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Repeated instillations of *Dermatophagoides farinae* into the airways can induce Th2-dependent airway hyperresponsiveness, eosinophilia and remodeling in mice
Effect of intratracheal treatment of fluticasone propionate

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Abstract

Dermatophagoides farinae are known to be a common environmental allergen causing allergic asthma; however, little is known about their pathophysiological effect via the allergenicities in vivo. Therefore, we first established a mouse model of asthma induced by repeated instillations of *D. farinae*. Second, to investigate whether the asthmatic responses are Th2-dependent, we examined the effect of the deficiency of interleukin-4 (IL-4) receptor α chain gene. Finally, we examined the effect of fluticasone propionate on this model. Mice were instilled with *D. farinae* without additional adjuvants into the trachea 8 times. After the final allergen instillation, the airway responsiveness to acetylcholine was measured, and bronchoalveolar lavage and histological examination were carried out. The instillation of the allergen-induced airway hyperresponsiveness, the accumulation of inflammatory cells and increases in the levels of Th2 cytokines and transforming growth factor-β_1 production in the bronchoalveolar lavage fluid dose dependently. The number of goblet cells in the epithelium and the extent of the fibrotic area beneath the basement membrane were also increased in the morphometric study. In contrast, the defect of IL-4/IL-13 signaling through IL-4 receptor α chain completely abrogated all these responses. Furthermore, the simultaneous instillation of fluticasone propionate with the allergen showed significant inhibition or an inhibitory tendency of these changes. These findings demonstrate that the repetitive intratracheal instillations of *D. farinae* can induce airway remodeling through Th2-type inflammation, and that fluticasone propionate inhibits *D. farinae*-induced airway remodeling in mice, and this model would be useful for studying mechanisms involved in the development of allergic asthma.

Keywords: Airway; Asthma; Corticosteroid; *Dermatophagoides farinae*; Eosinophil; Goblet cell; IL-13; Mouse; Subepithelial fibrosis

1. Introduction

Bronchial asthma is one of the most common health problems in the worldwide, especially within industrialized societies, and the prevalence rates have been increasing considerably over the last few decades (Mannino et al., 2002; Robertson et al., 2004; Verlato et al., 2003), for reason that are not yet completely understood. Changes in lifestyle and an increase in indoor allergen exposure caused by higher indoor temperature and humidity have been suggested as potential determinants, and it is reasonable to consider that environmental exposures to allergens are of primary importance for the prevalence and development of asthma, in genetically predisposed individuals, because genes

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controlling the inflammatory responses, IgE production, cytokine and chemokine production, airway remodeling as well as airway function, has not changed significantly in the last few decades. Among allergens like ragweed, pollens, house dust mite, and cockroach, house dust mites including *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are known to be principle allergen for the induction of asthma (Huss et al., 2001; Platts-Mills et al., 1997; Sporik et al., 1990), although the precise molecular mechanisms underlying the allergenicity of the allergens is not fully understood.

In contrast to the increased prevalence of allergic airway diseases, the control of bronchial asthma is becoming easier due to the wide use of inhaled corticosteroid. However, there are still some patients that are resistant to medical treatment. Airway remodeling, which is characterized by goblet cell hyperplasia/hypertrophy, subepithelial fibrosis and smooth muscle hyperplasia/hypertrophy (Aikawa et al., 1992; Heard and Hossain, 1973; Roche et al., 1989), is one of the causes of this problem. Although these structural changes have been considered to be characteristics of chronic and severe asthma, the latest clinical studies, using bronchial biopsy sampling, have demonstrated that they may exist even in patients with mild asthma and early in the disease process. Roche et al. reported that the deposition of collagen beneath the bronchial epithelium was also observed in young patients with mild atopic asthma (Roche et al., 1989), and Boulet et al. demonstrated that the degree of subepithelial collagen deposition in patients with mild asthma recently diagnosed was not significantly different from those of long-standing mild asthma (Boulet et al., 2000). Therefore, the comprehension of the mechanisms underlying airway remodeling is becoming an increasingly important problem, and the development of new anti-remodeling agents is strongly desired.

Animal models have been used for a long time to analyze the pathophysiology of human diseases and to seek new remedies for various diseases. In the case of bronchial asthma, guinea pigs, rats and mice have mainly been used (Pauluhn and Mohr, 2005). Although each animal has some good characteristics, recently, mice have been widely used because this species allows for the application in vivo of a broad range of immunological tools, including gene deletion technology (Kips et al., 2003). To date, in typical experiments using mice, they are systemically immunized to ovalbumin with a T helper type 2 (Th2)-skewing adjuvant, such as aluminum hydroxide gel, and challenged through airway application of the antigen in the form of an aerosol or an intranasal droplet aspiration. In fact, these murine models have been useful in understanding the immune responses, such as Th2 dominant phenotypes underlying allergic sensitization and airway eosinophilic inflammation (Wills-Karp, 2000), but it is still unknown how allergic sensitization via the mucosal surfaces of airways with aeroallergens, including house dust mite, is induced. Several studies have previously investigated aeroallergen-induced airway inflammation in vivo (Johnson et al., 2004; Sadakane et al., 2002; Yu et al., 1999). These studies demonstrated that repeated instillations of *D. farinae* without additional adjuvants can induce airway eosinophilic inflammation, probably through Th2-polarized responses in mice; however, it remains to be determined whether the repeated inoculation of *D. farinae* can induce local Th2 responses and airway remodeling, as well as airway inflammation and airway hyperresponsiveness, and whether the allergic responses induced by this allergen are Th2-dependent. Moreover, the effect of fluticasone propionate on *D. farinae*-induced airway eosinophilic inflammation, hyperresponsiveness to acetylcholine and remodeling in vivo has not been elucidated.

Therefore, to address these unanswered questions, we first established a *D. farinae*-induced airway inflammation using mice, which was locally immunized without any additional adjuvants. Then, we examined the characteristics of this model regarding the local Th1/Th2 balance, the development of airway remodeling and the peculiarity of the allergen by comparing with the characteristics of the ovalbumin-instilled mice. Furthermore, to clarify whether these allergic responses are Th2-dependent, we used interleukin (IL)-4 receptor α chain gene-deficient mice because the IL-4 receptor α chain is a common receptor for IL-4 and IL-13, which are both critical for Th2 polarization and the development. Finally, we examined the effect of fluticasone propionate, one of the strongest anti-inflammatory medicines available at the moment, on this model, especially on airway remodeling.

2. Materials and methods

2.1. Animals

Seven-week-old male BALB/c mice were purchased from Japan SLC (Shizuoka, Japan). IL-4 receptor α chain gene-deficient mice (IL-4Rα; BALB/c background) (Noben-Trauth et al., 1999; Noben-Trauth et al., 1997) were purchased from Immuno-Biological Laboratories, Co. Ltd. (Takasaki, Japan). The animals were housed in plastic cages in an air-conditioned room at 22±1 °C with a relative humidity of 60±5%, fed a standard laboratory diet and given water *ad libitum*. Experiments were undertaken following the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animals Science in 1987.

2.2. Agents

The following drugs and chemicals were purchased commercially and used: crude extract of *D. farinae* (LSL Co., Tokyo, Japan), ovalbumin (chicken egg white, grade V, Sigma, St. Louis, MO., USA), dimethyl sulfoxide (DMSO, Nacalai Tesque, Inc., Kyoto, Japan), phosphate-buffered saline (PBS, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), halothane (Takeda Chemical Industries, Ltd, Osaka, Japan), acetylcholine chloride (Nacalai Tesque, Inc.), bovine serum albumin (Seikagaku Kogyo, Tokyo, Japan), Türk solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan), pancuronium bromide (Sigma), sodium pentobarbitone (Abbott Lab., Chicago, IL, USA), disodium ethylenediaminetetraacetic acid (EDTA-2Na; Nacalai Tesque) and Diff-Quick solution (International Reagent Corp., Ltd., Kobe, Japan). Fluticasone propionate was kindly given to us by GlaxoSmithKline, Japan.