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EXPERIMENTAL MOUSE PNEUMONIA FOLLOWING LUNG INJURY WITH ONE PERCENT FORMALDEHYDE

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Abstract

Klebsiella pneumoniae or Serratia marcescens was inhaled in the mouse with intact or injured lung. Injury of the respiratory tract was realized by inhalation of 1% formaldehyde. By light and scanning electron microscopy, loss of the cilia and swelling of the epithelial cells were observed in the tracheo-bronchial tree, along with thickening of the alveolar septum accompanied by small round cell infiltration. By means of the inhalation of Klebsiella, typical pneumonia was provoked in the injured mouse, rarely in the intact mouse. Following the inhalation of Serratia, experimental pneumonia was not established in the intact mouse, nor in the injured mouse.

INTRODUCTION

Number of the cases accompanying isolation of Serratia marcescens from urine or sputum is increasing also in Japan.[1,2] As a rule, the patients possessed certain underlying diseases, and the infections occurred after admission to the respective hospitals. As the first step to approach more effective antibiotics for control of these accidental infections, we tried to establish experimental pneumonia in mice by means of the inhalation of Serratia, but pneumonia was not provoked even in the immuno-suppressive mice,[3] being treated with polyvinyl-pyrrolidone,[4] cyclophosphamide[5] or corticosteroid.[6] In the present paper, results of an experiment to produce pneumonia in mice with Serratia marcescens following injury of the lung with 1% formaldehyde are described, together with the results in Klebsiella pneumoniae.

MATERIALS AND METHODS

Albino ICR female mice weighing 24-30 g (4- to 9-week-old) were used for the experiment; they were raised on a pellet diet (CLEA). The bacteria

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adopted for the experiment were Serratia marcescens, strain S-1006, originally isolated from urine of a patient, and Klebsiella pneumoniae, strain B-54, originally isolated from sputum of a pneumonic patient. Injury of the respiratory tract was realized by applying 4 drops of 1% formaldehyde to the nasal cavity in each etherized mouse, which deeply inhaled drop after drop with the inspiration. For the inhalation of bacilli, a special instrument was adopted (Fig. 1); the mice were placed in a plastic box, which was filled with droplets of the respective bacilli by use of a jet nebulizer. In one experiment, 4 drops of Serratia suspension (4.4×10^7/ml) were applied to the mouse nasal cavity, as in the case with 1% formaldehyde.

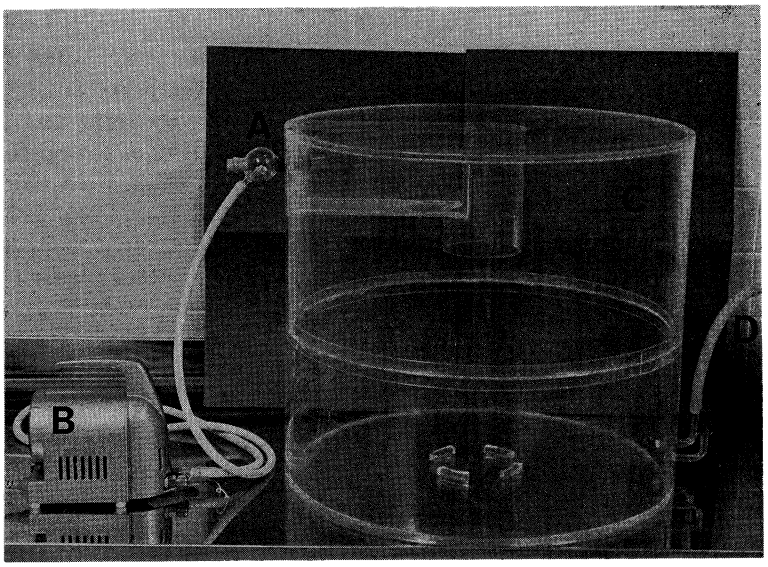


Fig. 1. Instrument for the inhalation of bacilli
A: jet nebulizer B: air compressor
C: plastic box for the mice
D: air aspirator, conducted to a water pipe

The animals were sacrificed by cervical dislocation, and their lungs, taken out aseptically, were adopted for culture of the bacilli and for preparation of the histological specimens. For the detection and quantification of bacilli, the removed lung was homogenized under sterile condition, and the emulsion of lung, diluted with physiological saline, was spread over the culture media (heart-infusion agar). Colonies of the bacilli on the agar were counted 24 hours after incubation at 37°C. The specimens for light microscopy were prepared by fixation in 10% formalin, embedding in paraffin and staining with

hematoxylin and eosin. The specimens for scanning electron microscopy were prepared in the following manner. Immediately after sacrifice, the trachea was released from the neighbouring tissues and ligated. After washing the air passage by infusion and sucking up of 1 ml of physiological saline via the trachea, 1.5 ml of 2.5% phosphate-buffered glutaraldehyde were injected into the air space from the trachea, and the trachea, bronchus and lung lobes were taken out carefully. Fixation was continued in the phosphate buffer for 24 hours, and the tissues were respectively cut into small pieces, which were post-fixed in 1% phosphate-buffered osmium tetroxide at 4°C for 2 hours, and dehydrated in an ascending ethanol series. The tissue pieces were transferred into an isoamyl alcohol-ethanol mixture, and finally into absolute isoamyl acetate. Each specimen was dried in a critical point apparatus (Hitachi), coated with thin layer of gold palladium in a vacuum evaporator, and examined under a scanning electron microscope (HHS-2R, Hitachi) at an accelerating voltage of 20 KV.

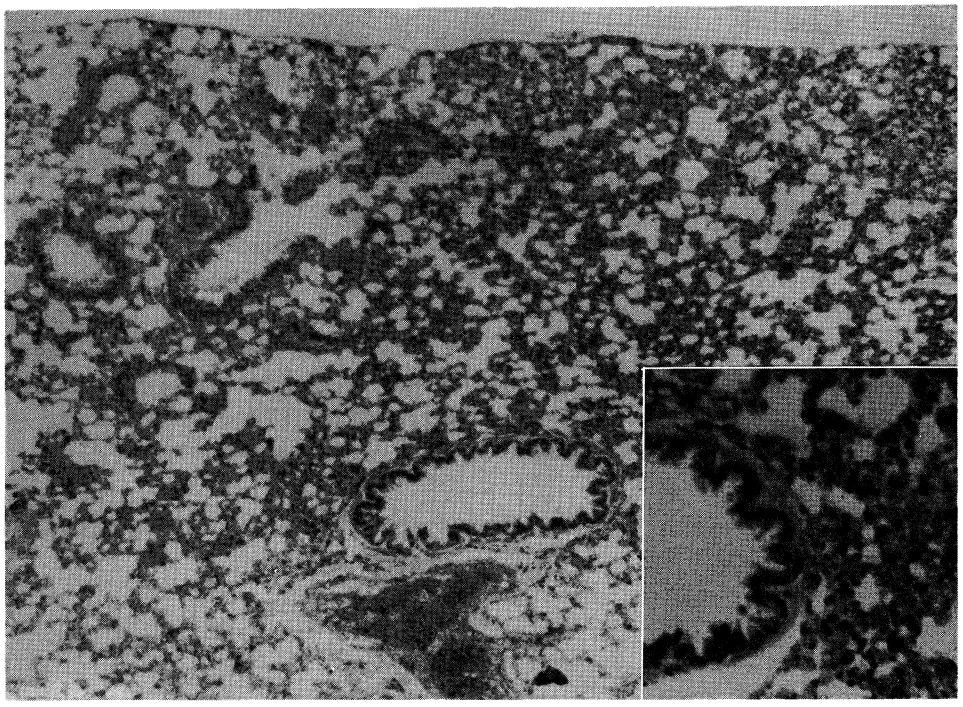


Fig. 2. Lung of a mouse four days after the transnasal application of 4 drops of 1% formaldehyde. Degeneration and exfoliation of the bronchial epithelium and thickening of the alveolar septum with small round cell infiltration are observed. H & E×40 (inset: ×100)

RESULTS

Following the inhalation of 4 drops of 1% formaldehyde, no mice died from injury of the tracheo-bronchial tree. Histological changes of the lung were most severe on the 4th day after inhalation, consisting of degeneration and exfoliation of the bronchial epithelium and thickening of the alveolar septum with small round cell infiltration (Fig. 2). Under the scanning electron microscope, most of the epithelial cells in the tracheo-bronchial tree were being denuded of the cilia, and the non-ciliated and patchily ciliated cells were swollen and deformed (Fig. 3). In the small bronchus, epithelial cells were slightly swollen, and their cilia were generally short, rough and reduced in number. The interalveolar septa were thickened and adopted more or less irregular pattern.

When the mice with injured or intact lung were subjected to the inhalation of suspension (6.1×10^7/ml) of Klebsiella pneumoniae for ten minutes with the jet nebulizer, all of the mice with injured lung died from pneumonia within 2 to 5 days, whereas some of the mice with intact lung died between the 4th day and the 8th day, and four mice out of ten survived (Fig. 4). Change in number of the bacilli cultured from the lung was examined in the injured, 17 or intact, 16 mice following the inhalation of suspension (4×10^7/ml) of Klebsiella for ten minutes with the jet nebulizer. As shown in Fig. 5, around 10^3 bacilli were cultured from the lung in both groups one hour after the inhalation, but no bacilli were demonstrated one day afterwards. From the 3rd day after inhalation, however, some of the remaining, 9 mice with injured lung began to die from pneumonia, and all of the surviving, 4 mice died between the 5th day and the 7th day; number of the bacilli cultured from the lung generally amounted to 10^7 or upwards. In the case of the mice with intact lung, only one died from pneumonia on the 7th day, presenting numberless bacilli from lung; in some of the remaining, 8 mice, 10^2 to 10^3 bacilli were cultured from the lung on the 4th day, but the bacilli disappeared after the 8th day and the hosts remained alive.

When the mice with injured or intact lung were subjected to the inhalation of Serratia marcescens by means of the transnasal application of 4 drops of suspension (4.4×10^7/ml), number of the bacilli cultured from the lung on the first day was around 10^3 on an average in both groups (Fig. 6). While number of the bacilli on the 4th day was still around 10^2 in the injured mice, it was less than 10 in the intact mice; on the 7th day after inhalation, no bacilli were cultured from the lung in both groups. When the mice with injured or intact lung were subjected to the inhalation of suspension (3.8×10^8/ml) of Serratia for ten minutes with the jet nebulizer, number of the bacilli cultured from the

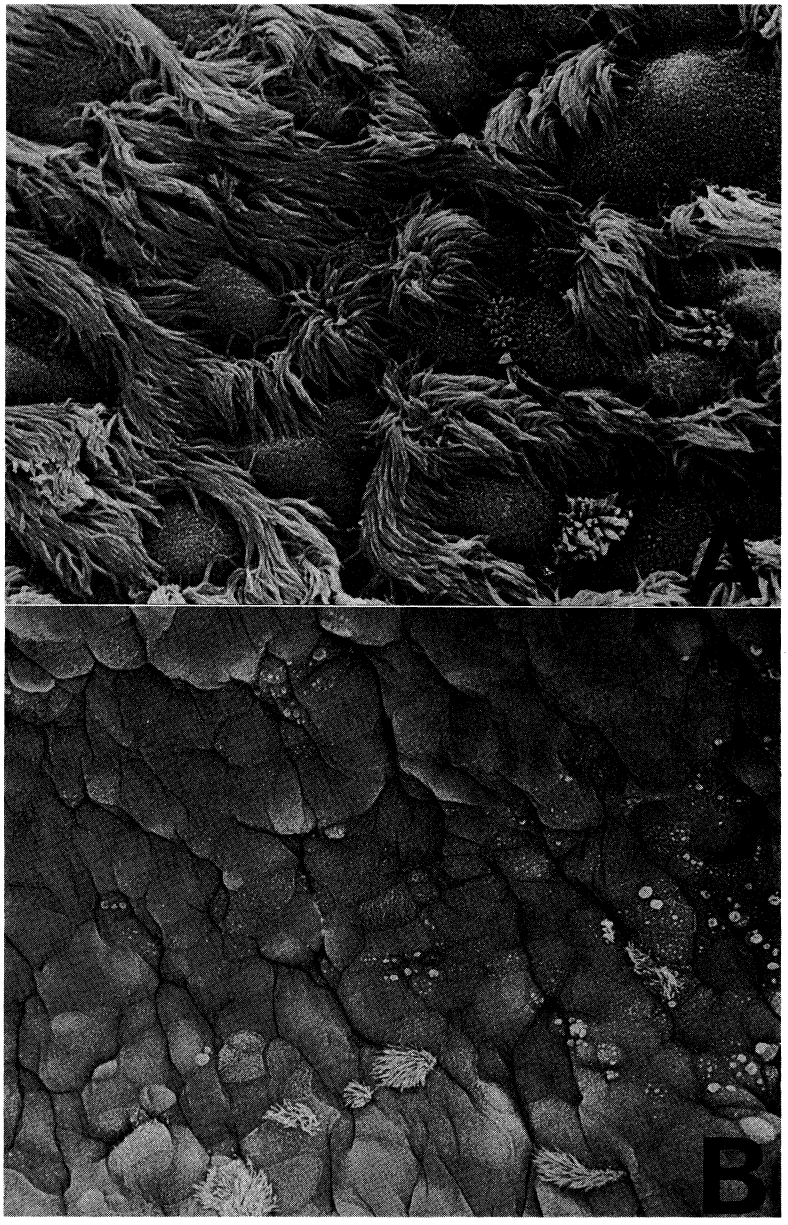
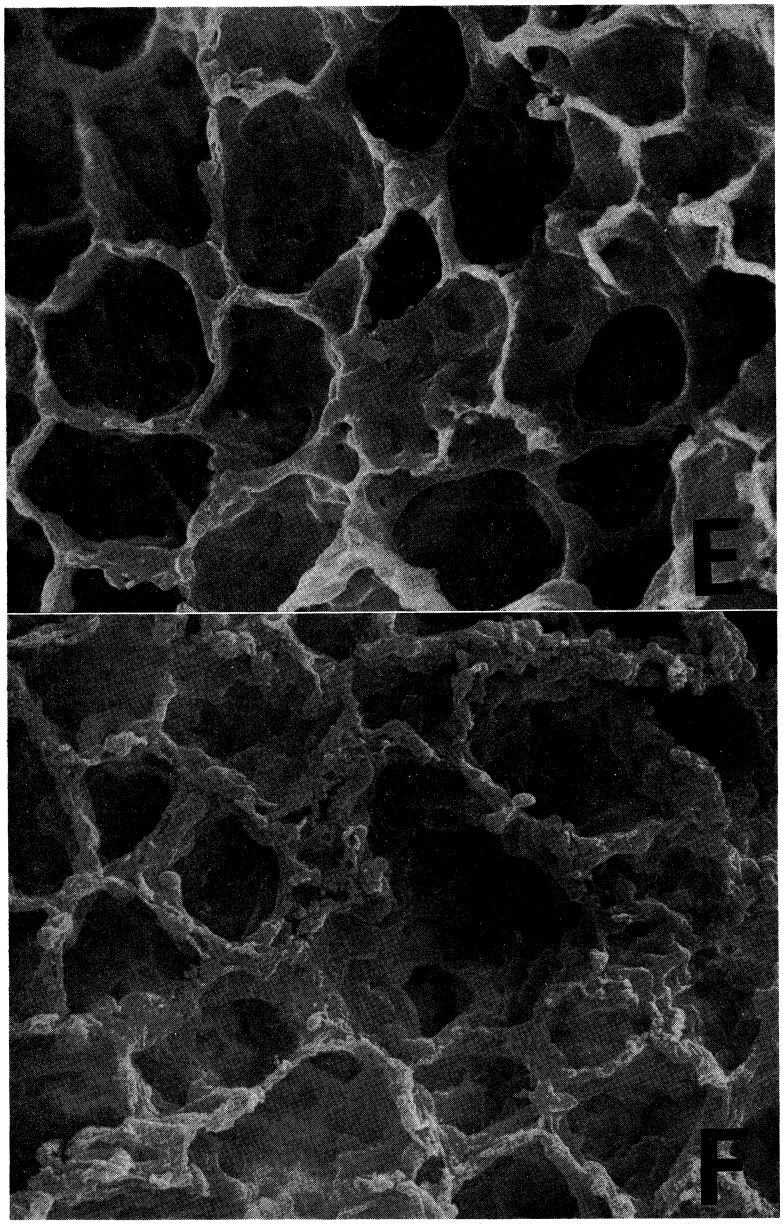


Fig. 3. Scanning electron micrograph of the trachea and lung in a mouse four days after the transnasal application of 4 drops of 1% formaldehyde. In the trachea (B), most of the epithelial cells are denuded of the cilia, and non-ciliated or patchily ciliated cells are swollen

(compare with A: control). In the small bronchus (D), epithelial cells are slightly swollen, and their cilia are generally short, rough and reduced in number (compare with C: control). In the alveolus (F), the



inter-alveolar septa are thickened and adopt more or less irregular pattern (compare with E: control). A, ×1,500; B, ×600; C, ×800; D, ×2,000; E, ×660; F, ×600

lung on the first day was around 10^2 on an average in both groups and decreased to around 10 on the 4th day; on the 8th day after inhalation, no bacilli were cultured from the lung in both groups (Fig. 7).

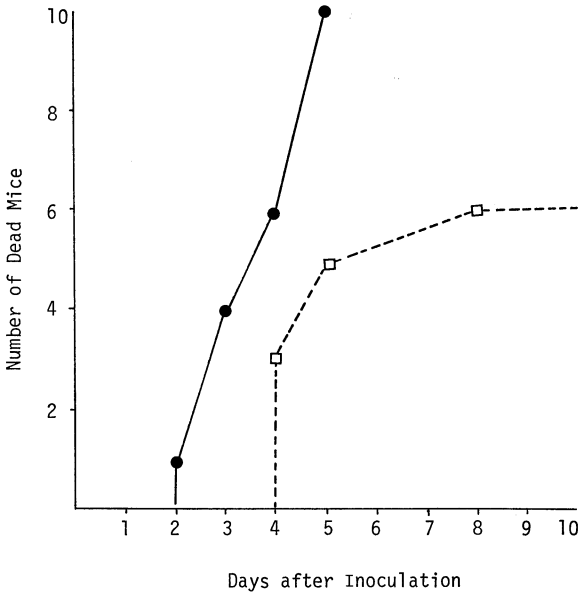


Fig. 4. Cumulative number of dead mice on successive days following the inhalation of suspension (6.1×10^7/ml) of Klebsiella pneumoniae, strain B-54, for ten minutes with the jet nebulizer (●——●: mice with the lung injured by 1% formaldehyde; □·····□: mice with the intact lung).

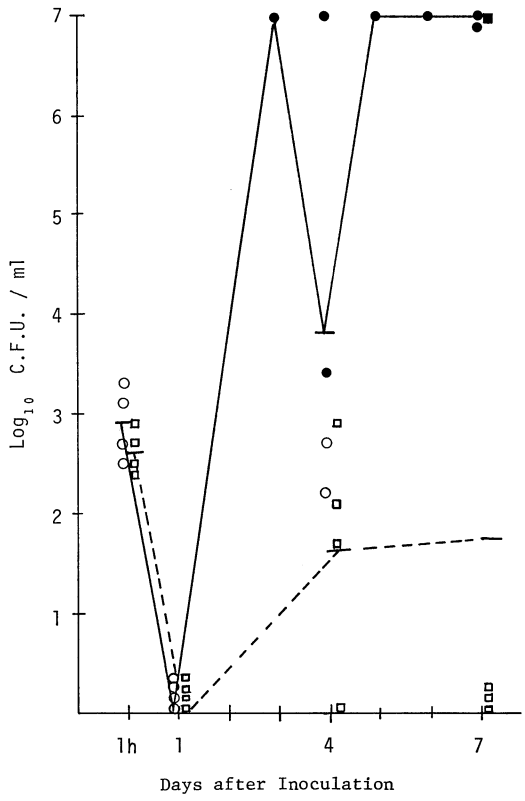


Fig. 5. Number of Klebsiella pneumoniae, strain B-54, cultured from the lung in mice following the inhalation of suspension (4×10^7/ml) of the bacilli with the jet nebulizer (○——●: mice with the lung injured by 1% formaldehyde; □------■: mice with the intact lung; ○ and □: alive and sacrificed; ● and ■: dead).

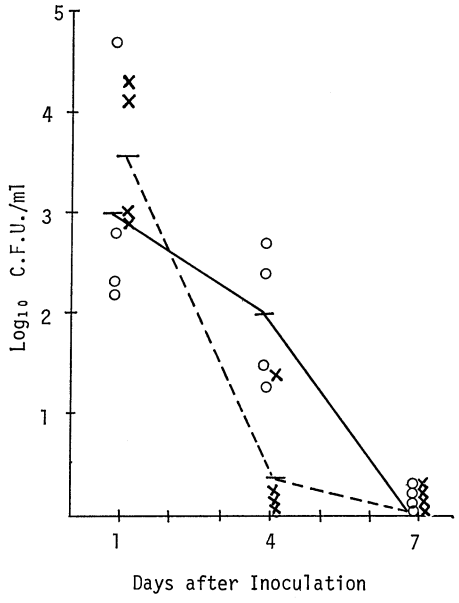


Fig. 6. Number of Serratia marcescens, strain 1006, cultured from the lung in mice following the transnasal aspiration of 4 drops of suspension (4.4×10^7/ml) of the bacilli (○——○: mice with the lung injured by 1% formaldehyde: ×-------×: mice with the intact lung).

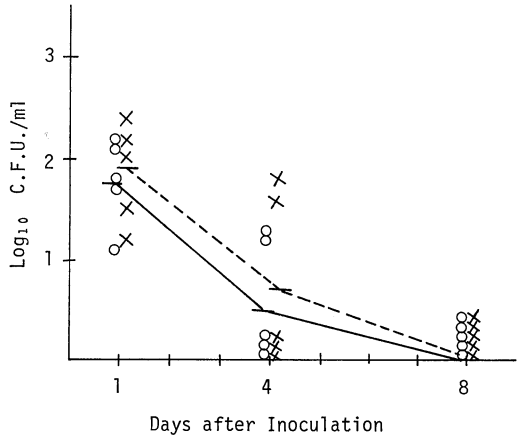


Fig. 7. Number of Serratia marcescens, strain 1006, cultured from the lung in mice following the inhalation of suspension (3.8×10^8/ml) of the bacilli with the jet nebulizer (○——○: mice with the lung injured by 1% formaldehyde; ×-------×: mice with the intact lung).

DISCUSSION

The wide-spread use of antimicrobial antibiotics during recent years has promoted a number of hitherto neglected, gram-negative bacilli to new pathogenic organisms in the compromised hosts. Among them, Serratia marcescens has been isolated from urine or sputum of the increasing number of patients, and deserves serious concern of the present day practioners.[1] This microorganism has been considered to be relatively avirulent,[7,8] but is being proved to be resistant to many antibiotics.[9,10] For the purpose of finding the best therapeutic means against Serratia infection, we tried to produce Serratia pneumonia in mice, but failed in healthy, non-treated animals.[3] Serratia pneumonia was not established even in the immuno-suppressive mice, which had received intraperitoneal injection of polyvinyl-pyrrolidone,[4] cyclophosphamide[5] or corticosteroid.[6] It is well-known that Serratia pneumonia occurs in the patients with severe underlying diseases,[11~14] including anti-neoplastic and other iatrogenic treatments and nosocomical accidents.[15]

In various kinds of bacterial pneumonia, local defence mechanism in the respiratory tree plays an important role,[16-18] and the tracheo-broncho-alveolar damage is believed to render the host susceptible to some kinds of gram-negative bacilli. In the present experiment, injury of the respiratory tract was realized by applying 4 drops of 1% formaldehyde to the nasal cavity in each etherized mouse. While about half of the mice died from acute pulmonary edema after inhalation of formaldehyde at concentrations above 3%[3], no mice died at this concentration. In all the mice examined, however, loss of the cilia and swelling of the epithelial cells were observed in the tracheo-bronchial tree by light and scanning electron microscopy, whereas the surface of normal tracheo-bronchial epithelium is almost completely covered with the cilia.[19,20]

Experimental Serratia pneumonia was not established even in these injured mice, although typical pneumonia was provoked in the injured mice following the inhalation of Klebsiella pneumoniae, rarely in the intact mice. We are planning to find another damaging factor upon the tracheo-bronchial tree to provoke Serratia pneumonia in mice.

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