Effect of T-Cell Deficiency on the Chronicity of Arthritis Induced in Mice by Mycoplasma pulmonis

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Mycoplasma pulmonis inoculated parenterally into athymic nude mice congenitally deficient in T cells caused a chronic arthritis of significantly greater magnitude than in immunologically normal mice. During the chronic phase of arthritis, M. pulmonis organisms were isolated from the joints and spleens of athymic nude mice more frequently and in larger numbers than from immunologically normal mice. The results support the concept that impaired T-cell function predisposes the mice to a severe degree of chronic arthritis as a result of their failure to eliminate the causative organisms.

Mycoplasma pulmonis induces a chronic arthritis in some mice in which the course and histopathology resemble that of rheumatoid arthritis. Evidence has accumulated which supports the concept that the chronicity of mycoplasma-induced disease is probably the result of organisms persisting within the joints of infected mice (2, 10). A better understanding of the immune mechanisms responsible for the eradication of M. pulmonis from the host is desirable because the reason for the inability of the host to eliminate the mycoplasmas from the joints is still unclear. The present study was therefore undertaken to investigate whether thymus-dependent (T) cells were involved in the elimination of M. pulmonis from an established joint infection in mice. To answer this question, we compared the development and persistence of arthritis in M. pulmonis-infected athymic nude mice congenitally deficient in T cells with that in similarly infected immunologically normal littermates.

MATERIALS AND METHODS

Cultivation of mycoplasmas. The J. B. strain of M. pulmonis was obtained from P. Hannan (Beecham Labs, England) as a broth culture from the original provided by J. G. Tully (National Institutes of Health, [NIH] Bethesda, Md.). Liquid medium for the isolation and growth of M. pulmonis consisted of Trypticase soya broth (BBL Microbiology Systems, 70 ml), unheated Burroughs Wellcome horse serum no. 6 (20 ml), 0.1% glucose, 0.002% phenol red, 1,200 thallium acetate, and penicillin G (1,000 U/ml). The pH of the medium was adjusted to 7.8.

Organisms for mouse inoculation were grown in thallium acetate-free medium and stored in portions at −70°C. The stock culture was diluted in Trypticase soya broth (BBL) to provide the required number of organisms for mouse inoculation.

Mice. Adult athymic nude mice and immunologically normal littermates, 5 weeks old, weighing 20 to 30 g, were used and kept in isolation. NIH, Nu/Nu Swiss outbred mice (from B. Gallie, Wellesley Hospital, Toronto) and Nu/Nu BALB/c mice (Microbiological Associates) were obtained and kept in an isolator through which HEPA-filtered air was drawn.

Mice were found to be free of detectable mycoplasmas in the nasopharynx before use, and they were inoculated in groups of at least five for each experiment.

Induction of arthritis and assessment of severity. Mice were inoculated intravenously with 0.2 ml of the mycoplasma stock culture or dilutions thereof containing 106 to 108 color-changing units (CCU).

Subjective assessment of the severity of the arthritis was made by scoring the swelling of each joint on a scale from 0 to 3 (12). Ankle, wrist, metacarpal, metatarsal, and digit joints were assessed, and a total score was obtained for each mouse. The mean arthritis score was obtained by taking the total score for all the mice in a group and dividing it by the number of mice within the group.

Mycoplasma isolation procedures. At the end of the study, mice were anaesthetized by intraperitoneal injection of sodium pentobarbitone and exsanguinated by severing the axillary vessels. Joints (bilateral ankles and wrists) and specimens of lung and spleen were removed from each mouse under sterile conditions. Surgical instruments were sterilized between sampling different organs. The solid tissues including joints were homogenized in Ten-Broek grinders with mycoplasma medium to give 10% (wt/vol) suspensions as described previously (10).

In some experiments, one-half of the ankle joint was examined for mycoplasmas and the other half was examined for histopathological changes.

Isolation of mycoplasmas and an estimation of their number were carried out by making serial 10-fold
dilutions of the specimen in 1.8-ml volumes of mycoplasma medium contained in 2-ml screw-capped vials. The vials were incubated at 37°C for at least 2 weeks during which time the medium was observed for a change in color from pink to yellow, this being an indication of organism multiplication. The highest dilution of the specimen at which a change was noted was considered to contain 1 CCU.

Mycoplasma identification. Isolated organisms were identified by means of the metabolism-inhibition test. The micro-technique of Taylor-Robinson et al. (12) was used with rabbit antiserum to M. pulmonis. Unheated guinea pig serum was incorporated at a final concentration of 1% (vol/vol).

Antibody to M. pulmonis. Complement-fixing antibody to M. pulmonis in mouse sera was measured by a microtechnique as described previously (9).

Histopathology. Joints were fixed in 10% Formal saline and decalcified overnight in 10% formic acid. After fixation, the tissues were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. The extent of the arthritis was quantitated histologically by assessing several measures of inflammation, which included subtenosynovial infiltration with polymorphonuclear leukocytes, synovial lining cell hyperplasia, cartilage erosion, and soft tissue necrosis. The severity of each measure was graded on a scale of 0 to 3, with 0 indicating no abnormality and 3 indicating severe histological abnormalities. The joint sections were read under code by the same observer (V.F.).

RESULTS

Arthritis in athymic and littermate control mice. In a preliminary study, 6 NIH athymic nude mice and 10 normal littermates were inoculated with 2 × 10^6 CCU of M. pulmonis. Although a mild degree of arthritis developed in both groups, the kinetics of the arthritis in the nude mice differed significantly from that in the littermate group (Fig. 1). In the normal littermate mice, the response was maximal within 6 days and declined gradually thereafter. In contrast, the arthritis score in the athymic mice was much greater and reached a plateau 16 days after inoculation, persisting at that level throughout the study.

Because only a low-grade arthritis was achieved in the first study, a second experiment was carried out with a larger dose of M. pulmonis; 10^6 CCU were inoculated into five NIH athymic and five littermate mice. The arthritis response was more severe in both the athymic mice and control littermates (Fig. 2) than previously, but the athymic mice again developed a more severe disease than did the controls. The kinetics of the acute phase of arthritis were similar for the two groups of mice, but they differed considerably during the chronic phase. Thus, the arthritis in the littermate group rapidly defervesced so that there was a low arthritis score 30 days after inoculation, whereas the arthritis in the athymic mice declined to a level which exceeded the peak response of the normal mice throughout the duration of the study. In this study two athymic mice died during the chronic phase of disease. In a third study undertaken with 10 Nu/Nu BALB/c athymic and littermate mice inoculated with 10^6 CCU of M. pulmonis, similar differences between the athymic and littermate mice were seen. As before, a more severe degree of chronic arthritis was maintained in the athymic mice than in the

![Fig. 1. Effect of T-cell deficiency on the development of arthritis in mice inoculated with 2 × 10^6 CCU of M. pulmonis. ●, Athymic nude mice; ○, normal littersmates. Each point represents the mean arthritis score per mouse from a group of five mice, and the error bars represent ± standard error.](http://iai.asm.org/)

![Fig. 2. Effect of T-cell deficiency on the development of arthritis in mice inoculated with 10^6 CCU of M. pulmonis. ●, Athymic nude mice; ○, normal littersmates. Each point represents the mean arthritis score per mouse from a group of five mice, and the error bars represent ± standard error. Each arrow represents the death of one mouse.](http://iai.asm.org/)
littermates throughout the course of the study.

Analysis of kinetic differences in the arthritis response between the athymic and littermate groups during the chronic phase of disease was accomplished by determining the slope of the model curves which best fit the portion of the kinetic curves after the peak arthritis score. The kinetic curves were studied in relation to linear, exponential, power, and logarithmic models. The results show that the slopes of the model curves involving the athymic mice were less steep than the slopes of curves involving the three normal groups studied (Table 1; Fig. 2 and 3). The peak arthritis response of the athymic group in the third study was not statistically different from that of the control strains. Therefore, it is unlikely that the more severe acute arthritis response observed in the athymic mice accounts for the kinetic differences observed during the chronic phase of disease.

M. pulmonis organisms in joints and other organs. The persistence of arthritis in the athymic mice could reflect either an inability of the host to eliminate M. pulmonis and therefore a continued response to them or an exaggerated inflammatory response to organisms which had been eliminated. To answer this question, we examined the joints and spleen of each mouse at the termination of the study for the presence of M. pulmonis organisms. The results showed that they were present in a greater proportion of the joints (Table 2) and spleens (Table 3) of the athymic mice than of the immunocompetent littermates. Moreover, the mycoplasmas were isolated in greater numbers from the joints and spleens of the athymic mice. These results indicate that T-cell-deficient athymic nude mice are unable to eliminate M. pulmonis organisms as efficiently as immunocompetent mice.

Histopathological observations. Differences in skin texture between the athymic and control mice might account for exaggerated joint swelling in nude mice, leading to periarticular fibrosis in the absence of inflammation. Thus, the clinical assessment may not accurately reflect the degree of joint inflammation, especially during the chronic phase.

To evaluate this possibility, we carried out histopathological studies on eight of the most clinically involved ankle joints from the athymic and littermate groups of mice in the third experiment. The athymic mice had a greater degree of periarticular cellular infiltration, synovial hyperplasia, cartilage erosion, and soft tissue necrosis than did the littermates (Table 4). Many scattered foci of aggregated polymorphonuclear leukocytes were seen in the joints of the athymic mice, but not in those of the littermates. Overall, therefore, the histological findings were consistent with the clinical and microbiological data.

Antibody responses. Since the antibody response to M. pulmonis during the chronic phase of arthritis is likely to be T-cell dependent, it is conceivable that the differences observed between the athymic and immunocompetent mice may be a reflection of a deficient humoral response to M. pulmonis in athymic mice. Serological studies on M. pulmonis-infected mice at the termination of the experiments revealed lower titers of complement-fixing antibody to M. pulmonis in athymic nude mice than in immunocompetent normal strains (Table 5). However, the relatively high titers of antibody to M. pulmonis in nude mice during the chronic phase of disease make it unlikely that the poorer humoral response alone accounts for the persistence of mycoplasmas.

**DISCUSSION**

The results of this study demonstrate that

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**TABLE 1. Effect of T-cell deficiency on the chronicity of arthritis in mice infected with M. pulmonis**

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Expt 2</th>
<th>Expt 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope*</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>Athymic</td>
<td>-0.23</td>
<td>0.82</td>
</tr>
<tr>
<td>Littermate</td>
<td>-1.86</td>
<td>0.93</td>
</tr>
<tr>
<td>C3H</td>
<td>ND*</td>
<td></td>
</tr>
</tbody>
</table>

* Slope of that portion of the kinetic curve after peak arthritis score.
* ND, Not done.
TABLE 2. Effect of T-cell deficiency on the isolation of M. pulmonis from joints of infected mice

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Immune status</th>
<th>No. of mice from which mycoplasma isolated/(\text{no. of mice inoculated})</th>
<th>No. of positive isolations/(\text{no. of isolation attempts})</th>
<th>No. of organisms isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal T cell deficient</td>
<td>2/10 3/6</td>
<td>2/40 3/24</td>
<td>1.5 ± 0.1 5.7 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>Normal T cell deficient</td>
<td>3/4 3/3</td>
<td>5/16 12/12</td>
<td>2.4 ± 0.9 5.8 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>Normal T cell deficient</td>
<td>1/10 5/6</td>
<td>1/40 12/24</td>
<td>2.5 ± 0.4 2.0 ± 0.0</td>
</tr>
</tbody>
</table>

* Geometric mean number of organisms isolated from positive cultures expressed as log_{10} CCU/ml ± standard error.

* Significant difference from normal groups at \(P \leq 0.05\).

TABLE 3. Effect of T-cell deficiency on the isolation of M. pulmonis from spleens of infected mice

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Immune status</th>
<th>No. of mice from which mycoplasma isolated/(\text{no. of mice inoculated})</th>
<th>No. of organisms isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal T cell deficient</td>
<td>0/10 1/6</td>
<td>0 ± 0.0</td>
</tr>
<tr>
<td>2</td>
<td>Normal T cell deficient</td>
<td>0/4 3/3</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>Normal T cell deficient</td>
<td>0/10 4/6</td>
<td>2.5 ± 0.3</td>
</tr>
</tbody>
</table>

* Geometric mean number of organisms isolated from positive cultures expressed as log_{10} CCU/ml ± standard error.

* Significant difference from normal groups at \(P \leq 0.05\).

TABLE 4. Effect of T-cell deficiency on the histopathology of arthritis in infected mice at 78 days after inoculation of M. pulmonis

<table>
<thead>
<tr>
<th>Histological abnormality</th>
<th>Histological grade (±1 SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal mice</td>
</tr>
<tr>
<td>Subsynovial cellular infiltration with polymorphonuclear leukocytes</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Synovial lining cell hyperplasia</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Cartilage erosion</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>Soft tissue necrosis</td>
<td>0.4 ± 0.3</td>
</tr>
</tbody>
</table>

* SE, Standard error.

* Significant difference from normal group at \(P \leq 0.05\).

TABLE 5. Effect of T-cell deficiency on the complement-fixing antibody response of M. pulmonis-infected mice

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Day of sacrifice after inoculation of M. pulmonis</th>
<th>Immune status</th>
<th>Antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>Normal T cell deficient</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>Normal T cell deficient</td>
<td>9.0 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>Normal T cell deficient</td>
<td>8.8 ± 0.5</td>
</tr>
</tbody>
</table>

* Antibody titer expressed as log_{10} ± standard error.

* Significant difference from normal groups at \(P \leq 0.05\).

Arthritis induced by M. pulmonis persists at near maximal severity in mice deficient in T cells. Moreover, the arthritis is associated with the persistence of a large number of mycoplasma organisms within the joints and other organs, which suggests that T cells are important in the elimination of M. pulmonis from these anatomical sites. This is in accordance with a previous study in which it was shown that T cells protected against M. pulmonis infection (11). Thus, Taylor et al. demonstrated a more severe degree of acute arthritis in thymectomized, sublethally irradiated, and bone-marrow-reconstituted mice than in normal controls (11). Similar kinetics of the acute response were demonstrated in the present study. It may be concluded, therefore, that the T-cell element of the immune response to M. pulmonis is important in both the prevention of and recovery from disease.

The enhanced susceptibility of nude mice to infection with M. pulmonis is consistent with impaired protective immunity to other microorganisms in T-cell-deficient mice (5). It should be pointed out, however, that the nude mouse constitutes a model of relative, not absolute, T-cell deficiency. Although nude mice are referred to as being athymic, they possess a small and dysplastic thymus (14). Moreover, they possess normal numbers of T-cell precursors (6) and a very small number of theta-bearing cells (7). The presence of the theta-antigen, which is acquired before the acquisition of T-cell competence, however, does not itself indicate full T-cell function (8). Indeed, with few exceptions, nude mice cannot perform normal T-cell func-
Our results raise doubts about the importance of cell-mediated hypersensitivity in the enhancement of disease chronicity. Harwick et al. (3) suggested the possibility of tissue injury being immunologically mediated by demonstrating cellular hypersensitivity to normal mouse synovial tissue in chronically arthritic mice. However, the fact that we observed severe chronic arthritis in the T-cell-deficient mice mitigates against the requirement for T-cell-dependent phylogistic mediators in the induction of significant chronic joint inflammation by *M. pulmonis*. Moreover, the strong correlation between the clinical, histological, and microbiological variables of chronic disease lend further support to the concept that the chronicity of *M. pulmonis* arthritis is a direct result of persistence of the organism within the inflamed joint. A recent study by us provides additional data to support this hypothesis (10).

The demonstration that cell-mediated immunity is suppressed in *M. pulmonis*-infected animals (4) might have relevance to the pathogenesis of *M. pulmonis*-induced arthritis in normal mouse strains. Because T cells appear to be important in the elimination of *M. pulmonis*, it is conceivable that the impairment of the T-cell response in the normal host as a consequence of *M. pulmonis* infection enables dissemination of the organisms to the joints. According to this hypothesis, one would predict that stimulation of the immune system before *M. pulmonis* inoculation might prevent the host from developing arthritis. Indeed, support for this concept has been provided by the demonstration that the mouse may be protected by pretreatment with *Corynebacterium parvum* (unpublished data).

The results of the present studies may be relevant to human disease. Impaired T-cell function has been demonstrated in patients with rheumatoid arthritis. Although the etiology of rheumatoid arthritis remains entirely speculative, it is conceivable that the chronicity of disease is due to the persistence of an infectious inciting agent (1). Our demonstration of the persistence of mycoplasma-induced arthritis in T-cell-deficient mice provides indirect support for such a hypothesis.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**