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EXPERIMENTAL STUDY ON THE SUSCEPTIBILITY TO *KLEBSIELLA PNEUMONIAE* IN THE MOUSE WITH INJURED LUNG

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Abstract

In order to confirm the enhancing effect of tracheo-broncho-pulmonary damage upon the susceptibility to infection, varying amounts of *Klebsiella pneumoniae*, strain B-54, were aspirated or inhaled to the mice having formaldehyde-injured lungs. As judged from higher death rates from pneumonia, these animals were found to be more susceptible to the transnasal inoculation than normal controls. For the demonstration of difference in the susceptibility between two groups, the dose of bacilli inoculated played a crucial role.

INTRODUCTION

The normal lung is equipped with a variety of nonspecific and specific mechanisms to resist development of many kinds of infections[1]. In the lung with tracheo-broncho-pulmonary damage accompanying disturbance of the physical barrier, however, recurrent infections of the respiratory tract may frequently develop, such as in bronchiectasis[2], chronic bronchitis[3] and diffuse panbronchiolitis[4]. We have attempted to develop an experimental pneumonia in mice by a low virulent bacillus, *Serratia marcescens*, following injury of their lungs by 1% formaldehyde, but the pneumonia was not established[5]. In the present paper, results of an experiment are described, which indicates an enhanced susceptibility of the mice with similarly injured lungs to *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Albino ICR, female mice weighing 24–28 gm (4-to 6-week-old) were used

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for the experiment. The bacillus adopted this time was *Klebsiella pneumoniae*, strain B-54, originally isolated from the sputum of a patient by Prof. K. Matsumoto, Nagasaki University, Japan ; its virulence is remarkably strong[6]. Method of the experimental injury upon respiratory tract by 1% formaldehyde and its effect, as well as method of the transnasal inoculation of the bacilli, were described in detail elsewhere[7].

RESULTS

In each of the experimental series, the Albino ICR mice received single transnasal inoculations of the bacterial emulsion, 4 days after injury of their lungs by 1% formaldehyde. Same numbers of the animals without pretreatment served as the controls.

Fig. 1 indicates survival rate of the mice receiving single transnasal aspirations of 4 drops (1/25 G needle) of 2.4×10^5/ml, $\times10^4$/ml and $\times10^3$/ml of the bacterial suspension. In the case of 2.4×10^5/ml, nine out of the ten mice with injured lungs died from pneumonia within 10 days, while six died out of the ten with intact lungs ; in the case of 2.4×10^4/ml, all ten mice belonging to the injured lung group died, whereas only three died out of the ten belonging to the control group ; in the case of 2.4×10^3/ml, nine mice of the former group died, but none in the latter.

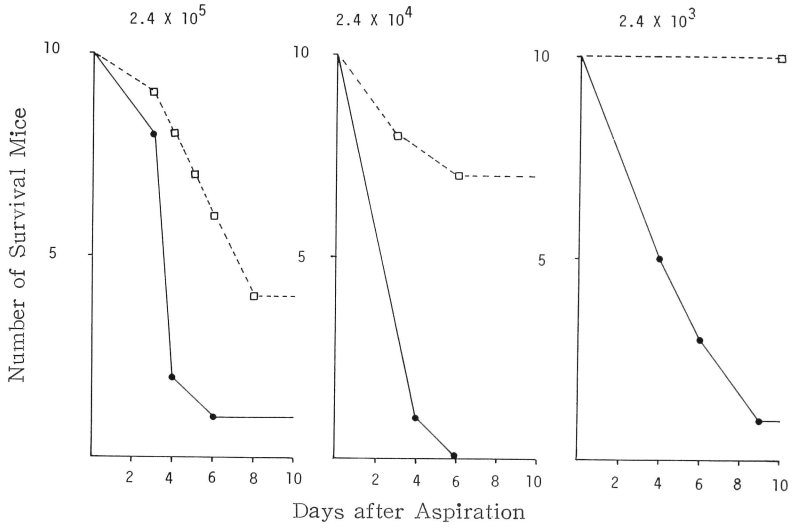

Fig. 1. Number of the survival mice following single transnasal aspirations of 4 drops of the suspension of *Klebsiella pneumoniae*, strain B-54 (•——• : mice with the lungs injured by 1 % formaldehyde ; □·····□ : mice with the intact lungs).

In the next place, 4 drops of the bacterial suspension (6×10^3/ml) were transnasally aspirated to three groups of four mice, 4 days after injury of their lungs by 1% formaldehyde, as well as to other three groups of four, non-treated mice. Thirty minutes, 24 and 72 hours after the inoculation, their lungs were respectively isolated, subjected to the homogenization and suspended in saline (a lung/ml). Fig. 2 indicates that, in the injured lung group, the number of bacterial colonies cultured from suspension of the lung in 1 ml saline steadily increased in parallel with the time after inoculation ; in the control group, a slight increase was apparent 24 hours after inoculation, owing to the presence of viable bacilli in one mouse, but the viable bacilli completely disappeared 72 hours after inoculation.

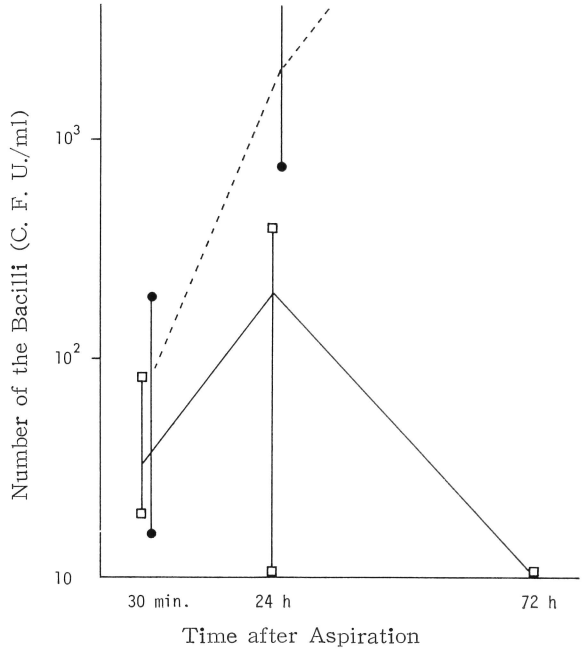

Fig. 2. Number of *Klebsiella pneumoniae*, strain B-54, cultured from suspension in 1 ml saline of a lung, which was obtained from each mouse following single transnasal aspirations of 4 drops of the bacterial suspension (6×10^3/ml).

Fig. 3 indicates the survival rate of the mice receiving single transnasal inhalations by a jet nebulizer of aerosols of the bacilli : 1.2×10^9, 10 min ; 6.1×10^7, 10 min ; 1.1×10^7, 5 min. As in Fig. 1, the number of survival mice was greater in the control group than in the injured lung group.

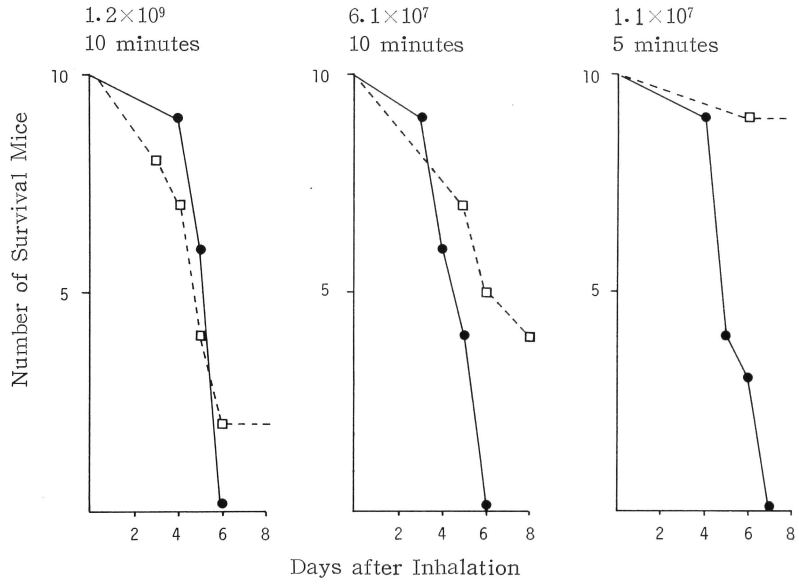

Fig. 3. Number of the survival mice following single transnasal inhalations of aerosols of *Klebsiella pneumoniae*, strain B-54, by a jet nebulizer (•——• : injured ; □·····□ : intact).

DISCUSSION

When low virulent bacilli are aspirated or inhaled to the lung in normal animals, they will be soon cleared up from the lung, and pneumonia cannot be usually established. For the purpose of establishing an experimental pneumonia by low virulent bacilli, we have aspirated *Serratia marcescens* to the mice, following injury of their lungs by 1% formaldehyde, but the pneumonia could not be produced[5]. In the present experiment, varying amounts of *Klebsiella pneumoniae*, strain B-54, have been aspirated or inhaled to the mice with similarly injured lungs, and have demonstrated an enhanced susceptibility in these mice. As may be apparent from Fig. 1 and 3, the difference in susceptibility between the injured lung group and the intact lung group was most pronounced, when the lowest concentration of bacterial suspension was adopted ; in Fig. 3, higher concentrations obscured the difference.

In the injured lung group, the susceptibility to bacterial infection seems to be brought about by morphological changes in the tracheo-broncho-pulmonary system. We have observed in the mice after NO_2 exposure that early inflammatory changes after inhalation of *Klebsiella pneumoniae* appeared in those portions of the alveoli, which are adjacent to respiratory bronchioles and which

are the most susceptible portion of the lung to NO_2 exposure[8]. Using the same experimental system, however, Niki[9] attributed the impairment of resistance against bacterial infection to the dysfunction of alveolar macrophages in intracellular disposal of the invading bacilli.

Mechanisms underlying the lowered or recurrent infectivity in tracheo-broncho-pulmonary damage should be examined more in detail hereafter. It has been reported that treatment by various antibiotics of pneumonia is more difficult in the mouse with injured lung than in the mouse with intact lung[10], from the standpoint of eradication of the bacilli and of the survival rate of the mouse.

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