Mycoplasmal Arthritis of the Mouse: Development of Cellular Hypersensitivity to Normal Synovial Tissue[1] (37635)

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The possible relationship between infectious agents and immune mechanisms in human rheumatoid arthritis has interested investigators for years (1). Availability of a model of chronic arthritis—the mouse infected with *Mycoplasma pulmonis*—has offered an opportunity to explore this concept.

Intravenous inoculation of 10^7–10^8 colony forming units (CFU) of *M. pulmonis* into mice is followed within two weeks by onset of chronic arthritis which in some animals migrates from joint to joint and can persist for the life of the mouse. The disease is characterized histologically by proliferative synovitis (2). In a previous experiment in which 140 mice were sacrificed in groups of ten from 1–46 weeks after inoculation, arthritis developed in 93% of the mice. Over the entire course of the experiment, *M. pulmonis* was recovered from only 27.2% of the inflamed joints. However, in those joints from which *M. pulmonis* grew, the number of mycoplasma colonies recovered correlated significantly with the degree of clinical arthritis (3). These findings suggested that the development of the disease might involve a more complex mechanism than a host response directed only at the mycoplasma.

The chronic nature of the disease and the histopathology of the arthritic joints indicated that cellular hypersensitivity might play a role in the pathophysiology of the disease. To investigate this possibility, evidence was sought that lymphoid cells from mice infected with *M. pulmonis* exhibited cellular hypersensitivity to either normal synovial tissue or the mycoplasma.

Methods and Materials. Cellular hypersensitivity was measured as the inhibition of peritoneal macrophage migration in the presence of an antigen compared to migration in the absence of that antigen. To prepare normal mouse synovium antigen, synovial tissue was dissected from the elbows, ankles, metatarsophalangeal joints and knees of normal HA/ICR mice (ARS-Gibco, Madison, Wisconsin), minced and filtered through gauze in Sorenson's phosphate buffer, pH 7.2; the pooled tissue was suspended in 0.5 ml of 0.25 *M* sucrose, iced, and sonicated in 20-sec bursts at 75 W in a Sonifier® cell disrupter until homogeneous. The tissue was then resuspended in 9.5 ml of 0.25 *M* sucrose containing 100 μg/ml methicillin and centrifuged at 10,000*g* for 30 min. The supernatant was frozen in 2 ml aliquots containing 8 mg protein/ml (4). *M. pulmonis* membrane antigen was prepared by freeze-thawing (5) and also frozen at a concentration of 8 mg protein/ml. Migration studies of peritoneal macrophages obtained from infected and control animals were performed in Sykes-Moore chambers using techniques similar to those of Friedman and Ceglowski (6). Migration areas were measured by tracing the projected image of each chamber. For each antigen, areas of macrophage migration from 24 HA/ICR mice which had been infected with *M. pulmonis* from 4–98 weeks previously were compared to migration areas from 24 normal mice of the same strain

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TABLE I. Area of Cell Migration in Response to Synovial and *Mycoplasma pulmonis* Antigens in Infected and Control Mice.

Group	Antigen	Number of capillaries[a]	Mean area of migration (± SE)	
			With antigen	Without antigen
Infected	synovial	24	48.37 ± 3.33 cm^2*	62.86 ± 4.83 cm^2*
Control	synovial	24	44.86 ± 3.63 cm^2	48.84 ± 3.39 cm^2
Infected	mycoplasma	24	56.20 ± 4.87 cm^2	65.26 ± 6.55 cm^2
Control	mycoplasma	24	43.30 ± 2.26 cm^2	44.37 ± 2.26 cm^2

[a] 2 capillaries from each mouse.
* $p < 0.02$ by t test.

matched in age to the infected mice. From each mouse a 10% suspension of peritoneal macrophages was prepared in medium 199 (Flow Laboratories). Two capillaries filled with the cell suspension were placed in the chamber containing antigen in medium 199, and two other capillaries were placed in a chamber containing the medium alone. *M. pulmonis* antigen was used in a concentration of 20 μg protein/ml and synovial antigen was used in a concentration of 250 μg protein/ml. These were determined by titrating antigen concentrations from 1–1,000 μg protein/ml using cells from normal mice of the same strain between 12–24 weeks of age. The concentrations used were the highest for each antigen which had no effect on the migration area.

Results. Infected and control mice were paired by age, and tests performed alternately with each antigen. Results were analyzed by a two-way analysis of variance for each of the two experimental and control groups. In each analysis the two variables were animal to animal variation and presence or absence of antigen. For each group, interaction between the two variables was negligible. Thus, the variables could be considered independently and individual comparisons made by t test. Mycoplasma antigen did not significantly affect migration of cells from either infected or control mice (Table I). Synovial antigen, however, did inhibit migration of cells from infected ($p < 0.02$) but not control mice. When duration of infection was taken into account, the effect of the synovial antigen was seen primarily in mice infected for longer than the median 14 weeks (Table II).

Discussion. Until relatively recently, human rheumatoid arthritis was known as chronic infective arthritis (7); yet no single microorganism has been isolated consistently from patients. In prior work with *M. pulmonis* induced arthritis in mice, a significant correlation between the degree of clinical arthritis and the number of mycoplasmas recovered from those joints which were culture positive was found. Yet no association between the number of culture positive arthritic joints and the total number of arthritic joints was found, regardless of the duration of infection (3). These seemingly contradictory findings suggested that a secondary mechanism might be involved. The development of cellular hypersensitivity (cell-mediated

TABLE II. Effect of Duration of Infection on Inhibition of Cell Migration by Synovial Antigen.

Duration of infection	Number of capillaries[a]	Mean area of migration	
		With antigen	Without antigen
≤14 weeks	24	58.32 ± 3.89 cm^2	65.48 ± 6.05 cm^2
>14 weeks	24	38.43 ± 4.64 cm^2*	60.23 ± 7.74 cm^2*

[a] 2 capillaries per mouse.
* $p < 0.02$ by t test.

immunity) to normal synovial tissue, suggested by this study, raises the possibility that lymphocytotoxic factors could be mediators of articular inflammation.

Failure to show statistically significant response to the mycoplasma antigen may have been due to the nature of the antigen preparation. Alternatively, interaction between the microorganism and synovium may cause development of new antigen more closely related immunologically to synovium than to *M. pulmonis*.

Discovery of a single infectious agent as the cause of human rheumatoid arthritis has defied intensive investigation for many years. Perhaps there is no single etiologic factor, but rather a multitude of agents capable, as Barland suggests (9), of altering the joint and resulting in an immunologic attack upon the articular tissues through mechanisms of cellular hypersensitivity.

Summary. Cellular hypersensitivity to normal mouse synovial tissue was found in arthritic mice infected by iv injection of *Mycoplasma pulmonis* but not in uninfected normal mice. Hypersensitivity was determined by inhibition of migration of peritoneal macrophages in the presence of an antigen made from normal mouse synovium. Since, in a previous study, no correlation was found between the presence of mycoplasmas in arthritic joints and the extent of arthritis, this data suggests that both microbiological and immunological mechanisms are involved in the pathogenesis of the disease.

Addendum. An additional experiment was performed to test the specificity of the reaction to synovial antigen. Cells from 12 mice infected 14 to 35 weeks and 12 age-matched controls were incubated in the presence or absence of an antigen prepared from normal mouse kidney by the method outlined for synovial antigen. Using 12.5 μg/ml (the highest concentration not affecting cells from normal mice) the migration area from the infected animals with antigen was 65.47 $\pm$ 7.97 cm^2; without antigen, 60.49 $\pm$ 5.58 cm^2; controls with antigen 40.74 $\pm$ 4.25 cm^2; without antigen 38.69 $\pm$ 3.05 cm^2. Thus no significant effect was seen in either infected or control animals.

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