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NOTES

Localization of *Mycoplasma pulmonis* in Cartilage

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Transmission electron microscopy of articular cartilage in *Mycoplasma pulmonis*-infected neonatal rats revealed the presence of mycoplasmas within the matrix and lacunae. The mycoplasmas appeared to have a tropism for the chondrocytes and induced lysis of both the chondrocytes and matrix of the cartilage.

The etiology of rheumatoid arthritis remains undefined; however, mycoplasma infection has been postulated to be the initiating event in the pathogenesis of this disease (2, 5). Interest in a possible causal relationship of mycoplasma is related to the known mycoplasma-induced arthritides in animals and fowl (4). *Mycoplasma pulmonis* is among the mycoplasmas that induce arthritis in animals. It can cause polyarthritis naturally in mice and experimentally in rats and rabbits (1, 7–9). Both the inflammatory reaction and the degenerative joint pathology associated with arthritis induced by *M. pulmonis* are similar to that present in rheumatoid arthritis. An important aspect that has not been adequately investigated, however, is the tissue tropism of *M. pulmonis* within an arthritic joint. Delineation of the articular site in which *M. pulmonis* replicates and initiates the events leading to degenerative joint disease may be a key in elucidation of the pathogenesis of the disease. Very basic to understanding *M. pulmonis*-induced arthritis in animals is whether the microorganisms remain in articular tissues in a sequestered site as viable organisms or as nonviable antigens. Moreover, in rheumatoid arthritis, the inability to regularly isolate mycoplasmas from affected joints has made tenuous the causal relationship of mycoplasmas with the disease. We initiated this study to determine, by transmission electron microscopy (TEM), the localization of *M. pulmonis* within arthritic joints of rats.

We chose to use neonatal rats, since previous work had shown that intracerebral inoculation with *M. pulmonis* consistently resulted in polyarthritis (7). Fourteen 2- to 3-day-old Sprague-Dawley rats (Timco Breeding Laboratories, Houston, Tex.) were inoculated intracerebrally with approximately 0.02 ml of an *M. pulmonis* saline suspension containing 8×10^8 colony-forming units per ml. A like number of littermates were inoculated intracerebrally with sterile broth medium and served as controls. The source of the *M. pulmonis* culture was a naturally infected rat. The identity and purity of inoculum and the mycoplasma isolated from infected joints were confirmed by epi-immunofluorescence. This test was performed by Microbiological Associates (Bethesda, Md.), using antiserum from mules inoculated with *M. pulmonis* PG34. To culturally recover *M. pulmonis* from the tibiotarsal joints, one *M. pulmonis*-inoculated and one control rat were killed on each of the following days after inoculation: 1, 2, 3, 5, 6, 7, 8, 10, 13, 15, 17, 20, 25, and 28. Specimens from the tibiotarsal joints were obtained at 7, 8, and 15 days for light microscopy and TEM. For TEM, cartilaginous tissues were dissected and immediately placed in decalcifying-fixing solution containing 0.1 M disodium EDTA and 3% buffered glutaraldehyde. Tissues were postfixed in 1% buffered osmium tetroxide. Thick sections stained with toluidine blue were made to help locate portions of abnormal cartilage and synovial tissues. Thin sections were made of selected areas.

M. pulmonis was consistently isolated from the tibiotarsal joints of inoculated rats from day 2 postinoculation through the last day tested, day 28. None were isolated from control animals. Polyarthritis was clinically apparent from days 8 through 28. Tibiotarsal and tarsal joints became ankylosed in two rats killed after 17 days. Light microscopy showed an intense polymorphonuclear-cell inflammatory response within the synovial spaces and membranes and in the periarticular tissues. Hydrocephalus was induced in 11 of the 14 rats inoculated with *M. pulmonis*.

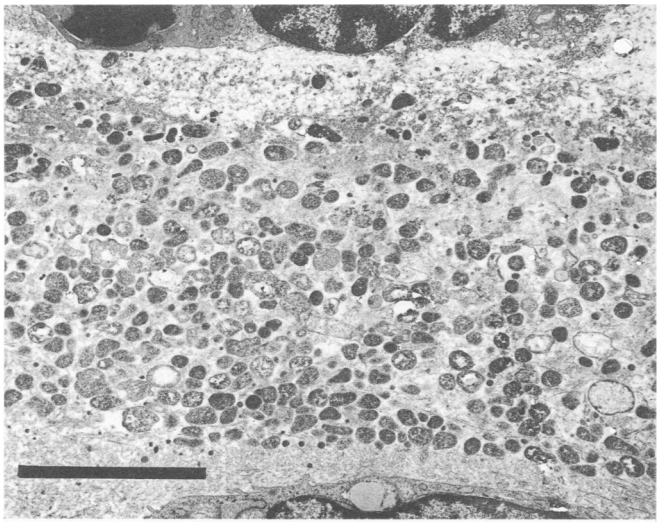

FIG. 1. TEM of interface of articular cartilage with synovial space. Note the peripheral zone of cartilage filled with many mycoplasmas. Bar = 5 μm.

TEM of articular cartilage and synovial tissues showed mycoplasmas located in wide bands at the periphery of the articular cartilage (Fig. 1). Within the cartilage, mycoplasmas were frequently observed in the matrix but more prominently in association with lacunae. In this latter site, *M. pulmonis* localization was associated with degeneration of chondrocytes (Fig. 2A and B). In some instances, the lacunae were devoid of chondrocytes and impacted with mycoplasmas, whereas in some instances, portions of degenerated cells still remained (Fig. 3). With-

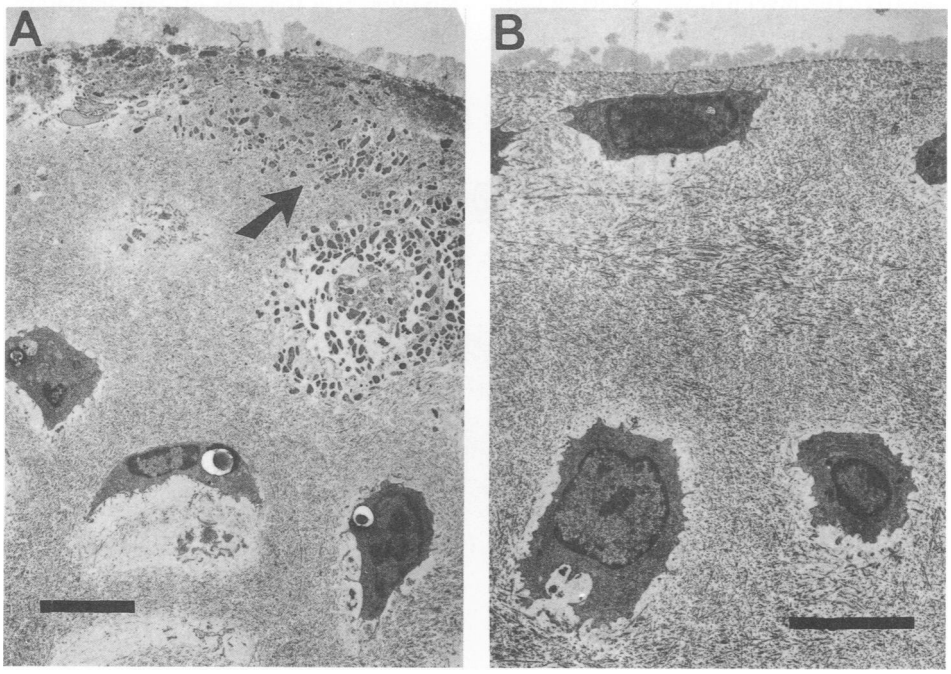

FIG. 2. (A) TEM of *M. pulmonis*-infected cartilage showing remnants of a chondrocyte surrounded by numerous mycoplasmas. Organisms are also present within the matrix (arrow). Bar = 5 μm. (B) TEM of noninfected articular cartilage showing normal-appearing chondrocytes and matrix. Bar = 5 μm.

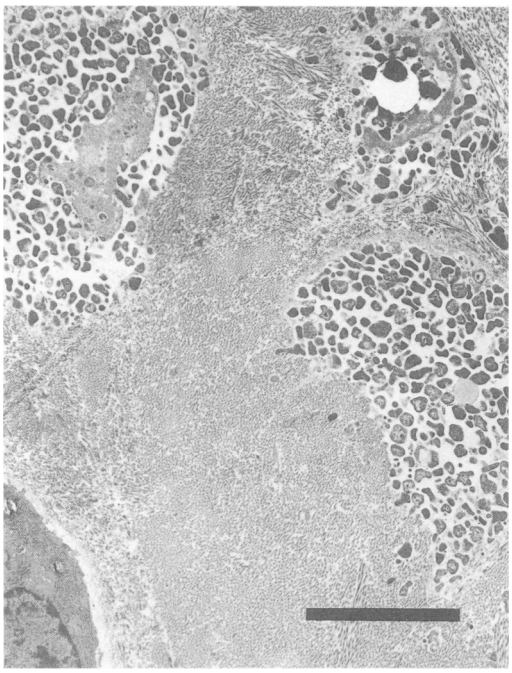

FIG. 3. TEM of mycoplasmas which fill the space within two lacunae. A normal-appearing chondrocyte remains in a nearby lacuna. Bar = 5 μm.

in the matrix, aggregations of *M. pulmonis* were associated with dissolution of the reticular fibers of the matrix. The mycoplasmas observed within the cartilage had diameters of 0.4 to 0.8 μm and were pleomorphic, but most had the typical round or elongated shapes of *M. pulmonis*. A few organisms displayed electron-dense, tapered, or stalked ends (Fig. 4). Mycoplasmas were differentiated from degeneration products and lipid particles of host origin by the above characteristics and staining dissimilarities.

The presence of *M. pulmonis* in articular cartilage is unique, since this microorganism is considered to be a cell surface pathogen of ciliated epithelia (3). The mechanism by which the mycoplasmas are able to penetrate the surface of the articular cartilage is unknown. However, lysosomal enzymes from the polymorphonuclear cells within the synovial spaces may alter the integrity of the articular surface to permit entry of the microorganism. *M. pulmonis* is motile, and it is probable that this capability is associated with the apparent inward movement of the organism through the matrix. Mycoplasmas are dependent upon the host as a source of sterols. Within articular cartilage, it has been shown that the lipid-rich portions are at the surface and pericellular (6). This may explain the observed preponderance of mycoplasmas at both of these sites.

To our knowledge, this is the first report to show that mycoplasmas penetrate and apparently replicate within articular cartilage. Finding this infection of cartilage may assist in more fully elucidating the pathogenesis of mycoplasma-induced arthritides. However, since neonatal animals were used, it is yet to be determined whether this observed mycoplasma-cartilage interaction is a function of only immature cartilage.

In most instances, previous studies have focused on lesions induced initially in synovial tissues rather than in cartilage. The degenerative

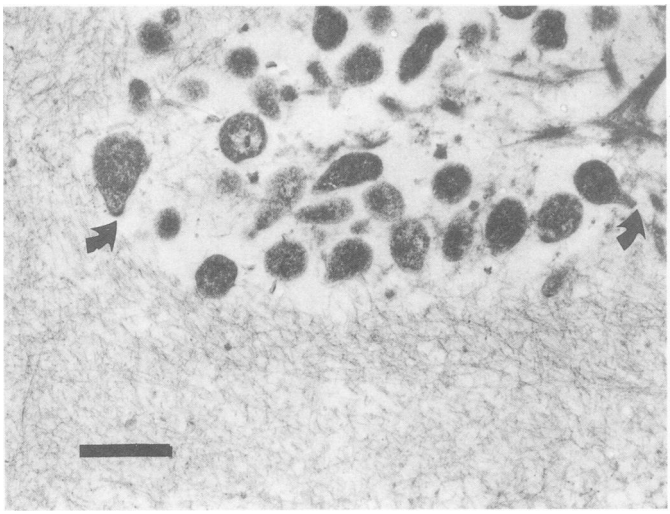

FIG. 4. TEM of numerous mycoplasmas in a vacuolated area of the matrix. Several of the mycoplasmas have the tapered or stalked ends which are frequently associated with *M. pulmonis* (arrows). Bar = 1 μm.

lesions observed by TEM suggest that lysis of the chondrocytes and matrix is a direct effect of the microorganism. Our study documents the presence of viable-appearing mycoplasma within the cartilage for at least 15 days, but long-term studies will be needed to determine the chronicity of their presence. However, it can be postulated that the chronic inflammatory nature of mycoplasmal arthritides may be mediated by either viable mycoplasmas or their antigens sequestered within cartilage. Alternatively, the induced lesions observed by TEM may result in an altered antigenicity of both cellular and matrix components, and these components might become a source for continued autoimmune stimulation. We suggest that our preliminary data justify the need for more studies dealing with mycoplasma-cartilage interaction.

LITERATURE CITED

1. **Barden, J. A., and J. G. Tully.** 1969. Experimental arthritis in mice with *Mycoplasma pulmonis*. J. Bacteriol. **100**:5–10.
2. **Bennett, J. C.** 1978. The infectious etiology of rheumatoid arthritis. Arthritis Rheum. **21**:531–538.
3. **Cassell, G. H., J. K. Davis, W. H. Wilborn, and K. S. Wise.** 1978. Pathobiology of mycoplasmas, p. 399–403. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
4. **Cole, B. C., and G. H. Cassell.** 1979. Mycoplasma infections as models of chronic joint inflammation. Arthritis Rheum. **22**:1375–1381.
5. **Cole, B. C., and J. R. Ward.** 1979. Mycoplasmas as arthritogenic agents, p. 367–398. *In* J. G. Tully and R. F. Whitcomb (ed.), The mycoplasmas. II. Human and animal mycoplasmas. Academic Press, Inc., New York.
6. **Ghadially, F. N., G. Meachim, and D. H. Collins.** 1965. Extra-cellular lipid in the matrix of human articular cartilage. Ann. Rheum. Dis. **24**:136–146.
7. **Kohn, D. F., B. E. Kirk, and S. M. Chou.** 1977. Mycoplasma-induced hydrocephalus in rats and hamsters. Infect. Immun. **16**:680–689.
8. **Sabin, A. B.** 1939. Experimental proliferative arthritis in mice produced by filtrable, pleuropneumonia-like microorganisms. Science **89**:228–229.
9. **Washburn, L. R., B. C. Cole, M. I. Gelman, and J. R. Ward.** 1980. Chronic arthritis of rabbits induced by mycoplasmas. I. Clinical, microbiologic, and histologic features. Arthritis Rheum. **23**:825–836.