Clinical Complications of *Mycoplasma pneumoniae* Disease—Central Nervous System

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Received January 4, 1983

The mechanism of the neurologic complications associated with primary atypical pneumonia is unknown. To examine the ability of *Mycoplasma pneumoniae* to enter the brain of experimental animals, the organism was inoculated into adult and suckling mice by various routes. After intranasal infection, *M. pneumoniae* was isolated from brains and lungs of both groups of mice. After intracerebral inoculation, the high levels of the mycoplasma persisted for two months or more in the brains of suckling mice. In addition, after intravenous infection, the systemic spread of infection occurred in the mice treated with high doses of cyclophosphamide. Our results suggest that *M. pneumoniae* may be able to reach the brain via blood and it may occur with relative ease in compromised hosts.

INTRODUCTION

Central nervous system complications of *Mycoplasma pneumoniae* infection include various symptoms such as encephalitis, meningitis, myelitis, polyradiculitis, or acute psychosis. These neurologic complications are usually preceded by respiratory symptoms although some patients give no history of the symptoms. The incidence of central nervous system complications among *M. pneumoniae* infections was reported to be about 0.1 percent. However, the incidence among hospitalized patients was as high as 7 percent [1,2].

The overall incidence of current *M. pneumoniae* infections among patients with neurological syndromes was 5 percent, with a maximum of 10 percent during the epidemic [3].

It is suggested that the complications may result from a direct invasion of *M. pneumoniae* into the brain, a neurotoxin produced by the organism, cross-reacting antibodies to antigen(s) shared by mycoplasma and brain, or from vascular microthrombi with the organism [1]. However, the mechanism of the neurologic disease associated with *M. pneumoniae* is not fully understood. The present study was undertaken to examine the ability of *M. pneumoniae* to enter the brain of experimental animals. Adult and suckling mice were challenged with the organism by various routes. In addition, cyclophosphamide-treated mice were infected with *M. pneumoniae* to examine the susceptibility of compromised hosts to the infection.

MATERIALS AND METHODS

*Animals*

Specified pathogen-free ICR female mice, three to four weeks old, and suckling mice of the same strain of both sexes were used in these experiments.

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Organism

*M. pneumoniae* strain 104166 was used as the inoculum for challenge of all experimental groups.

Challenge Procedure

Adult mice were infected with 25 μl of a PPLO broth suspension of *M. pneumoniae* by intranasal instillation. Adult and suckling mice were exposed in a desiccator with a nebulizer for 30 to 50 minutes to aerosol of the broth suspension. Mice were also challenged with *M. pneumoniae* by the intracerebral route. Control animals received sterile broth medium.

Cyclophosphamide (CY) Treatment

Adult mice were administered 260 mg CY per kg weight, intraperitoneally, once daily for a total of four injections. The animals were then challenged intravenously with *M. pneumoniae*.

Enumeration of Organisms

At appropriate intervals after infection, mice were killed to detect *M. pneumoniae* present in lungs, brains, spleen, and blood. Each organ was homogenized in a glass homogenizer containing 2 to 5 ml of PPLO broth, and blood (100 μl) was collected in a test tube containing 2 ml of the broth. After centrifugation, the supernatant fractions obtained from individual animals were plated on PPLO agar plates.

RESULTS

Recovery of *M. pneumoniae* from Lungs of Adult and Suckling Mice Infected by Exposure to Aerosol

Young adult mice, 28 days old, and suckling mice, one to seven days old, were infected with the mycoplasma by exposure to the aerosol. Nine ml of the broth culture containing $4 \times 10^7$ CFU/ml was nebulized in a desiccator for 50 minutes. As shown in Table 1, *M. pneumoniae* was not isolated from any of the lungs of 28-day-old mice under this experimental condition. The susceptibility of one- to four-day-old suckling mice to *M. pneumoniae* was much higher than that of six- to seven-day and 28-day-old mice, from the numbers of mycoplasmas isolated from lungs.

| Days after infection | Days after birth |
|---------------------|-----------------|
|                     | 28              | 7    | 6   | 4   | 3   | 2   | 1   |
| 0                   | 0               | 2.6 x 10^4 | 3.5 x 10^4 | 1.3 x 10^4 | 1.5 x 10^4 | 3.6 x 10^4 | 2.2 x 10^4 |
| 7                   | 0               | 2.1 x 10^4 | 5.6 x 10^4 | 1.6 x 10^4 | 2.7 x 10^4 | 2.6 x 10^4 | 4.8 x 10^4 |
| 0                   | 1.9 x 10^4      | 8.9 x 10^4 | 4.3 x 10^4 | 3.7 x 10^4 | 4.5 x 10^4 | 2.4 x 10^4 |
| 14                  | 0               | 0     | 5.5 x 10^4 | 1.2 x 10^4 | 4.5 x 10^4 | 3.1 x 10^4 | 4.0 x 10^4 |
| 0                   | 0               | 3.5 x 10^4 | 9.4 x 10^4 | 1.1 x 10^4 | 1.5 x 10^4 | 8.9 x 10^4 |
| 0                   | 0               | 0     | 7.1 x 10^3 | 2.0 x 10^3 | 5.4 x 10^3 | 1.4 x 10^4 |

* Nine ml of the broth culture containing $4 \times 10^7$ CFU/ml was nebulized in a desiccator for 50 minutes.
Recovery of M. pneumoniae from Lungs, Brains, and Blood After Aerosol or Intranasal Instillation

Young adult mice, 28 days old, were infected with 10^6 CFU of *M. pneumoniae* by intranasal instillation under anesthesia. The mycoplasma was isolated from the lungs and brains six days after the infection (Table 2). One-day-old suckling mice were infected with the mycoplasma by exposure to the aerosol. At autopsy 13 days later, the organisms were isolated from the lungs of all animals and from the brains of two of four animals (Table 2). The numbers of organisms isolated from brains were less than the numbers isolated from lungs. *M. pneumoniae* was not detected in the blood of either the adult or suckling mice at any time of autopsy. The mycoplasma was not isolated from any specimens of the control animals given sterile broth medium throughout these experiments.

Recovery of *M. pneumoniae* from Brains and Lungs After Intracerebral Inoculation

Adult and suckling mice were infected with *M. pneumoniae* by intracerebral inoculation. In suckling mice, the numbers of mycoplasmas in the brains decreased in the early stage after infection, then increased on day 20 (Exp. 1 in Table 3) and the

| Days post-infection | Adult mice* | Suckling mice* |
|---------------------|-------------|----------------|
| Lung (CFU/organ)    | Brain (CFU/organ) | Lung (CFU/organ) | Brain (CFU/organ) |
| 6 1.8 x 10^4 | 3.0 x 10^1 | 2.3 x 10^4 | 0 |
| 1.7 x 10^4 | 7.0 x 10^1 | 1.8 x 10^4 | 0 |
| 1.3 x 10^4 | 9.3 x 10^4 | 1.2 x 10^4 | 0 |
| 7.0 x 10^1 | 4.0 x 10^1 | 2.0 x 10^1 | 0 |
| 13 | 7.0 x 10^1 | 0 | 6.0 x 10^4 | 0 |
| 1.0 x 10^1 | 0 | 2.7 x 10^4 | 2.0 x 10^1 |
| 0 | 0 | 1.1 x 10^4 | 5.0 x 10^1 |
| 0 | 0 | 3.1 x 10^1 | 0 |
| 21 | 4.0 x 10^1 | 0 | 2.8 x 10^2 | 0 |
| 0 | 6.0 x 10^1 | 2.1 x 10^2 | 0 |
| 0 | 7.0 x 10^1 | 5.0 x 10 | 0 |
| 0 | 0 | 2.0 x 10 | 0 |
| 29 | 0 | 0 | ND | ND |
| 0 | 0 | 0 | 0 |
| 43 | 0 | 0 | ND | ND |

*Infected intranasally with 10^6 CFU

*Infected by nebulization—Five ml of the broth culture containing 3.9 x 10^4 CFU/ml was nebulized in a desiccator for 30 minutes.

*ND, Not done
TABLE 3
Recovery of *M. pneumoniae* from Three-Week-Old and One-Day-Old Mice
Infected by Intracerebral Inoculation

| Time post-infection | 3-week-old mice<sup>a</sup> | 1-day-old mice |
|---------------------|----------------------------|----------------|
|                     | Exp. 1<sup>+</sup> | Exp. 2<sup>+</sup> |
| 30 minutes          | 1.0 × 10⁶ | 1.4 × 10⁶ \( \text{ND} \) |
|                     | 9.8 × 10⁵ | 1.3 × 10⁶ |
|                     | 7.0 × 10⁴ | 1.2 × 10⁶ |
| 3 days              | ND         | 5.3 × 10⁴ |
|                     | 0          | ND          |
| 6 days              | 7.9 × 10⁵ | 2.3 × 10⁴ |
|                     | 0          | 3.0 × 10⁴ \( \text{ND} \) |
| 13 days             | 5.2 × 10⁴ | 3.7 × 10⁴ |
|                     | 0          | 3.0 × 10⁴ |
| 20 days             | 3.2 × 10³ | 3.6 × 10⁴ |
|                     | 5.0 × 10² | 1.8 × 10⁴ \( \text{ND} \) |
|                     | –          | 1.3 × 10⁴ |
| 26 days             | ND         | 1.4 × 10⁴ |
|                     |            | 4.5 × 10⁴ \( \text{ND} \) |
|                     |            | 7.4 × 10³ |
| 35 days             | ND         | ND          |
|                     |            | 6.6 × 10³ \( \text{ND} \) |
|                     |            | 2.2 × 10⁴ |
|                     |            | 1.3 × 10⁴ |
| 50 days             | ND         | ND          |
|                     |            | 7.2 × 10³ |
|                     |            | 4.5 × 10³ |
|                     |            | 0          |
| 70 days             | ND         | ND          |
|                     |            | 1.2 × 10³ \( \text{ND} \) |
|                     |            | 3.4 × 10³ |

*In inoculated with 0.025 ml of PPLO broth containing 2 × 10⁷ CFU

<sup>a</sup>Inoculated with 1 × 10⁶ CFU

<sup>c</sup>ND, Not done

High levels of the organism persisted two months or more (Exp. 2 in Table 3). The recovery from adult mice which had been inoculated with the same number of mycoplasmas was lower than that from suckling mice. On the other hand, the organism was not isolated from any lung of either the suckling or adult mice.

*Treatment with Cyclophosphamide*

Twenty-eight-day-old mice were subjected to treatment with CY to determine if such treatment led to systemic infection. Severe leukopenia was caused by the treatment. In such mice, *M. pneumoniae* was found not only in the lung but also in brain, spleen, and blood after intravenous infection. Persistence of higher levels of
## Table 4

| Time post-infection | Lung (CFU/organ) | Brain (CFU/organ) | Spleen (CFU/organ) | Blood (CFU/ml) |
|---------------------|------------------|------------------|-------------------|----------------|
| Control CY          | Control CY       | Control CY       | Control CY        | Control CY     |
| 5 minutes           | 4.2 × 10⁴        | 1.6 × 10⁵        | 1.0 × 10          | 5.0 × 10⁴      |
|                     | 1.0 × 10⁵        | 2.8 × 10⁵        | 7.7 × 10⁴         | 2.0 × 10⁵      |
| 4 hours             | 5.4 × 10⁴        | 2.4 × 10⁵        | 5.2 × 10⁴         | 7.9 × 10⁴      |
|                     | 1.5 × 10⁵        | 2.2 × 10⁵        | 7.9 × 10⁴         | 8.0 × 10⁴      |
| 1 day               | 0                | 8.2 × 10⁴        | 2.9 × 10⁵         | 3.5 × 10⁵      |
|                     | 0                | 6.0 × 10⁴        | 2.2 × 10⁵         | 1.1 × 10⁵      |
|                     | 0                | 9.3 × 10⁴        | 7.0 × 10⁴         | 1.6 × 10⁵      |
| 3 days              | 1.0 × 10          | 0                | 9.0 × 10⁵         | 6.1 × 10⁵      |
|                     | 0                | 2.2 × 10⁵        | 2.5 × 10⁵         | 2.0 × 10⁵      |
|                     | 0                | 1.9 × 10⁵        | 2.0 × 10⁵         | 1.0 × 10⁵      |

*ND, Not done

**Cyclophosphamide (CY, 260 mg per kg, once daily) was injected intraperitoneally for four days before challenge with *M. pneumoniae*.**

**Mean value for CFU in five animals.**
the mycoplasma was noted in those organs during the three-day observation period. On the other hand, in the infected mice which had not been treated with CY, the mycoplasma was not found in the brain at four hours post-infection and disappeared rapidly from the lung, spleen, and blood (Table 4).

**DISCUSSION**

*M. pneumoniae* has been isolated from cerebrospinal fluid of patients with the meningitis or meningoencephalitis associated with primary atypical pneumonia. This fact suggests that *M. pneumoniae* can enter the central nervous system. We reported at the Third IOM Congress that *M. pneumoniae* reached the brain of experimental animals, including adult hamsters and mice. Suckling animals as well as adults were used in the present study.

*M. pneumoniae* was isolated from lungs and brains of the suckling mice infected by exposure to the aerosol of the organism. The susceptibility of suckling mice to the infection was much higher than that of adults, from the numbers of mycoplasmas isolated from lungs.

To examine the possibility of reaching the brain via blood, mice were inoculated intravenously with the mycoplasma. The systemic spread of infection occurred in the mice treated with high doses of cyclophosphamide. In addition, the high levels of *M. pneumoniae* persisted for two months or more after the intracerebral inoculation. This fact suggests that the organism can grow in the brain. However, there is no evidence that the organism passes the blood-brain barrier after intravenous or intranasal infection.

Finally, our results suggest that *M. pneumoniae* may be able to reach the brain via blood and it may occur with relative ease in compromised hosts. However, it seems difficult for the mycoplasma to enter the parenchyma of brain of normal hosts because the isolation rate and titers of the organism from brains were low in intravenously or intranasally infected animals throughout the present experiments. If the injury of blood-brain barrier is produced by a cause such as metabolic disorder, mixed infection, or unknown factor, *M. pneumoniae* will be able to enter the parenchyma of brain and to grow there.

**REFERENCES**

1. Levine DP, Lerner AM: The clinical spectrum of *Mycoplasma pneumoniae* infections. Med Clin N Amer 62:961–978, 1978
2. Lerer RJ, Kalavsky SM: Central nervous system disease associated with *Mycoplasma pneumoniae* infection: Report of five cases and review of the literature. Pediatrics 52:658–668, 1973
3. Lind K, Zoffman H, Larsen SO, et al: *Mycoplasma pneumoniae* infection associated with affection of the central nervous system. Acta Med Scand 205:325–332, 1979