Experimental Arthritis in Mice with

*Mycoplasma pulmonis*

JERRI A. BARDEN¹ AND JOSEPH G. TULLY

Laboratory of Infectious Diseases, and Laboratory of Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

Received for publication 6 June 1969

A *Mycoplasma pulmonis* strain, recovered from the arthritic joints of mice employed in the serial passage of a chemically induced tumor, was found to be arthritogenic for mice under experimental conditions. Some joint involvement occurred in all mice challenged intravenously with this strain, and *M. pulmonis* was recovered frequently from the enlarged joints. The arthritis was migratory, appearing first in the radiocarpal joints and later in the tibiotarsal joints. There was little evidence of a generalized mycoplasmal infection as a consequence of the experimental challenge. Histopathologically, the early stages of the infection in the joints was characterized by an inflammatory response in the synovium and periarticular tissues. Exudate in the joint space contained about equal numbers of polymorphonuclear and mononuclear cells. The polyarthritis resolved slowly, but some residual joint enlargement was noted for as long as 4 months. Two other *M. pulmonis* strains were also observed to be arthritogenic for mice. Rats were not susceptible to *M. pulmonis* challenge. Characteristics of the nonsuppurative *M. pulmonis* arthritis in mice were compared to *M. arthritidis* joint infections in rats.

Arthritis is a common manifestation of many natural and experimentally induced mycoplasmal infections in animals. *Mycoplasma arthritidis*, a natural pathogen of rats, has been shown to produce synovitis and periartthritis in this species under experimental conditions as well as in natural outbreaks of the disease (4, 7, 13, 25). An acute, nonsuppurative, polyarthritis, usually in conjunction with other systemic involvement, has been observed in chickens and turkeys infected with *M. gallisepticum* or *M. synoviae* (1, 11, 12, 19) and in swine infected with *M. hyorhinis* or *M. granularum* (14, 15, 22). Although many of these acute infections resolve and the hosts recover, some animals show periods of exacerbation and remission of the arthritis. The pathological response in the joints to the avian and swine mycoplasmal infections, the observed predisposing factors to disease, and the chronic nature of the infections tend to suggest some similarities to human rheumatoid arthritis (9). *M. arthritidis* invasion of joints and other tissues of the rat has been described more frequently as a septic infection (20, 25). The earliest lesions are observed in the synovium and periarticular tissue, and some purulent exudate, large numbers of polymorphonuclear cells, and microabscess formation can usually be seen in those areas. However, the early lesions may also contain only lymphocytes and mononuclear cells (21).

Sabin (16-18) described a progressive, proliferative, migratory arthritis in mice following the intravenous inoculation with a *Mycoplasma* strain (type B) isolated from the brain and nasal mucosa of normal mice. Arthritis was seen less often when the mycoplasmas were given intraperitoneally, but, by either route, the challenge culture localized only in the joints where it ultimately induced a chronic infection and ankylosis after 2 to 5 months. Pathological changes, apparently limited to the joints, consisted primarily of a cellular proliferation in the synovial membrane, capsule, and perichondrium, in addition to some necrosis of the articular cartilage. Joint exudate in the early stages of the infection contained about equal numbers of mononuclear and polymorphonuclear leukocytes. The mycoplasmas retained their pathogenicity through 50 or more broth passages, but the culture was eventually lost in the early 1940's.

The recovery of a *Mycoplasma* strain from an outbreak of arthritis in mice receiving tumor-passage material and the subsequent typing of this strain as *M. pulmonis* provided an opportunity to examine the ability of this and other
**M. pulmonis** strains to induce infectious arthritis in mice.

**MATERIALS AND METHODS**

**Recovery of arthritogenic mycoplasmas.** *M. pulmonis* strain JB was recovered from the arthritic joint of a mouse that had received methylcholanthrene-induced tumor material. The initial introduction of the drug into mice was made intramuscularly into the hind leg. Tumors were subsequently observed at the site of inoculation and in the lung, the latter apparently representing metastasis. Tumor suspensions prepared from leg and lung were passed intramuscularly into the hind leg of new mice. Tumor material obtained only from the leg was again passed into new mice. Three months after the second tumor passage, all mice developed joint enlargement of the rear ankle. Several mice were sacrificed, the hind legs were severed at the hip, and the skin was removed. The ankle joint was cut, and fluid expressed from the cut surface was plated on a solid medium consisting of 70% Mycoplasma Broth Base (Difco), 20% horse serum (BBL), 10% fresh yeast extract (Microbiological Associates, Inc., Bethesda, Md.), and bacterial inhibitors (6). The mycoplasmas recovered were cloned and typed as *M. pulmonis* by indirect and direct fluorescent-antibody techniques (2, 23). The fermentation of glucose and the colonial morphology of the isolates were also compatible with the characteristics of other *M. pulmonis* strains (23).

**Experimental *M. pulmonis* infection.** Pools of the JB strain at the 3rd, 10th, and 33rd broth passages were prepared in a medium similar to that described above, except that the broth base was increased to 89% and 1% serum fraction (Difco) was substituted for the horse serum. Bacterial inhibitors were omitted, except for penicillin (500 units/ml). The cultures were incubated aerobically for 2 to 4 days until the broth possessed a slight turbidity. Plate counts of challenge inocula indicated that approximately 10^7 colony-forming units (CFU)/ml were present in 96-hr cultures of the pass 3 material, and 10^6 CFU/ml were present in 48-hr cultures of pass 10 and pass 33. Female NIH general purpose mice (9 to 11 g) were given 0.5 ml of the undiluted culture intravenously. Nine other *M. pulmonis* strains (PG-34, PG-22, Kon, M1, WRAIR, Kenny 47, Negroni, and 880) were also inoculated intravenously into mice in 0.5-ml quantities of undiluted 48-hr broth cultures. Osborne-Mendel rats weighing 70 to 80 g were also challenged intravenously with either 0.5 or 1.0 ml of a 48-hr culture of the 3rd and 15th passage of the JB strain. Control groups of mice or rats receiving the same quantity of uninoculated broth by the intravenous route were also included. The amount of joint involvement in challenged animals was graded on a 1 to 4+ basis, with erythema and minimal enlargement of any one joint designated 1+.  

**Mycoplasma isolation and identification techniques.** Mice with and without arthritis were sacrificed at various intervals after challenge, and their tissues were cultured for mycoplasmas. Blood drawn by cardiac puncture was added directly to 10 ml of broth medium. The brain, lung, liver, spleen, and joints were removed aseptically and minced into 18 ml of broth containing 20% horse serum and bacterial inhibitors. The tissue suspensions were diluted immediately in fresh medium to 1:10 and 1:50 and incubated at 37 C, some aerobically and others in an atmosphere of 95% nitrogen, 5% carbon dioxide. Subcultures were made at 4, 7, 10, and 14 days to both mycoplasma agar plates and broth. Some joints were also cultured directly on mycoplasma agar plates in a manner similar to that reported above, and the plates were incubated either aerobically or anaerobically. All recovered mycoplasmas were identified as *M. pulmonis* by their agar colony fluorescence when treated with a 1:15 to 1:30 dilution of fluorescein-tagged antisera to the PG-34 strain of *M. pulmonis* (2). Comparable joints obtained from other mice at the time of autopsy were fixed in 10% buffered Formalin and stained with hematoxylin and eosin for histological examination.

**RESULTS**

**M. pulmonis arthritis.** One week after intravenous inoculation with third passage JB strain, all mice showed erythematous and swollen forefeet with an apparent involvement of the radiocarpal (wrist) joint (Fig. 1). In some animals only a single limb showed arthritis, but in others both wrists were enlarged. However, the arthritis did not appear to extend to the interphalangeal joints. At the end of the 2nd week after inoculation, enlargement of the tibiartarsal (ankle) joint was noted, whereas the arthritis in the wrist seemed to be subsiding. Enlargement of the ankle joint continued to occur during the next week, and in some mice the leg eventually appeared to be two to three times the normal size (Fig. 2). At 4 to 6 weeks after inoculation, the ankle joint enlargement had generally receded, but the swelling and enlargement of the wrist joint returned, usually.

![Fig. 1. Arthritis developing in mouse 1 week after inoculation with *M. pulmonis* JB (pass 3). Note more severe involvement (3+) of lef't wrist joint over right wrist (1+).](http://jb.asm.org/)
to a lesser extent than was initially observed. Continued decrease of joint swellings occurred over the next 2 or 3 weeks, but some animals exhibited residual enlargement of the ankle joint for as long as 4 to 5 months after challenge.

Mice receiving the 10th or 33rd passage JB strain responded, not only with more rapid development of arthritis, but the joint enlargement was greater (Table 1). This enhanced response is consistent with the increased number of mycoplasmas present in the challenge inocula at these later passage levels. Arthritis was again present in all mice inoculated with the *M. pulmonis* culture, and the migratory characteristics of the infection were confirmed.

In only one instance was there evidence of systemic disease arising from the challenge. One mouse receiving the third passage material exhibited chills, weight loss, and ruffled fur during a 2-week period after inoculation. *M. pulmonis* was isolated from the spleen and the ankle joint of this animal. No other mice showed evidence of systemic involvement; they remained well, except for the joint infection.

**Recovery of mycoplasmas.** *M. pulmonis* was recovered frequently from the joints of mice exhibiting arthritis. With exception of the mycoplasmas recovered from the spleen of the animal described above, there were no isolations from the organs of other mice. However, organ cultures were not taken earlier than 2 weeks and prior to onset of joint disease. The joints of some mice that earlier had shown arthritis, and where culture attempts were made after the joint swelling had subsided, did not yield mycoplasmas. However, *M. pulmonis* was recovered from the arthritic joints of some mice for as long as 6 weeks. It is also important to note that *M. pulmonis* was recovered from the joints of the methylcholanthrene-tumor passage mice 1 year after the onset of arthritis. At the time cultures were taken, the joints exhibited only a minimal amount of enlargement.

There were no apparent differences in the recovery rate of mycoplasmas from infected joints of mice when cultures were incubated aerobically or anaerobically, or when direct primary plating to agar plates or fluid broth cultures were employed. Rats given a 0.5- to 1.0-ml intravenous challenge of the 3rd or 15th passage of the JB strain did not develop arthritis during a 4-week observation period. All control animals receiving

**FIG. 2. Arthritis in a mouse 2 weeks after inoculation with *M. pulmonis* JB (pass 3).** Note extensive (3-4+) enlargement of both ankle joints and absence of arthritis extension to the tarsal-metatarsal joints.

**Table 1. Mycoplasma pulmonis arthritis in mice**

<table>
<thead>
<tr>
<th><em>M. pulmonis</em> challenge (strain and origin)</th>
<th>Passage level</th>
<th>No. arthritis/no. inoculated</th>
<th>Joint enlargement (grade 1+ to +++)</th>
<th><em>M. pulmonis</em> recovery from joints</th>
</tr>
</thead>
<tbody>
<tr>
<td>JB (mouse joint)</td>
<td>3</td>
<td>5/5</td>
<td>3+</td>
<td>+ (3 weeks)</td>
</tr>
<tr>
<td>JB (mouse joint)</td>
<td>10</td>
<td>10/10</td>
<td>4+</td>
<td>+ (6 weeks)</td>
</tr>
<tr>
<td>JB (mouse joint)</td>
<td>33</td>
<td>10/10</td>
<td>4+</td>
<td>ND</td>
</tr>
<tr>
<td>WRAIR (rat blood)</td>
<td>11 + 5</td>
<td>6/10</td>
<td>1+</td>
<td>+ (6 weeks)</td>
</tr>
<tr>
<td>WRAIR (rat joint)</td>
<td>13 + 5</td>
<td>2/10</td>
<td>1+</td>
<td>- (5 weeks)</td>
</tr>
<tr>
<td>Negroni (tissue culture)</td>
<td>? + 10</td>
<td>2/10</td>
<td>1+</td>
<td>+ (7 weeks)</td>
</tr>
<tr>
<td>Negroni (tissue culture)</td>
<td>? + 13</td>
<td>5/10</td>
<td>1+</td>
<td>ND</td>
</tr>
<tr>
<td>PG-34 (rat lung)</td>
<td>? + 60</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PG-22 (mouse lung)</td>
<td>? + 53</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Kon (rat lung)</td>
<td>? + 60</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M1 (mouse lung)</td>
<td>? + 53</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Kenny 47 (rabbit oral)</td>
<td>? + 20</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Nelson A (mouse middle ear)</td>
<td>8 + 11</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>880 (tissue culture)</td>
<td>? + 18</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* First number represents passages from initial isolation, and second number represents passages in this laboratory (LM); (?) indicates unknown number of passages prior to receipt.

a Not done.
uninoculated broth intravenously remained well and did not develop arthritis.

**Histopathology.** Sections of the joints in the early stages of the arthritis showed an inflammatory response in the synovium and periarticular tissues. The exudate in the joint space consisted of about equal numbers of polymorphonuclear and mononuclear cells (Fig. 3). There was no evidence of a generalized purulent infection or microabscess formation. Histological examination of joints after the joint enlargement had receded showed the disappearance of polymorphonuclear cells and decreased amounts of exudate in the joint space. The histopathological response was primarily confined to the tibiotarsal (ankle) and radiocarpal (wrist) joints and associated periarticular tissues (Fig. 4).

**Response of mice to other M. pulmonis strains.** Arthritis was observed in only two other groups of mice receiving various *M. pulmonis* strains (Table 1). The WRAIR strain is a recent isolate initially recovered from the blood and arthritic joint of a rat with a *Plasmodium berghei* infection (R. M. Coan et al., Walter Reed Inst. Res. Annu. Rep., 1968). The response in the joint to this strain appeared similar to that noted with the JB strain, although the incidence of arthritis in the former group was less. The *M. pulmonis* isolate from rat blood produced arthritis in about 60% of the mice inoculated, and the organism was recovered from the joint for as long as 6 weeks after challenge. The Negroni strain of *M. pulmonis* was originally isolated from tissue cultures inoculated with leukemic tissues (3). Arthritis occurred in 20 to 50% of mice receiving this *M. pulmonis* strain, and it was also retained in the joints for at least 7 weeks. Some mice receiving the Negroni strain did not show migratory arthritis, and joint enlargement was confined to the wrist throughout the observation period.

**DISCUSSION**

The clinical and histopathological characteristics of the arthritis developing in mice after intravenous inoculation of selected *M. pulmonis* strains are similar to the type B mycoplasmal joint infections described by Sabin (16-18). The properties attributed to the type B strain, including its origin in normal mouse brain and nasal mucosa, the ability to ferment glucose, and its vacuolated colonial morphology, are more compatible with the characteristics of *M. pulmonis* than with those of *M. neurolyticum* or *M. arthritidis*, the other rodent mycoplasmas (24). From these similarities, it would appear that the type B mycoplasmas were *M. pulmonis* strains. Some additional support for this interpretation was also obtained in earlier antibody studies. Samples of three individual rabbit antisera prepared against the type B strain were made available through the kindness of A. B. Sabin. All three antisera possessed fluorescent-antibody titers of 1:128 to 1:256 against *M. pulmonis* (PG-34) antigen but were not reactive to *M. neurolyticum* (Type A) or *M. arthritidis* (PG-6) antigens (Tully, unpublished data).

*M. pulmonis* has been implicated in infectious catarrh of mice and rats, and the organism has been isolated frequently from the nasal cavity, brain, and lung of laboratory rodents with respiratory disease. It is also present in a latent state in similar tissues of normal mice and rats, and the onset of respiratory disease is most fre-
quently associated with host stress. However, systemic infections arising from *M. pulmonis* invasion of other tissues have rarely been observed. That mycoplasmas associated with infectious catarrh are capable of invading the joints of mice was first noted by Nelson (10). Intraperitoneal inoculation of these strains into female mice showed a very selective localization in the ovaries, and many animals developed arthritis as well. Nelson's catarrhal strains were identified later as *M. pulmonis* (23).

The arthritis observed in mice with *M. pulmonis* was notable for its migration from front to back joints. There was little evidence that a generalized infection occurred in these animals, and the localization of *M. pulmonis* appeared confined to the joints and adjacent periarticular tissue. On the other hand, *M. arthritidis* joint infections of the rat, particularly those observed in natural outbreaks of the disease, have been associated frequently with subcutaneous abscesses or purulent infections in the joints or in other organs of this host. Multiple joint involvement without the migratory characteristics has been the usual finding in the rat polyarthritis syndrome. Arthritis in rats has also been noted to follow repeated animal-to-animal passages of malignant rodent tissue, suggesting that *M. arthritidis* is latent in these animals. However, latent infection with this particular serotype has not been established with any certainty. Microscopically, the joint involvement in the early stages of *M. pulmonis* and *M. arthritidis* infections may show some similarities, not only in the specific tissues invaded but also in the type of cellular response. However, some histopathological studies of the joints of rats with *M. arthritidis* infections have shown localized abscesses and a very extensive polymorphonuclear cell response (4, 7, 13, 20, 25). This type of response was not observed in the joints of mice in this study, although extensive histological studies were not made on the joints at various stages of arthritis to exclude the possibility of a more vigorous polymorphonuclear cell response. Some observations on experimental arthritis in rats induced with tumor-associated *M. arthritidis* show that the relative proportion of mononuclear and polymonucleated cells in the joint exudate depends upon the challenge level and route (8). Thus, the joint response with these two mycoplasmas may be very similar.

The apparent host specificity for mice of the arthritogenic *M. pulmonis* strains might also appear to offer distinction from rat polyarthritis. However, there is some evidence that this particular host response to *M. pulmonis* may also occur in rats. As noted above, rats frequently carry *M. pulmonis* in the lung and, under appropriate stress, mycoplasmas may increase in sufficient numbers to seed the circulation, localize in the joint, and produce arthritis. The recovery of *M. pulmonis* (WRAIR strain) from the blood and infected joint of a rat with a *P. berghei* infection is perhaps an example of this specific response in the joint to the stress of an experimental infection. The ability of rat-derived *M. pulmonis* strains to induce experimental arthritis in rats should also be explored. Natural *M. arthritidis* joint infections, on the other hand, have been limited solely to rats, and this serotype has not been reported to occur spontaneously in mice. Some strains of *M. arthritidis* can produce abscesses in mice upon subcutaneous or intraperitoneal inoculation, and arthritis has been reported in some instances (4, 5, 7). It would appear that both *M. pulmonis* and *M. arthritidis* might produce joint infections in either rats or mice under appropriate conditions of stress and latency.

Experimental *M. pulmonis* arthritis in mice might provide a model to study host and microbial factors that lead to chronic, nonsuppurative arthritis. Little specific information is now available as to why certain mycoplasma evoke a nonsuppurative joint response in some animal hosts. However, tropism for joint and adjacent tissue appears to be lost rapidly by mycoplasmas cultivated outside the host. The nature of the factors responsible for the arthritogenic properties of mycoplasmas might be more easily determined in this model.

ACKNOWLEDGMENTS

We thank Leon Sokoloff for his advice in the interpretation of the histological sections of the joint. We are also grateful to R. T. Rusten for preparing the tissue sections and to C. W. Renshawe and N. G. Ramsburg for technical assistance.

LITERATURE CITED