.

Among the Runyon group IV acid-fast organisms that cause disease in man, *Mycobacterium fortuitum* is the only species taxonomically well characterized. *M. abscessus* was first described in 1953, when Moore and Frerichs (7) reported the case of a 63-year-old white woman in whom infection of the knee was followed by subcutaneous, abscess-like lesions of the gluteal region. Recently, pulmonary granulomatous disease in man caused by this species has also been reported (4, 15, 16). *M. runyonii*, a rapidly growing, acid-fast bacillus, was first described by Bojalil and associates in 1962 (1). Tsukamura and co-workers (15) and Saito et al. (10) have disclosed, on the basis of Adansonian classification methods (13), that *M. runyonii* is a synonym of *M. abscessus*. Although *M. abscessus* has many properties identical to *M. fortuitum*, *M. abscessus* can be distinguished from *M. fortuitum* by biological and biochemical tests (11, 14). Although *M. fortuitum* is known to cause kidney lesions and spinning disease in mice (5, 17), pathogenicity studies of *M. abscessus* have not been recorded. The present study was undertaken to ascertain the possible pathogenicity of *M. abscessus* for mice and to determine whether this species and *M. fortuitum* can be distinguished by this property. A brief report on pathogenicity of some of the strains used here was published previously (9).

MATERIALS AND METHODS

Cultures. Stock cultures of 12 strains of *M. abscessus* and 8 strains of *M. fortuitum*, maintained on 1% Ogawa egg medium (8), were transferred into Tween-albumin broth. At least two passages were made in this liquid medium before the organisms were used in infection experiments. The cultures were incubated at 37 C for 3 to 4 days before animal inoculation. The size of the infecting dosage was determined by colony counts of appropriate dilutions of the cultures inoculated onto 1% Ogawa egg medium.

Animal inoculation. Tween-albumin broth cultures of each organism tested were adjusted to an optical density of 0.15 at 540 nm on a Coleman Junior Spectrophotometer. An 0.1-ml portion of this suspension was injected into the tail vein of each of five male mice (ddY-F) weighing approximately 20 g. The mice were weighed weekly and observed daily for the appearance of spinning disease as described by Gorrill (3).

Autopsy was carried out immediately after spontaneous death or upon termination of the study period, which lasted until 6 weeks after infection. Observations of gross disease were made on the lungs, liver, spleen, and kidneys at necropsy, and portions of each visceral organ were removed for culture. The homogenates of each organ were treated with 1% sodium hydroxide and inoculated into two tubes of 1% Ogawa egg medium. The number of culturable organisms per milligram of lung, liver, spleen, or kidney was then determined. Portions of the visceral organs of mice infected with the *M. abscessus* strain 481 and with *M. fortuitum* ATCC 6841 were fixed in 10% Formalin. In addition, the skulls of two mice

with spinning disease, selected from each of these groups, were also fixed in 10% Formalin for histological studies. Tissue sections were made from all fixed tissues and stained with either hematoxylin and eosin or by the Ziehl-Neelsen method.

RESULTS

All eight strains of *M. fortuitum* produced the characteristic spinning disease in which the mice held their heads to one side and often showed shaking and twitching movements of the heads; such mice, suspended by their tails, rotated vigorously. With the exception of strains 380 and T-42-2, similar behavior was also observed in mice infected with *M. abscessus*. The overall incidence of spinning disease was 42% of the total *M. abscessus*-infected mice and 78% in the *M. fortuitum*-infected animals (Table 1). Of the mice, the 84% that exhibited spinning disease did so by the 20th day, whereas 74% of the *M. fortuitum*-infected mice did so by the 10th day. None of the animals infected with *M. abscessus* showed spinning disease within 10 days after inoculation. The probable cause of spinning disease is considered in the discussion.

Of the 60 mice inoculated with *M. abscessus*, 17 (28%) died within 38 days, and the average survival time was 26.5 days. The surviving 43 were sacrificed 42 days after infection. On the other hand, of the 40 mice inoculated with *M. fortuitum*, 6 (15%) died within 36 days, with an average survival time of 21.3 days; the remaining 34 were sacrificed 42 days after infection.

TABLE 1. *Spinning disease and gross postmortem findings in mice infected with M. abscessus and M. fortuitum*

Finding	*M. abscessus* (60 mice)	*M. fortuitum* (40 mice)
Spinning disease		
No. of strains producing spinning disease in some mice	10 of 12 tested	8 of 8 tested
Per cent of inoculated mice showing spinning disease	42%	78%
Kidney lesions		
No. of strains producing grossly evident kidney lesions	10 of 12 tested	8 of 8 tested
Per cent of inoculated mice showing grossly evident kidney lesions	64%	98%
Other lesions		
Gross lung, liver, or spleen disease	0	0

Whether the mice were infected with *M. abscessus* or *M. fortuitum*, gross disease was apparent only in kidneys, with no evidence of lesions in the lungs, liver or spleen. All but two strains of *M. abscessus* (Yamamoto and Sato) and all of the strains of *M. fortuitum* produced abscess-like lesions of the kidney in some or all of the mice inoculated. The overall incidence of renal lesions was 64 and 98% of the mice inoculated with *M. abscessus* and with *M. fortuitum*, respectively. In most of the animals, renal involvement was limited, since 22 of 38 animals (61%) inoculated with *M. abscessus* and 31 of 39 (79%) inoculated with *M. fortuitum* showed only a few lesions. Extensive renal lesions were encountered in only 16% of the animals inoculated with *M. abscessus*.

The 12 strains of *M. abscessus* yielded cultures from 41% of the lungs, 47% of the livers, 64% of the kidneys, and 63% of the spleens of 59 mice inoculated with these strains. Thirty per cent of the lungs, livers, and spleens, and 73% of the kidneys in the 40 mice inoculated with the eight strains of *M. fortuitum* were positive on culture. The number of organisms recovered was generally greatest in the kidney, regardless of whether the mice were injected with *M. abscessus* or *M. fortuitum*.

The visceral organs of five mice inoculated with *M. abscessus* strain 481 and four inoculated with *M. fortuitum* ATCC 6841 were selected for histological observations (Table 2). In one animal infected with *M. abscessus*, bronchopneumonia was found. In the lung of one animal infected with *M. fortuitum*, a microabscess was observed. With the exception of one mouse infected with *M. abscessus*, no acid-fast organisms could be demonstrated in the tissues of the lungs. In the liver of the mice inoculated with *M. abscessus*, discrete foci, consisting of cellular infiltration by mononuclear cells, were revealed at the periphery of acini and in the Glison's sheath. A microabscess and widespread necrosis were also ob-

TABLE 2. *Histopathology of visceral organs and cochlea in mice infected with M. abscessus and M. fortuitum*

Histopathology	*M. abscessus* strain 481 (5 mice)	*M. fortuitum* strain 6841 (4 mice)
Kidney lesions	++++	+
Lesions in other viscera	+	+
Skull lesions (cochlea)	++[a]	+[a]

[a] Two mice.

served in two other animals. The livers of the mice inoculated with *M. fortuitum* showed only cellular infiltration in the Glison's sheath by polymorphonuclear leukocytes and mononuclear cells. With the exception of one mouse infected with *M. abscessus*, acid-fast bacilli were not detected in sections from the livers. Regardless of which organism was inoculated, the spleens of infected mice revealed atrophy of the splenic nodules and the following two types of lesions, either alone or in combination: diffuse infiltration of mononuclear phagocytes and polymorphonuclear leukocytes with accompanying Langhans giant cells and reticulum cell hyperplasia associated with Langhans giant cells. Acid-fast organisms could not be demonstrated in the lesions. The kidneys (Fig. 1) of animals inoculated with *M. abscessus* invariably possessed cortical abscesses, in which enormous numbers of acid-fast bacilli were observed. Medullary microabscesses were less frequently encountered, and the capsule was rarely affected. In striking contrast to this very extensive renal involvement associated with *M. abscessus* was the degree of disease produced by *M. fortuitum*. In the latter case, microscopic disease, when present, as indicated by serous exudation in the glomeruli and infiltration of mononuclear cells into the periglomerular connective tissues.

The skulls of two animals infected with *M. abscessus* strain 481 and two animals infected with *M. fortuitum* ATCC 6841 were decalcified, and tissue sections were prepared for histological studies. In the animals infected with *M. abscessus*, the most characteristic lesions were the massive abscesses with necrosis of the cochlea (Fig. 2). Short acid-fast rods and a loose network of long acid-fast filaments were demonstrable in these lesions. An abscess with numerous acid-fast bacilli was also detected in the cerebrum of one mouse. The mice infected with *M. fortuitum* also had involvement of the cochlea. However, histologically a more striking difference was observed. Unlike the lesions in *M. abscessus*-infected mice, the cochlear lesions produced by *M. fortuitum* showed a tendency toward granulomas, and abscesses were rarely encountered (Fig. 3). Acid-fast stains of the tissues revealed large numbers of bacilli. In the leptomeninges, close to the inner ear, a focal collection, consisting predominantly of lymphocytes, was observed. Organisms were demonstrated in moderate numbers in the lesions. However, none of the animals infected with either *M. abscessus* or *M. fortuitum* showed cerebellar involvement.

DISCUSSION

Spinning disease in mice was produced by all 8 strains of *M. fortuitum* and by 10 of 12 strains of *M. abscessus* studied. This interesting neurological condition was very similar in appearance for both mycobacterial species. However, in the *M. fortuitum*-infected mice, the overall incidence of spinning disease was much higher and generally appeared much earlier than in the *M. abscessus*-infected animals. The development of conditions

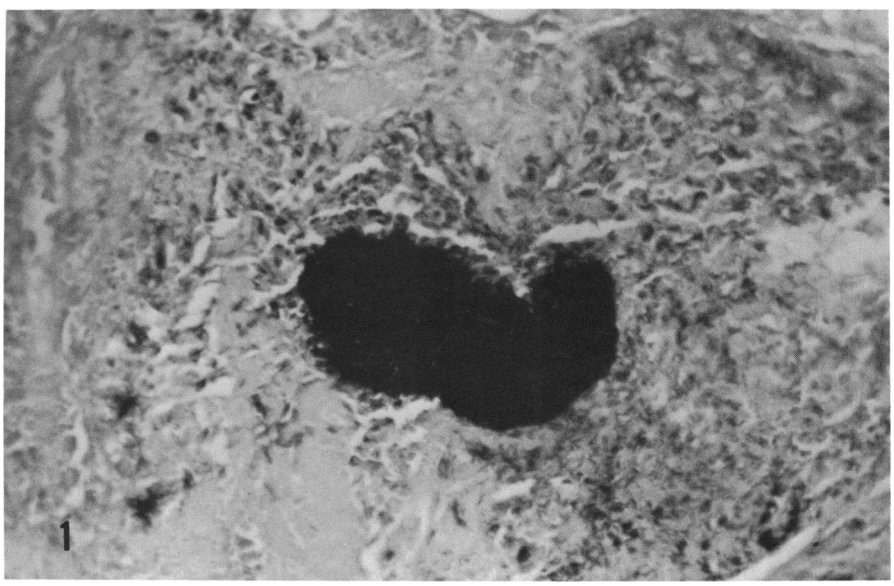

FIG. 1. *Acid-fast stain of kidney lesions in an M. abscessus-infected mouse showing masses of organisms. Ziehl-Neelsen stain. × 400.*

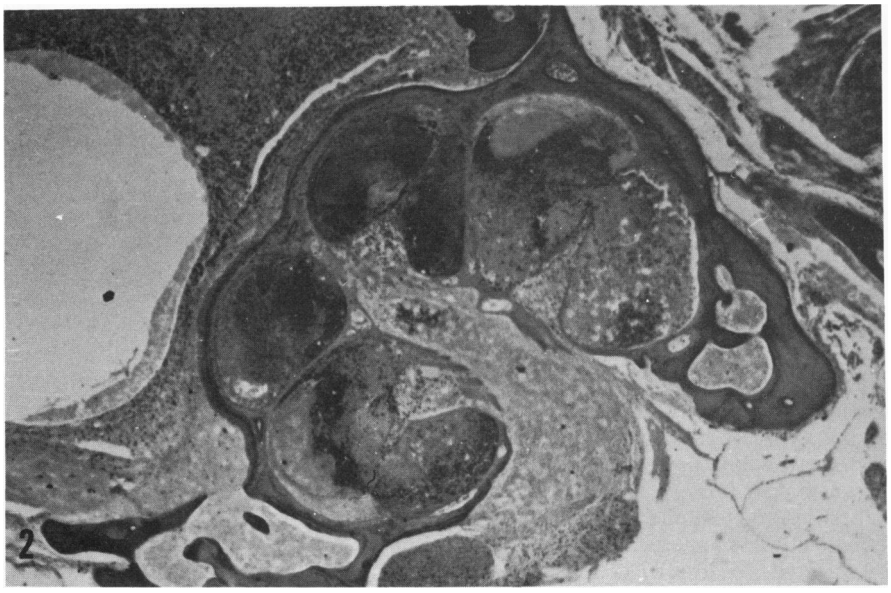

FIG. 2. *Cochlea of an M. abscessus-infected mouse showing abscesses. Hematoxylin and eosin stain.* × *40.*

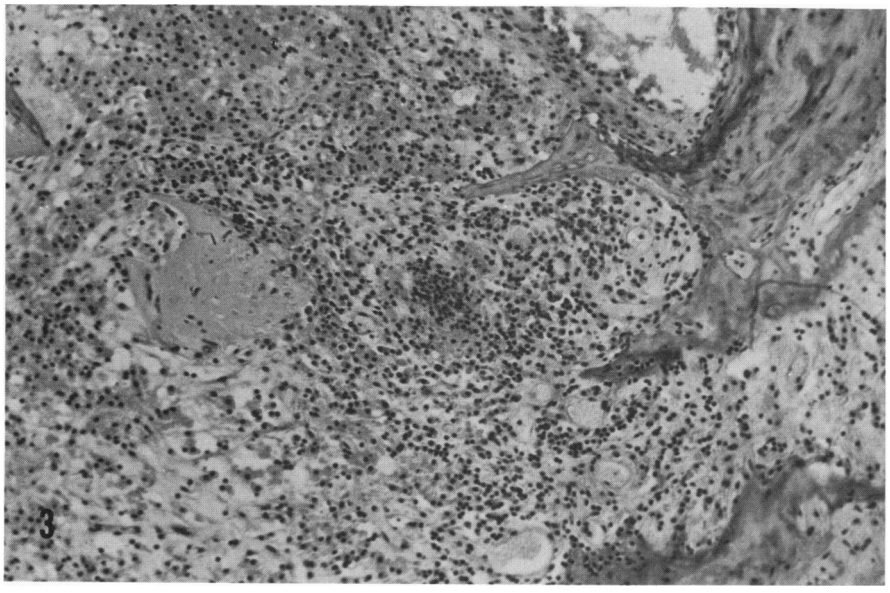

FIG. 3. *Cochlea of an M. fortuitum-infected mouse showing diffuse infiltration of epithelioid-like cells and a microabscess. Hematoxylin and eosin stain.* × *200.*

like spinning disease after intravenous injection with acid-fast organisms has already been reported (5, 6, 12, 17). Levaditi and co-workers (6) showed that the outstanding histological feature in mice with spinning disease is the presence of miliary abscesses in the brain, but these workers did not include any account of the inner ear. Saito (12) noticed granulomas in the cerebrum or cerebellum and mild cellular infiltration of the inner ear in mice which developed spinning disease after injection with saprophytic mycobacteria. Gorrill (3) observed spinning disease in some of the mice injected intravenously with *Pseudomonas pyocyanea* and found that the mice with spinning disease had some involvement of the inner ear. In view of the present limited

studies, the occurrence of spinning disease, caused either by *M. abscessus* or by *M. fortuitum*, might be accounted for by severe inner ear infection, although limited observations were made. Some differences in histological findings of the lesions caused by each organism were present. The lesions caused by *M. abscessus* were uniformly abscesses, whereas the lesions caused by *M. fortuitum* had many granulomatous characteristics, and only rarely did discrete abscesses occur.

Intravenous injection of mice with either *M. abscessus* or *M. fortuitum* resulted in gross abscess-like lesions in the kidneys, whereas other organs apparently remained free from disease. This organ specificity had previously been noticed with *M. fortuitum*, *P. pyocyanea*, and staphylococci (2, 5, 17). The lesions in the kidneys of mice infected with *M. abscessus* strain 481 were uniform abscesses similar to those observed by Wells and co-workers (17) and Kushner and associates (5) in animals infected with *M. fortuitum*. However, the lesions in the kidneys produced by *M. fortuitum* ATCC 6841 were, at most, merely mild cellular infiltrations by mononuclear cells. This discrepancy between our results and the results of Wells and co-workers (17) and of Kushner and associates (5) may depend on the number of organisms injected. Further studies on this point may be necessary.

ACKNOWLEDGMENTS

We thank Humio Hirose and Yoshio Sugihara for their instruction in histopathological findings. We also thank Kaoru Urabe for helpful suggestions in the preparation of this manuscript.

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