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A NOVEL INHALATION CHALLENGE SET TO STUDY ANIMAL MODEL OF ALLERGIC ALVEOLITIS

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Abstract: A novel inhalation challenge set for the study of experimental allergic alveolitis (hypersensitivity pneumonitis) in mice was designed. A finely dispersed aerosol of allergenic extract generated by the commercial ultrasonic nebulizer "TAJFUN MU1" (produced by Medbryt, Warsaw, Poland) was transported to the airtight inhalation chamber. In the chamber were placed 15 perforated containers made of transparent plastic, each containing one mouse. They were coupled in 3 units, each consisted of 5 containers. The constant flow of aerosol through the chamber was assured by commercial vacuum pump "PL 2/3" (AGA LABOR S.C., Warsaw, Poland). The applied set enabled the natural exposure of mice via the inhalation route to known quantities of allergen (usually microbial) suspended in saline, and then dispersed in form of fine aerosol by ultrasonic nebulizer. This method assures the penetration of allergen into the deep parts of lungs, alveoli and bronchioli. The detailed study of histopathological and biochemical changes in the lungs of exposed animals will be the subject of further publications. So far, the retention of endotoxin in the lungs of mice exposed to the extract of a Gram-negative bacterium *Pantoea agglomerans* and appearance of positive serologic reactions to this extract indicate the effectiveness of the method.

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INTRODUCTION

Exposure to organic dusts may be a cause of various diseases of the respiratory tract, conjunctiva and skin, of which one of the most important is allergic alveolitis (hypersensitivity pneumonitis), evoked mostly by allergens of bacteria and fungi contaminating the dusts [4, 11]. This is a lymphocyte-mediated disease leading to granuloma and lung fibrosis [7, 11]. To study the complex pathogenesis of the disease, of importance is the experimental reproduction of pathological processes in the lungs of laboratory animals exposed to various etiological agents of allergic alveolitis, such as a thermophilic actinomycete *Saccharopolyspora rectivirgula* (synonyms: *Micropolyspora faeni*, *Faenia rectivirgula*) [1, 12], or Gram-negative bacterium *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) [6]. In hitherto conducted studies various species of laboratory animals were used, including mice, rats, guinea pigs, and rabbits [1, 3, 5, 6, 9, 10, 12]. The animals were exposed to suspensions of the disease agents by various methods: tracheal instillation [12], nasal instillation [1], or exposure to inhalation of aerosol from dust or fluid comprising etiological agent(s), generated by various devices (such as DeVilbiss ultrasonic nebulizer, Pitt 3 aerosol generator) [5, 6, 9, 10, 15]. More recently, mostly mice have been used for experiments [1, 12], hence the murine model of allergic alveolitis became the standard for description of the disease process.

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The aim of the present work was to design an inhalation challenge set enabling natural exposure of mice via inhalation route to known quantities of microbial allergen suspended in saline, and then dispersed in the form of a fine aerosol by ultrasonic nebulizer.

MATERIALS AND METHODS

Design of a novel inhalation challenge set. A novel inhalation challenge set for mice was constructed, composed of commercially available and newly designed elements.

Testing the effectiveness of inhalation exposure. The C57BL/6J 3-months-old mice were exposed for 28 days, one hour daily, to the extract of Gram-negative bacterium *Pantoea agglomerans* associated with organic dusts and known to cause allergic alveolitis [8] and to produce biologically active endotoxin [2]. The lyophilized extract of *P. agglomerans* cells [14] was dissolved in PBS and used for inhalation in the concentration of 1 mg/ml. The mean concentration of the extract in the air of the chamber (x ± SD) was 67.6 ± 31.3 μg/m^3. As determined by the *Limulus* (LAL) test [13], the extract contained 2.5% (25 μg/mg) biologically active endotoxin (BAE). The presence of BAE inhaled in the lungs of the mice was assumed as a marker of exposure. Excisions of the lungs of the mice killed and sectioned before inhalation (4 mice) and after 7 and 28 days of inhalation (6 mice each) were examined for the presence of BAE by the *Limulus* (LAL) test.

Moreover, pooled blood serum samples of mice taken before inhalation and after 28 days of inhalation were tested by the Ouchterlony double diffusion precipitin test [14] for the presence of antibodies against antigen of *Pantoea agglomerans*.

RESULTS AND DISCUSSION

Description of a novel inhalation challenge set. An inhalation challenge set consists of three basic subunits (Fig. 1):

1) Ultrasonic aerosol generator "TAJFUN MU1" (1) produced commercially by Medbryt, Warsaw, Poland. This device, originally assigned for inhalation treatment, nebulizes fluids in form of a finely dispersed aerosol composed of particles measuring on the average 1.77 μm.

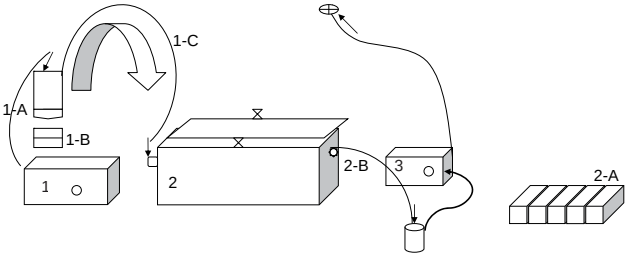

Figure 1. Scheme of a novel inhalation challenge set for mice.
1) ultrasonic aerosol generator, 1-A) upper vessel of the nebulizing chamber, 1-B) lower vessel of the nebulizing chamber with piezoelectric converter, 1-C) notched aerosol pipe transporting aerosol, 2) airtight inhalation chamber, 2-A) perforated containers for mice, 2-B) mounted filter, 3) vacuum pump.

Figure 2. An inhalation challenge set for mice before inhalation.
1) ultrasonic aerosol generator, 2) inhalation chamber, 3) vacuum pump.

Figure 3. An inhalation challenge set for mice during inhalation. The chamber is filled with nebulized allergen extract.
1) ultrasonic aerosol generator, 2) inhalation chamber, 3) vacuum pump.

2) Cuboid airtight inhalation chamber (2) made of transparent plastic, 54 cm long, 40.7 cm wide, and 22 cm high. In the chamber were placed 15 perforated containers made of transparent plastic, of the dimensions 12.6 cm × 4.0 cm × 6.2 cm, each harboring one mouse. They were coupled in 3 units, each consisting of 5 containers.

3) Vacuum pump "PL 2/3" (3) produced commercially by AGA LABOR S.C., Warsaw, Poland. This assures the constant flow of aerosol through the chamber at the velocity of 9 l/min.

The tested solution, obtained by dissolving of the lyophilized allergen in saline, usually at the concentration of 1 mg/ml, was poured through a pipe to the upper vessel of the nebulizing chamber of the aerosol generator (1-A). The piezoelectric converter placed at the bottom of the lower vessel of the nebulizing chamber (1-B) generated ultrasonic waves which were transferred to the allergen solution through intermediate fluid (distilled water) located in the lower vessel, and dispersed it in the air in the

Table 1. Effects of inhalation exposure of C57BL/6J mice to aerosolized antigen of *Pantoea agglomerans*: presence of endotoxin in lung tissue and serological response.

	Numbers of positive/examined (percent)		
	Before inhalation	After 7 days of inhalation	After 28 days of inhalation
Presence of endotoxin in lung tissue	0/4 (0)	0/6 (0)	3/6 (50)
Positive precipitin response to *Pantoea agglomerans* (pooled sera)	Negative	N. t.	Positive

N. t. = Not tested

form of a fine aerosol. Aerosolized allergen, thus generated by "ultrasonic fountain", was blown away from nebulizing chamber and transported by notched aerosol pipe (1-C) into the airtight inhalation chamber (2) containing the mice kept in the perforated containers (2-A) (Figs 2, 3). The aerosol generator was additionally equipped with a built-in electro-acoustic converter which caused vibration of the aerosol and increased the velocity of its particles and their sedimentation in the respiratory tract of the exposed animals. Aerosolized allergen was sucked into the vacuum pump (3) with a constant velocity through the outlet orifice of inhalation chamber in which a polypropylene filter was mounted (2-B) for monitoring of allergen concentration in the air of inhalation chamber.

Effectiveness of inhalation exposure. The results of the *Limulus* test for determination of endotoxin content in the lungs of mice exposed to aerosolized extract of *Pantoea agglomerans*, and the results of serological examination of exposed mice for the presence of precipitating antibodies to *P. agglomerans,* are presented in Table 1. Endotoxin was not detected in the lungs of mice before inhalation and after 7 days of inhalation, but was detected in 50% of exposed mice after 28 days of inhalation. The concentration of endotoxin in lung tissue was between 0.55–0.93 ng/g. It was found that the exposed mice produced specific antibodies against *P. agglomerans*.

Discussion. Various methods of exposure applied for the study of experimental allergic alveolitis in mice show advantages and disadvantages. The use of tracheal or nasal instillation allows for exact measurement of dose, but does not mimic the natural process of acquiring of disease agents by breathing. Such a process is followed by exposure to free inhalation of the aerosol from dust or fluid comprising etiological agent(s), but does not always assure the exact measurement of the dose.

The novel inhalation challenge set applied in the present work enabled the natural exposure of mice via the inhalation route to known quantities of microbial allergen suspended in saline, and then dispersed in the form of a fine aerosol by ultrasonic nebulizer. This method assures also the penetration of allergen into the deep parts of lungs, alveoli and bronchioli. The detailed study of histopathological and biochemical changes in the lungs of exposed animals will be subject of further publications. So far, the retention of endotoxin in the lungs of mice exposed to the extract of *Pantoea agglomerans* and the appearance of positive serologic reactions to this extract indicate the effectiveness of the method.

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