〈Regular Article〉

Development of two types of mite-allergen induced murine models of chronic asthma with different severity

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ABSTRACT Asthma is an allergic disease characterized by chronic airway inflammation, hyper-responsiveness (AHR), and reversible obstruction. The main inflammatory changes are induced by infiltration of eosinophils into the airway. Few animal models resemble the spontaneous history of asthma due to variations in the selection of the mouse strain, appropriate antigen, and exposure methods. Here, we prepared two different mouse models in which the mechanism was close to that of human asthma.

We transnasally administered mite *Dermatophagoides farinae (Df)* allergen to BALB/c mice 10 times (Df-2) or 25 times (Df-5). After comparison with mice administered phosphate-buffered saline, the AHR and immediate asthmatic response were evaluated, in addition to the number of eosinophils in the bronchoalveolar lavage fluid (BALF). Df-specific IgE and IgG1 levels in the serum, and Th2 cytokines (interleukin [IL]-5, IL-13) in the BALF were measured by enzyme-linked immunosorbent assay. Immediate asthmatic response and AHR were enhanced in mite allergen-treated mice (Df-2 and Df-5) compared to PBS-treated mice. The number of eosinophils and IL-13 levels in the BALF, and specific IgE in the serum were greater in Df-5 than in Df-2 mice. We established two different murine chronic asthma models, in which the severity depended on the number of exposures to Df . Greater intranasal exposure to a Df allergen resulted in more severe asthma in a BALB/c mouse model.

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INTRODUCTION

Asthma is a chronic inflammatory disease characterized by airway hyper-responsiveness (AHR) and an increase in airway mucus-secreting cells related to airway remodeling. The main pathogenesis is chronic airway inflammation caused by the infiltration of eosinophils[1]. Diverse animal models of asthma have been established to research the pathologic mechanisms and potential therapies[2]. Ovalbumin is traditionally used to create a sensitized animal model. Ovalbumin is not a proper antigen for human asthma pathogenesis,

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however, and the intraperitoneal route for sensitization is not appropriately representative of the spontaneous history of human asthma onset. If exposure to an exogenous antigen elicits airway inflammation with eosinophils and AHR, many features of the condition may be in common with those of human asthma[3]. The preparation of a basic model in mice with a BALB/c background may be useful for elucidating the pathogenesis of allergic asthma and drug effectiveness. The severity of the airway inflammation almost certainly depends on the process of airway allergen exposure, repeated allergen administration, dose, and dosing interval (i.e., long period and short interval). Study results must therefore be carefully interpreted[4].

In the present study, we prepared two mouse models of human asthma by repeated transnasal exposure (either 2 or 5 times per week for 5 weeks) with the usual allergen in human asthma, the mite *Dermatophagoides farinae* (Df), in BALB/c mice.

MATERIALS AND METHODS

Animal Model

BALB/c mice (female, 7 weeks old) were obtained from Charles River Laboratory (Yokohama, Japan). The mite Df allergen (LSL Co., Tokyo, Japan) was dissolved with distilled water and adjusted to 1 mg/ml with phosphate-buffered saline (PBS). The mice were sensitized by transnasal administration of Df allergen, as described in Fig. 1. The allergen (40 μl of 1 mg/ml Df) was administered via the nasal cavity two or five times a week for 5 weeks (sensitization group; Df-2, Df-5, respectively). PBS was similarly administered at the same volume (40 μl) and schedule (twice per week for 5 weeks, control group; PBS). All experiments in this study were performed according to the protocol approved by the Institutional Animal Care and Use Committee of Kawasaki Medical School (Protocol Numbers: 13-006/15-010).

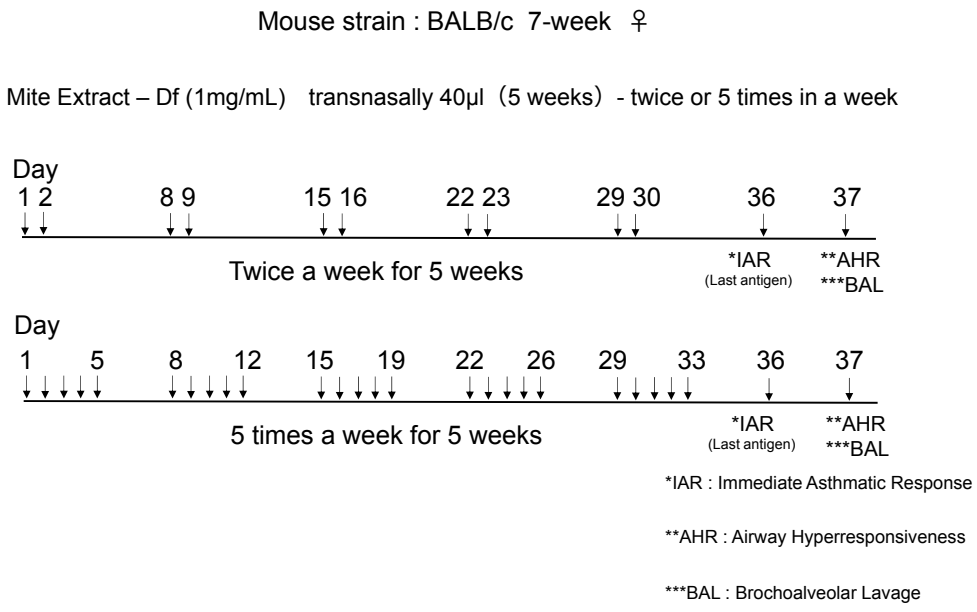

Fig. 1 Development and evaluation of mite allergen-induced murine model of chronic asthma. BALB/c mice underwent airway sensitization two or five times per week for five weeks by intranasal administration of mite-allergen or PBS as described in the Materials and Methods. IAR was evaluated for 5 min after the final allergen challenge. AHR was evaluated 24 h after the final allergen challenge. BAL was performed after examination of AHR as described in the Materials and Methods. IAR: Immediate asthmatic response, AHR: Airway hyper-responsiveness, BAL: Bronchoalveolar lavage.

Airway hyper-responsiveness

To investigate AHR, mice were forced to inhale PBS or acetyl-β-methylcholine chloride (Mch; Sigma-Aldrich, St. Louis, MO) at concentrations of 3 or 6 mg/ml. The airway resistance (sRaw) in awake mice was measured with a two-chambered, double-flow plethysmograph system (Pulmos; M.I.P.S, Osaka, Japan), as described previously[5]. Force inhalation was administered for 3 min. After a 1-min rest, airway resistance was measured for 2 min.

Immediate asthmatic response (IAR)

The airway resistance was measured after transnasal Df administration to evaluate the rate of increase (ΔsRaw) in comparison with the PBS exposure airway resistance. sRaw was monitored for 5 min after Df challenge.

Collection of blood, bronchoalveolar lavage fluid, and lung tissue

After confirming respiratory arrest, blood and bronchoalveolar lavage fluid (BALF) were collected. Lung tissue specimens were removed for hematoxylin and eosin (HE) staining, and Periodic acid-Schiff (PAS) staining for pathologic evaluation. BALF was obtained by washing the lungs four times with 1 mL PBS and centrifuging the collected wash solution. The supernatant of the first wash was stored at -80℃ until use. Cell pellets of all washes were collected and re-suspended in 1 ml PBS. The number of BALF cells was counted using a cell counter. Cytospin slides were stained with Diff-Quik (Sysmex, Kobe, Japan). Differential cell counts were performed on at least 400 cells.

Enzyme-linked immunosorbent assay

Amounts of interleukin (IL-5) and IL-13 (R&D Systems, Minneapolis, MN) in the BALF were measured using an enzyme-linked immunosorbent assay. The detection limits were 15.6 for IL-5 and 7.8 pg/mL for IL-13. Df-specific serum IgE and IgG1 were measured by enzyme-linked immunosorbent assay as previously described[6], using biotin-conjugated antibodies against IgE (Serotec, Raleigh, NC), IgG1 (Bethyl, Montgomery, TX), and streptavidin-horse radish peroxidase (Invitrogen, Carlsbad, CA).

Statistical analysis

All data are expressed as mean ± standard deviation (SD) . Statistical analysis was performed using GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA). The results were compared among the three groups using the Kruskal-Wallis test. A P value of less than 0.05 was considered significant.

RESULTS

We first evaluated the IAR after exposure to a specific allergen. The airway resistance change from PBS (%) at 5 min after exposure (IAR) was significantly higher in the Df administration groups (Df-2, Df-5) than in the PBS group ($p<0.01$; Fig. 2). We evaluated AHR 24 h after intranasal Df challenge using double-flow plethysmography. Df challenge induced a significant increase in airway hyperresponsiveness to 6 mg/ml Mch in the Df administration groups (Df-2, Df-5) compared with the PBS control group ($p<0.01$; Fig. 2).

The total number of leukocytes in the BALF of the Df administration groups (Df-2, Df-5) was significantly higher than that in the PBS administration group (1.96 ± 0.86, 2.70 ± 1.11, $0.75 \pm 0.22 \times 10^6/\mu l$, respectively, $p<0.01$). The number of eosinophils in the BALF was significantly higher in the Df-5 group than in the Df-2 group ($p<0.0001$; Fig. 3). The BALF of the PBS group contained no eosinophils. The numbers of neutrophils ($p<0.01$) and lymphocytes ($p<0.01$) in the Df groups were significantly higher than that in the PBS group (Fig. 3).

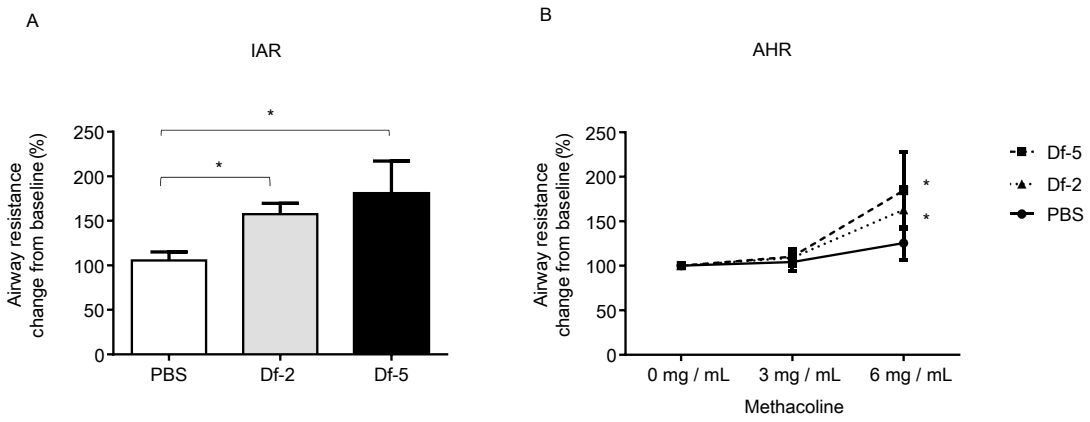

Fig. 2 Evaluation of physiologic function.
A. Immidiate Asthmatic Response (IAR) . IAR was evaluated as described in Materials and Methods. PBS: PBS-sensitized group, Df-2: Df-sensitized twice a week for 5 weeks group, Df-5: Df-sensitized five times a week for 5 weeks group. Results are shown as airway resistance change from baseline. Data represent means ± SD. * $p< 0.01$ compared with the PBS group
B. Airway-Hyper Responsiveness (AHR). AHR was evaluated as described in the Materials and Methods. Each group was exposed to PBS and methacoline-adjusted concentrations (3 and 6 mg/ml) before measuring airway resistance. Data represent means ± SD. * $p< 0.05$ compared with the PBS group

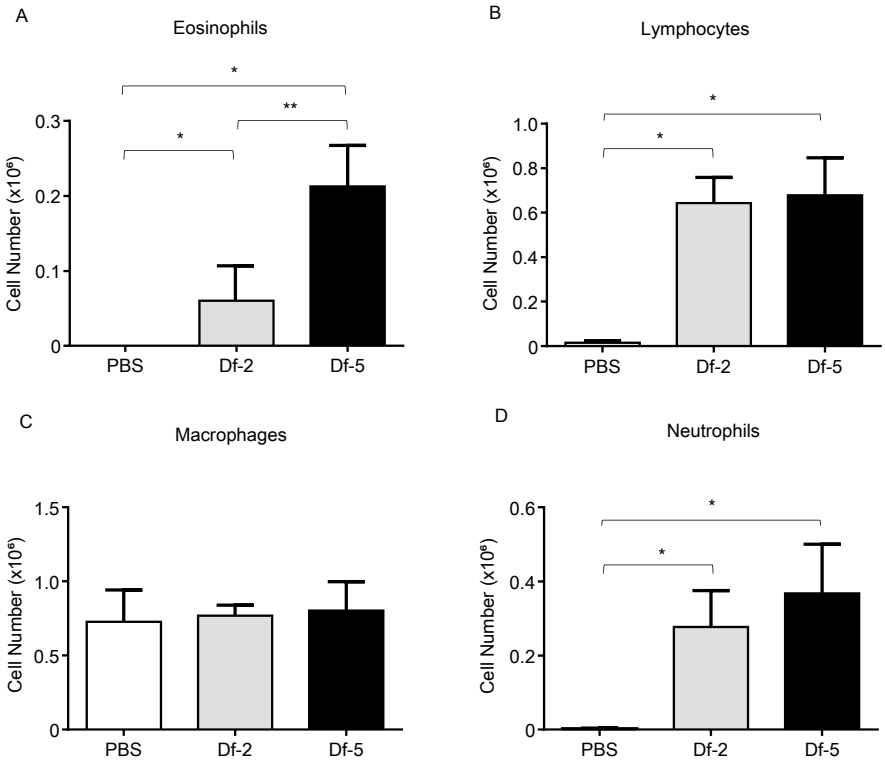

Fig. 3 Evaluation of inflammatory cell numbers in the BALF
Eosinophils (A), lymphocytes (B), macrophages (C), and neutrophils (D). Data represent means ± SD. * $p< 0.01$ compared with the PBS group. ** $p<0.05$ compared with Df-2 group. PBS:PBS-sensitized group, Df-2: Df-sensitized twice a week for 5 weeks group, Df-5: Df-sensitized 5 times a week for 5 weeks group.

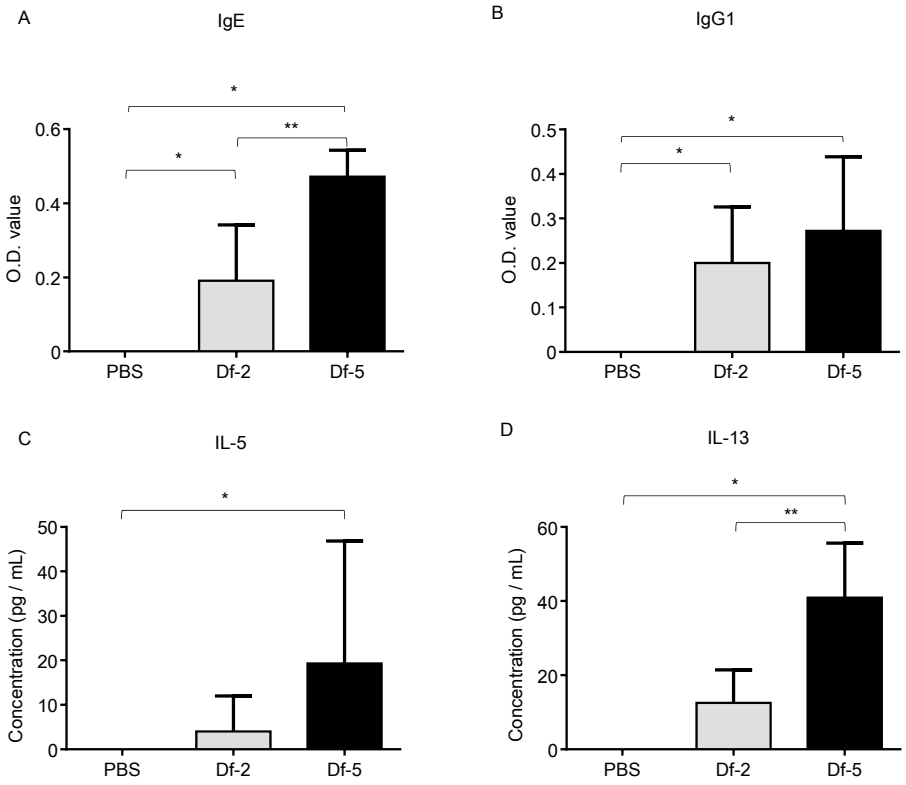

Fig. 4 Antigen-specific IgE and IgG1 in serum, and IL-5 and IL-13 in BALF
Df-specific IgE (A), and Df-specific IgG1 (B) in serum, and IL-5 (C) and IL13 (D) in BALF. Data represent means ± SD. * $p< 0.05$ compared with the PBS group. ** $p<0.05$ compared with the Df-2 group. PBS: PBS-sensitized group, Df-2: Df-sensitized twice a week for 5 weeks group, Df-5: Df sensitized 5 times a week for 5 weeks group.

The serum levels of Df-specific IgE and IgG1 were significantly increased in the Df administration groups (Df-2, Df-5) compared with the PBS administration group (IgE, $p<0.0001$, IgG1, $p<0.01$; Fig. 4A,B). The serum levels of Df-specific IgE were significantly higher in the Df-5 group compared with the Df-2 group ($p<0.01$). The IL-5 and IL-13 levels in the Df groups were significantly higher than in the PBS group ($p<0.05$). IL-13 levels in the Df-5 group were significantly higher than in the Df-2 group. We compared the lung tissue specimens after HE staining and PAS staining. In the Df-5 group, the infiltration of eosinophils around the bronchus, mucus-secreting cells, blood vessels, and airway wall thickness were more severe than in the Df-2 group. There was no infiltration of inflammatory cells in the PBS group (Fig. 5).

DISCUSSION

In previous studies involving the preparation of a mouse model of asthma, the sensitization and intra-airway allergen administration methods varied, and the grade of inflammation depended on the frequency of allergen administration, dose, interval, and interval from the final dosing until analysis. Therefore, the results of those studies must be interpreted carefully. As described by Walsh *et al.*, the requirements to acquire AHR depend on the mouse strain[3]. Therefore, a mouse model reflecting the development of asthma in humans is necessary

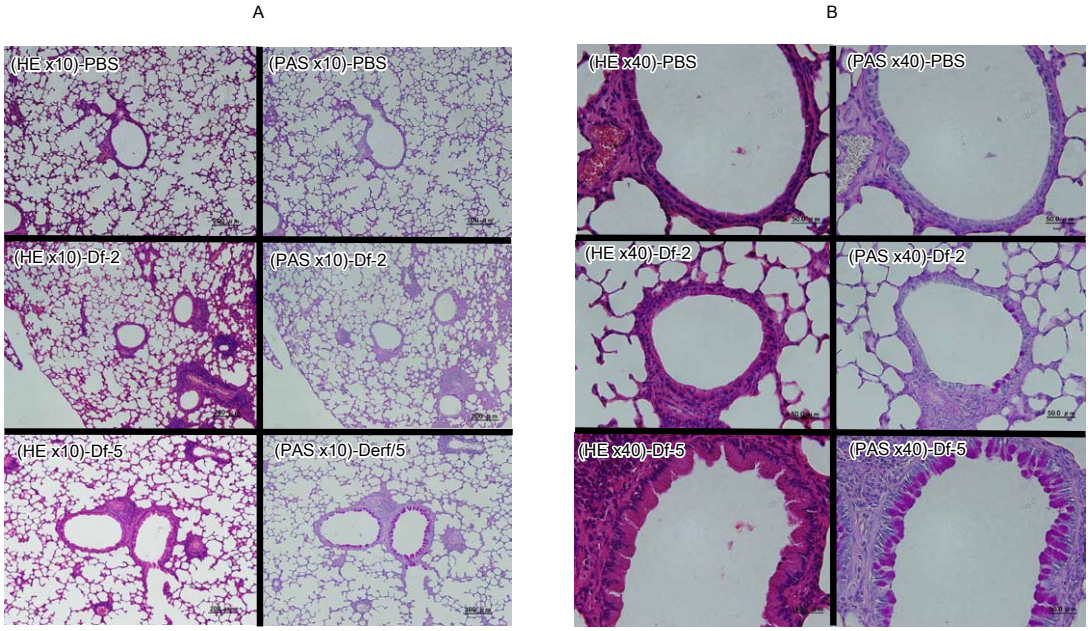

Fig. 5 Pathologic lung tissue
Histologic examination of formalin-fixed lung sections stained with hematoxylin and eosin (HE) (A) (original magnification: × 10), and stained with periodic acid-Schiff (PAS) (B) (original magnification: × 40)

to clarify the pathogenesis of asthma and establish remedies. In the present study, we developed two mouse models of asthma with different severities after repeated transnasal Df administration (Df-2 and Df-5). The Df met the following conditions: IAR on a specific antigen exposure (ΔsRaw), AHR on Mch inhalation, increases in the serum levels of Df-specific IgE and IgG1, and an increase in the number of eosinophils in the BALF, confirming that the characteristics of asthma in humans could be reproduced. This mouse model may reflect the pathogenesis of human asthma, because the type of allergen, mouse strain, and route of sensitization differ from those in conventional models such as an ovalbumin-sensitized model and that using BALB/c mice.

Eosinophils are main inflammatory cells with many pathophysiologic functions, inducing airway contraction, mucus secretion, and enhancement of vascular permeability[5]. Mast cells were not found at the sites of lung inflammation and in the BALF. Mast cells may not play an important role in the development of AHR and airway inflammation in this model. Df-specific IgE levels were higher in the Df-5 group than in the Df-2 group. IL-13 levels in the BALF of the Df-5 group were also higher than that in the Df-2 group. IL-13 might influence the production of Df-specific IgE.

One of the pathophysiologic features of asthma is airway remodeling. Airway remodeling consists of increased airway wall thickness due to fibrosis and increased mucus-secreting cells evaluated by PAS staining. In the present study, Df-5 group had more sever pathologic feature in the airway than Df-2 group. IL-13 might play an important role for airway remodeling in mucus secretion. Airway remodeling is associated with asthma severity and treatment resistance.

In conclusion, these two models may reproduce not only the characteristics of human asthma, such as eosinophilic inflammation of the airway, increased AHR, and specific allergen exposure-

related obstruction of the airway, but also the chemotaxis of eosinophils to local areas and airway remodeling. In this study, we successfully established two mouse bronchial asthma models with different severity reflecting the mode of onset in humans in comparison with conventional models, using Df allergen and BALB/c mice. Analyses of these models may be useful for clarifying the pathogenesis of human asthma and the development of effective drugs.

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CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

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