



Atopic cough-like cough hypersensitivity caused by active sensitization with protein fraction of *Aspergillus restrictus* strain A-17

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Abstract

We examined the sensitivity of the cough reflex to inhaled citric acid in guinea pigs that had been actively sensitized with the protein fraction of *Aspergillus restrictus* strain A-17. The number of coughs elicited by an aerosol of 5% citric acid was significantly increased in the sensitized group compared to the non-sensitized group. The number of citric-acid-induced coughs in sensitized guinea pigs was dose-dependently and significantly reduced to the level in non-sensitized guinea pigs when animals were pretreated with fexofenadine, a selective histamine H1 receptor antagonist, 60 min before citric acid inhalation. The bronchial responsiveness to inhaled methacholine or histamine in the sensitized group was not significantly heightened compared to the non-sensitized group. These results suggest that active sensitization with the protein fraction of *A. restrictus* by itself, that is without subsequent allergen challenge, enhances the excitability of cough receptors to tussive stimuli, and the physiologic features of this animal model are consistent with those of atopic cough.

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1. Introduction

Chronic cough is a common and distressing symptom. In these disorders, the sensitivity of the cough reflex is heightened while patients are coughing and become normal upon successful treatment [1]. Allergic reactions and cough hypersensitivity may be induced by chemical mediators such as histamine [2], prostaglandins [3], thromboxane A2 [4], and platelet activating factor, which are released from mast cells activated by IgE antibody and/or the production of Th2 cytokines [5].

Fungus is one of the main allergens that causes allergy diseases, such as asthma, along with house dust and mites. However, fungal antigens have not yet been identified, in contrast to house dust and mites because of their low IgE positivity [6]. One of the main reasons for this lack of information is the difference in the composition of fungal

allergens obtained by cultivation sampling, and the characteristics of fungi that make them important allergens have not yet been identified. Large amounts of xerophilic fungi have been found to be widely distributed in our inhabited environment [7]. The typical xerophilic fungus *Aspergillus restrictus* (*A. restrictus*) SMITH has been very frequently detected in house dust culture on dichloran 18% glycerol agar medium [8]. In mycofloristic analyses of house dust samples from the dwellings of asthmatic patients in Japan, xerophilic fungi *A. restrictus*, *Cladosporium sphaerospermum* PENZIG, *Wallemia sebi* and *Penicillium* sp. were detected as main fungal flora [9]. Therefore, xerophilic fungi might be the primary causative fungi for various allergenic diseases, such as asthma. Furthermore, it is also reasonable to speculate that xerophilic fungi might modulate cough hypersensitivity for some chronic cough diseases. To clarify these points, we examined the sensitivity of the cough reflex in response to inhaled citric acid in guinea pigs that had been actively sensitized with the protein fraction of *A. restrictus* strain A-17 [10].

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2. Methods

2.1. Animals

Male Hartley guinea pigs (Tokyo Animal Laboratory, Inc., Tokyo, Japan) weighing about 300–350 g were used. The animals were housed in groups of four per cage under a 12-h light/12-h dark cycle with food and water available continuously. These studies were carried out in accordance with the Guidelines for the Use of Laboratory Animals as adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Cough sensitivity and antitussive assay

The cough reflex was induced as previously described [11,12]. Briefly, animals were exposed to a nebulized solution of citric acid under conscious and identical conditions using a body plethysmograph. Citric acid was dissolved to a concentration of 5% in a saline solution. The guinea pigs were exposed to citric acid for 5 min. For the assay of the antitussive effect of fexofenadine, the animals were exposed for 5 min to citric acid before drug treatment to determine the frequency of control coughs. The animals were also exposed for 5 min to citric acid 60 min after treatment with fexofenadine. The number of coughs produced after drug treatment was compared to the number of control coughs.

2.3. Preparation of *A. restrictus* antigens

A. restrictus strain A-17 [10] was cultured at 25 °C for 21 days in Petri dishes containing 20 ml M40Y agar medium (40% sucrose, 2% malt extract, 0.5% yeast extract and 2% agar) in each dish. After 3 weeks of incubation, the spores and mycelium were separated from the agar medium by scratching with the addition of distilled water. The spores, including the mycelium, were suspended in distilled water with continuous stirring for 16 h at 4 °C. The supernatant was separated by centrifugation ($10,000 \times g$) for 30 min and filtered to remove the spores and the mycelium, and then dialyzed in distilled water to remove small molecules. The filtrate was ultra-centrifuged ($109,000 \times g$) for 60 min and filtered off by a membrane filter (0.45 μm) to remove water-insoluble substances, and then concentrated by ultra-filtration to give *A. restrictus* antigenic extract solution. The amount of protein contents in this extract was determined by using BioRad Protein assay kit.

2.4. Sensitization of animals

One milligram of ovalbumin was emulsified in 0.4 ml of *A. restrictus* antigenic extract solution (protein content was 7 mg/ml) together with 0.8 ml of *Bordetella pertussis*

(2×10^{10}) and injected intraperitoneally into each guinea pig at multiple sites. A booster dose (1 mg of ovalbumin was emulsified in 0.6 ml of *A. restrictus* antigenic extract solution together with 0.6 ml of *B. pertussis* (2×10^{10})) was prepared and administered intramuscularly 5 days later. Each animal was used for experiment 3 days after the booster dose. In control animals, ovalbumin was emulsified with normal saline, instead of *A. restrictus* antigenic extract solution, together with *B. pertussis* (2×10^{10}) and injected in the same schedule as sensitized animals.

2.5. Measurement of specific airway resistance

Specific airway resistance (sRaw) was measured by comparing air flow sensors attached to the front and rear chambers according to the method described by Pennock et al. [13]. Briefly, a conscious guinea pig was positioned with its neck extending through the partition of a two-chambered, whole-body plethysmograph. Each chamber was fitted with identical wire-screen pneumotachographs and identical differential pressure transducers (model DP-45; Validyne, Northridge, CA). These transducers were connected to a respiratory analyzer (Pulmos-I, MIPS, Osaka, Japan), and a personal computer was used for the on-line breath-by-breath measurement of sRaw. Airway responsiveness was evaluated by the airway response to histamine and methacholine.

2.6. Drugs

Histamine dihydrochloride and methacholine chloride were purchased from Sigma Chemical Co. Fexofenadine hydrochloride was generously supplied by Sanofi-Aventis K.K. (Tokyo, Japan). All drugs were dissolved in saline (0.9% NaCl). Fexofenadine was administered p.o. 60 min before citric acid inhalation. The doses of fexofenadine were determined as previously described [11].

2.7. Statistics

Data are expressed as the means $\pm$ S.E.M. The statistical significance of differences was assessed by the Mann–Whitney *U*-test. A level of probability of 0.05 or less was considered significant.

3. Results

3.1. Cough sensitivity

Fig. 1 shows the number of coughs induced by inhaled citric acid in non-sensitized and sensitized guinea pigs. The number of coughs elicited by an aerosol of 5% citric acid was significantly increased in the sensitized group (17.3 ± 1.6 coughs/5 min, $n = 6$) compared to that in the non-sensitized group (10.8 ± 0.6 coughs/5 min, $n = 6$).

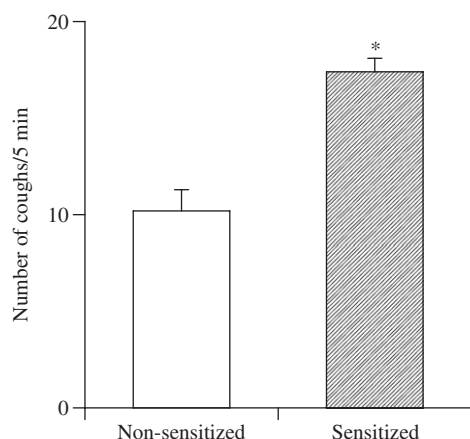


Fig. 1. Number of citric-acid-induced coughs in the guinea pigs that had been actively sensitized with the protein fraction of *A. restrictus* and control guinea pigs. Data are presented as the mean $\pm$ S.E.M. ($n = 6$). * $P < 0.05$ vs. non-sensitized group.

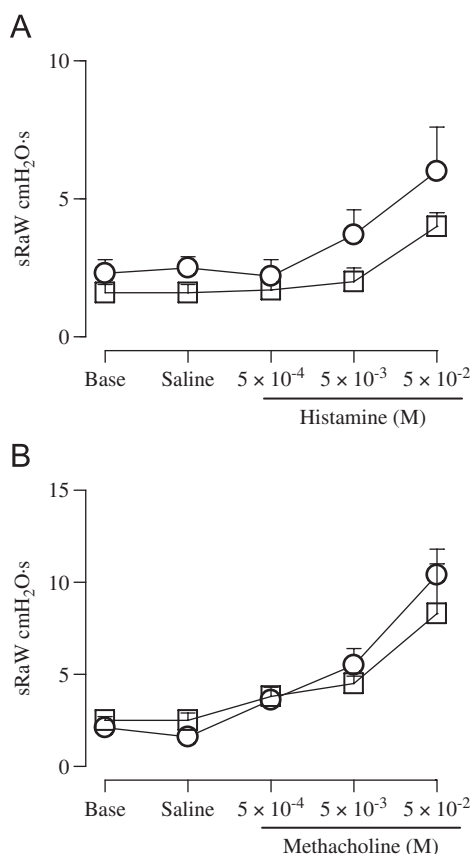


Fig. 2. Bronchial responsiveness to histamine (a) and methacholine (b) in guinea pigs that had been actively sensitized with the protein fraction of *A. restrictus* (circle) and control (square) guinea pigs. Data are presented as the mean $\pm$ S.E.M. ($n = 5$).

3.2. Bronchial responsiveness

Bronchial responsiveness to inhaled methacholine or histamine in sensitized guinea pigs is shown in Fig. 2. In both groups, specific airway resistance (sRaw) was dose-

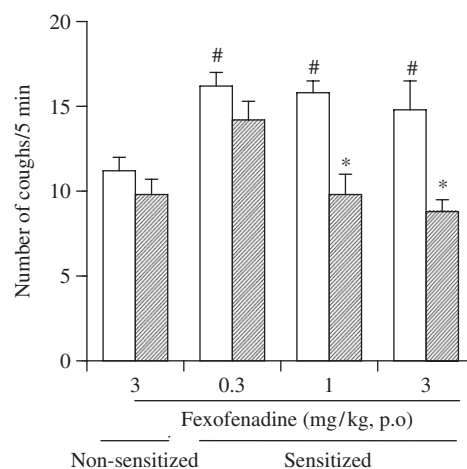


Fig. 3. Effects of fexofenadine on the increased number of citric-acid-induced coughs in guinea pigs that had been actively sensitized with the protein fraction of *A. restrictus*. Animals were pretreated with fexofenadine, at doses of 0.3–3 mg/kg, p.o., 60 min before citric acid inhalation. Data are presented as the mean $\pm$ S.E.M. ($n = 6$). # $P < 0.05$ vs. value before drug treatment in the control group. * $P < 0.05$ vs. respective value before drug treatment.

dependently increased by both methacholine (5×10^{-4} – 5×10^{-2} M, $n = 5$) and histamine (5×10^{-4} – 5×10^{-2} M, $n = 5$). The bronchial responsiveness to inhaled methacholine or histamine in the sensitized group was not significantly heightened compared to the respective value in the control group ($n = 5$).

3.3. Effect of fexofenadine, a histamine H1 antagonist, on cough sensitivity

As shown in Fig. 3, the increased number of citric-acid-induced coughs in sensitized guinea pigs was dose-dependently and significantly reduced to the level in control guinea pigs when animals were pretreated with fexofenadine, at doses of 0.3–3 mg/kg, p.o., 60 min before citric acid inhalation (0.3 mg/kg, before treatment: 16.2 ± 0.8 coughs/5 min, after treatment: 14.2 ± 1.1 coughs/5 min, $n = 6$; 1 mg/kg, before: 15.8 ± 0.7 coughs/5 min, after: 9.8 ± 1.2 coughs/5 min, $n = 6$; 3 mg/kg, before: 14.8 ± 1.7 coughs/5 min, after: 8.8 ± 0.7 coughs/5 min, $n = 6$). However, fexofenadine (3 mg/kg, p.o.), by itself, had no significant effect on the number of citric-acid-induced coughs in control animals (before: 11.2 ± 0.8 coughs/5 min, after: 9.8 ± 0.9 coughs/5 min, $n = 6$).

4. Discussion

In the present study, we demonstrated that in guinea pigs that had been actively sensitized with the protein fraction of *A. restrictus* strain A-17 by itself, that is, without subsequent allergen challenge, significantly enhanced the number of citric-acid-induced coughs. This enhanced cough reflex in sensitized guinea pigs was significantly inhibited by a histamine H1 receptor antagonist, fexofenadine. However, the bronchial responsiveness to

inhaled methacholine or histamine in the sensitized group was not significantly greater than the respective value in the control group. Gastroesophageal reflux-associated cough, postnasal drip-induced cough, angiotensin-converting enzyme inhibitor-induced cough and cough variant asthma are known causes of non-productive cough [14]. Fujimura et al. [15] proposed that a bronchodilator-resistant cough associated with an atopic constitution is another cause of non-productive cough. The pathological and physiological characteristics of atopic cough include eosinophilic tracheobronchitis without bronchoalveolar lavage eosinophilia and cough hypersensitivity without bronchial hyperresponsiveness, respectively [1,16,17]. Furthermore, atopic cough responded to anti-histamine drugs, but not to bronchodilators. Conversely, cough variant asthma appears to be similar to asthma: mild bronchial hyperresponsiveness, eosinophilic inflammation of central and peripheral airways, normal cough sensitivity and cough that responds to bronchodilators [1,18]. On the other hand, there is some clinical evidence that hypersensitivity to several fungal antigens can cause atopic cough [19,20]. These results and our present results strongly suggest that this animal model mimics some of the key features of atopic cough.

In conclusion, our present results suggest that active sensitization with the protein fraction of *A. restrictus*, by itself, that is without subsequent allergen challenge, enhances the excitability of cough receptors to tussive stimuli, and this animal model mimics some of the key features of atopic cough.

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