MODELS OF INFECTION

Establishment of a model of penicillin-resistant Streptococcus pneumoniae pneumonia in healthy CBA/J mice

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Examination of strain differences in the susceptibility of mice to experimental respiratory tract infection with penicillin-resistant Streptococcus pneumoniae TUM19 revealed that a fatal infection model could be induced in immunocompetent CBA/J mice, but not in C3H/HeN, C57BL/6 or ICR mice. After intranasal instillation of c. $10^6$ cfu of S. pneumoniae, the bacterial counts in the lungs of CBA/J mice increased from $10^5$ to $10^7$ cfu after 3-5 days, and gradually increased thereafter. The challenge organisms localised mainly in the lungs until 14 days after infection. Mice began to die c. 7 days after infection, and by 3 weeks most of the mice had died. Histopathologically, infiltration of neutrophils and lymphocytes around bronchi was observed from 1 day after infection, and fibrin deposition was seen in alveolar and bronchial spaces from 5 days. This model may be useful for investigating therapy of respiratory tract infection caused by penicillin-resistant S. pneumoniae because its pathological features resemble those observed in the human disease.

Introduction

Streptococcus pneumoniae is a common cause of community-acquired pneumonia [1, 2]. Because this organism was originally highly susceptible to penicillin (MIC < 0.1 mg/L), penicillin was the drug of choice for treatment. However, penicillin-resistant strains have been isolated with increasing frequency in virtually every part of the world in recent years [3], and successful treatment with penicillin is no longer guaranteed. In addition, penicillin-resistant strains may be resistant to a wide range of β-lactam and macrolide antibiotics [4, 5] and new strategies for treating these infections should be investigated.

A suitable infection model that closely resembles the features of human disease is necessary to investigate the optimal treatment of infections caused by penicillin-resistant strains. Azoulay-Dupuis et al. [6] and Moine et al. [7] described a murine model of pneumonia caused by penicillin-resistant S. pneumoniae in leucopenic mice, but the strain was avirulent in immunocompetent mice. Their model represented acute infection, with a high incidence of bacteraemia in the early phase of infection, and most mice died within 2 days. However, S. pneumoniae frequently causes community-acquired pneumonia in healthy individuals and only 20-30% of pneumococcal pneumonia is accompanied by bacteraemia [8]. There have been no reports of experimental pneumonia caused by penicillin-resistant S. pneumoniae in healthy immunocompetent mice.

Strains of mice differ in susceptibility to pneumococcal infection [9] and these differences might be useful for establishing a new infection model with penicillin-resistant S. pneumoniae. Therefore, the comparative susceptibility of various strains of mice to experimental respiratory tract infection with penicillin-resistant S. pneumoniae strain TUM19 was examined.

Materials and methods

Mice

Five-to-seven-week-old female CBA/J, C57BL/6 and ICR mice were obtained from Charles River Japan Inc., Kanagawa, Japan. Six-to-seven-week-old female C3H/
HeN mice were obtained from SLC Japan Inc., Shizuoka, Japan. Mice were caged in groups of five-to-ten and given food and water *ad libitum*.

**Organisms**

A clinical isolate of *S. pneumoniae* serotype 19 (strain TUM19), maintained as a stock culture in the Department of Microbiology, Toho University School of Medicine, Tokyo, Japan, was used. The MIC of benzylpenicillin (Meiji Seika Kaisha Ltd, Tokyo, Japan) against the organism, determined by the broth microdilution method according to the reference procedure recommended by the Japanese Society for Chemotherapy 10 was 2 mg/L. The organisms were incubated at 37°C for 20 h on Mueller-Hinton Agar (MHA; Difco Laboratories) supplemented with defibrinated horse blood 5% suspended in sterile physiological saline.

**Respiratory tract infection procedure**

Mice were anaesthetised by intraperitoneal injection of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, ILL, USA) 50 mg/kg of body weight and challenged intranasally by instillation of 40 μl of bacterial suspension.

**Bacteriological examination**

Mice were anaesthetised by intraperitoneal injection of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, ILL, USA) 50 mg/kg of body weight and challenged intranasally by instillation of 40 μl of bacterial suspension.

**Histopathological examination**

The lungs of the mice were fixed in neutral buffered formalin 10% and embedded in paraffin. Sections were stained with haematoxylin and eosin, phosphotungstic acid haematoxylin solution, Gram's stain and azan stain, and examined by light microscopy.

**Results**

**Susceptibility of various strains of mice to infection**

After intranasal instillation of $2 \times 10^7$ cfu of *S. pneumoniae* TUM19, eight of 11 CBA/J mice died within 7 days, whereas all other strains of mice (C3H/HeN, C57BL/6 and ICR) survived (Table 1). Most of the mice that survived had no gross pulmonary lesions and eliminated the organisms from their lungs. Therefore, CBA/J mice were chosen for the following experiments.

**Effect of challenge dose**

With challenge doses of $5.6 \times 10^6$ and $1.1 \times 10^6$ cfu/mouse, most mice died c. 7 days and 11 days after infection, respectively (data not shown). With a challenge dose of $2.2 \times 10^5$ cfu/mouse, all five mice survived for 14 days after infection; bacteria were not recovered from the lungs of two of these mice. Thus, $\geq 10^6$ cfu/mouse were required to cause uniform infection.

**Bacteriological examination**

After challenge with c. $10^6$ cfu/mouse, the bacterial counts in the lungs increased slowly from $10^4$ to $10^7$.

![Fig. 1. Bacterial counts in the lungs (●) and blood (○) of CBA/J mice infected with *S. pneumoniae* TUM19 by intranasal instillation. Each point represents the value for a mouse.](image_url)

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<tr>
<th>Table 1. Susceptibility of various strains of mice to intranasal instillation with <em>S. pneumoniae</em> TUM19*</th>
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<td><strong>Mouse strain</strong></td>
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<tr>
<td>CBA/J</td>
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<tr>
<td>C3H/HeN</td>
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<td>C57BL/6</td>
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<td>ICR</td>
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<td>NT, not tested.</td>
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<td><em>c. $2 \times 10^7$ cfu/mouse.</em></td>
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10^5 cfu/lung by 3 days after infection and increased rapidly to 10^7 cfu at 5 days. The number of bacteria in the lungs increased gradually thereafter (Fig. 1). Some mice developed bacteraemia when the bacterial counts in the lungs reached > 10^8 cfu from 7 days after infection. Mice began to die c. 7 days after infection, and by 3 weeks 83% (48 of 58) had died.

**Histopathological changes**

Histopathological examination of lung from infected mice revealed the existence of bronchopneumonia. Infiltration of neutrophils and lymphocytes around bronchi and hyperplasia of alveolar epithelia were observed at 1 day (Fig. 2A); haemorrhage and necrosis of alveolar epithelia were seen from 3 days after infection.

![Histopathological changes in the lungs of CBA/J mice infected with *S. pneumoniae* TUM19 by intranasal instillation. Stained with haematoxylin and eosin.](image)

A, Neutrophil and lymphocyte infiltration around bronchi and hyperplasia of alveolar epithelia 1 day after infection (x400). B, Haemorrhage and necrosis of alveolar epithelia 3 days after infection (x400). C, Fibrin deposition (arrow) in the bronchial and alveolar spaces 5 days after infection (x400). D, Proliferation of fibroblasts (arrow head) and infiltration of foam cells (arrow) 14 days after infection (x400).
infection (Fig. 2B). Similar lesions extended throughout the lungs thereafter (Fig. 2C, D). In addition to these inflammatory responses, fibrin deposition in the bronchial and alveolar spaces was found at 5 days (Fig. 2C), and fibrosis accompanied by proliferation of fibroblasts and infiltration of foam cells was observed at 14 days (Fig. 2D). Fibrin and collagen fibres were clearly identified by phosphotungstic acid, haematoxylin stain and azan stain, respectively. The infecting organisms in the bronchial and alveolar spaces were detected by Gram’s stain after 5 days of infection (data not shown).

Discussion

It has been thought difficult to establish an experimental respiratory tract infection model with penicillin-resistant S. pneumoniae in immunocompetent mice. Clinical isolates of penicillin-resistant S. pneumoniae predominantly belong to serotypes 6, 14, 19 and 23 [3,4], which are naturally avirulent for mice [11]. Several investigators have failed to induce pneumonia with any penicillin-resistant strains belonging to these serotypes in immunocompetent C57BL/6 and Swiss mice [6, 7]. This study also failed to induce pneumonia in C3H/HeN, C57BL/6, and ICR mice by intranasal challenge with serotype 19 strain (TUM19). However, CBA/J mice showed high susceptibility to the infection: most mice died after intranasal challenge with $\geq 10^6$ cfu of the organism. Genetic defects in the host defences against bacterial infection have not been reported in CBA/J mice and it is unclear why they are susceptible to pneumococcal infection.

Intranasal inoculation of CBA/J mice with $10^6$ cfu of S. pneumoniae TUM19 induced a fatal infection. The challenge organisms localised mainly in the lungs without causing bacteraemia, at least in the first week of infection, while the bacterial counts in the lungs increased from $10^4$ to $10^7$ cfu. Histopathological features such as intense infiltration of inflammatory cells and fibrin deposition in alveolar spaces resembled the features of pneumococcal pneumonia in man [12].

As the course of the infection is long and lung lesions develop gradually, this model might be useful for examining the effect of antibiotics at the various stages of pneumonia caused by penicillin-resistant S. pneumoniae. Furthermore, it is also possible to investigate methods for the suppression of inflammation induced by pneumococcal infection.

References