

A Novel Mouse Model of Experimental Asthma

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Key Words

Recombinant cockroach · Dust mite · Novel mouse model · Inner city asthma

Abstract

Background: Animal models that mimic the pulmonary features observed in human asthma are important tools to study the mechanism(s) of allergen-induced asthma. Cockroach and dust mite allergens are two common allergens found in the 'inner city' environment. In this study, we examined the interaction between recombinant cockroach (r Bla g 2) and dust mite (r Der f 1) allergens in inbred mouse strain (A/J). The tested hypothesis was that there are enhanced effects of exposure to r Bla g 2 and r Der f 1 allergens in the airway inflammatory response in A/J mice. **Methods:** Five groups of mice (male, 6–8 weeks) were examined: vehicle (saline) controls; adjuvant (alum) controls; r Bla g 2 immunized (0.01–10 µg/mouse), r Der f 1 immunized (0.01–10 µg/mouse), and combined immunization with r Der f 1 (0.05 µg/mouse) and r Bla g 2 (0.05 µg/mouse). Mice were immunized at days 0 and 7, challenged by orotracheal inhalation with r Der f 1 and/or r Bla g 2 allergen at day 14, and were studied and sacrificed on day 17.

Airway hyperreactivity was measured by peak airway pressure and airway pressure time index (APTI). Differential cell analysis and total proteins in bronchoalveolar lavage returns were used to assess airway inflammation and epithelial injury. **Results:** Dose-related statistically significant increases in peak pressure, APTI, total cells, eosinophils, epithelial cells, but not total proteins, were induced by r Bla g 2 challenge in r Bla g 2-immunized mice. Similar allergen-induced dose-related increases in airway total cells, eosinophils, epithelial cells and total proteins were observed in r Der f 1 immunized mice. Compared to either allergen alone, enhanced airway inflammation and epithelial damage, but not airway reactivity, were detected in the combined group. **Conclusion:** This novel mouse model will allow investigation of the immunopathogenesis of human asthma and should provide insight into the common form of 'inner city asthma'.

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Introduction

Asthma is a common disease which has been increasing in incidence. In the USA low socio-economic groups residing in inner cities appear to be particularly at risk for severe asthma as judged by treatment requirement, acute episodes requiring emergency treatment, hospitalization and mortality. In these groups, exposure to cockroach

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allergen is common [1] and has been associated with sensitization [2–3] and onset [4] and severity [5] of asthma. Among African-American children residing in these environments, the combination of sensitization to cockroach and exposure in their homes has been shown to be an important risk factor for emergency room visits [6] and hospitalizations [5] related to asthma. It is suggested that interactions among indoor allergens like dust mite and cockroach allergens in susceptible individuals, rather than any one factor by itself may cause the disproportionate occurrence and burden of asthma in the inner city environment [3, 7, 8].

To mimic bronchial asthma in animals, a wide variety of animal models have been developed [9–11].

Conventional models of allergic airway inflammation involve administration of ovalbumin. Each model has its own inherent advantages and shortcomings and unfortunately, no model is identical to the conditions found in human asthma [12]. Nonetheless, the development of relevant animal models continues in anticipation that they will aid in the examination of underlying processes that contribute to asthma, or will assist in the identification of novel therapies that can be used to treat allergic asthma.

Airway responses in A/J mice has been well characterized and found that allergen susceptibility in this model correlates with the strain-dependent expression of experimental asthma in response to ovalbumin challenge [9]. The profile of allergen sensitivity in a population clearly shows that not all allergens are equally potent [13]. Indeed some allergens such as the protease containing mite-derived allergens show such a preponderance of activity as sensitizing agents that there must be predisposing functional properties of the allergen and this needs to be modeled [7]. While the attractions of using ovalbumin as an allergen might be clear from immunologic view, naturally occurring respiratory disease provoked by ovalbumin is not a major concern, even in mice [14].

German cockroaches produce several important allergens, including Bla g 2, Bla g 4, and Bla g 5, that are secreted and accumulate in the environment [15]. Previous studies have shown that Bla g 2 is a potent allergen that could identify 60–80% of patients allergic to domestic cockroach [16]. Most cockroach and dust mite allergens have been cloned and expressed as recombinant proteins, and are comparable with those of their natural counterparts [17–18]. The advantage of using recombinant allergen is the high degree of purity [14]. A recombinant form of dust mite allergen, r Der f 1 is a major source of the allergen and its potency is comparable to the native form [14].

In this study, we hypothesized that interaction of recombinant cockroach and dust mite allergen may enhance the airway inflammatory response in mouse model of experimental asthma. To test this hypothesis, we determined the dose-effect of exposure to recombinant cockroach and dust mite allergens on airway hyperreactivity and inflammation. The results indicate that recombinant cockroach and dust mite allergens cause dose-dependent increase in airway inflammation. Moreover, at low doses of immunization, further airway exposure to both allergens *enhanced* the airway inflammation. The results also demonstrated association between airway inflammation and airway reactivity with exposure to r Bla g 2 and dissociation between these subphenotypes with exposure to r Der f 1 alone or combined r Der f 1 and r Bla g 2.

Materials and Methods

General

Male (6 to 8 weeks, 20 to 28 g) A/J mice were purchased from Jackson Laboratories (Bar Harbor, Me., USA). An inbred strain was chosen to control for genetic variability. Mice were housed in the animal care facilities of the Johns Hopkins Bloomberg School of Public Health, at 22°C with a constant photoperiod of 14L:10 D. Water and mouse chow (Ag-way Pro-Lab RMH 1000; Waverly, N.Y., USA) were provided ad libitum. Sentinel animals in the room were examined periodically to ensure that the animals were free of infection. Mice were handled in accordance with the standards established by the U.S. Animal Welfare Acts set forth in National Institutes of Health guidelines and the Johns Hopkins University Animal Care and Use Committee.

Airway Reactivity

Airway reactivity was measured as previously described [19]. Briefly, mice were anesthetized with 1.5% halothane (Halocarbon Laboratories, N. Augusta, S.C., USA) in an inhalation chamber. After initial anesthesia, mice were administered halothane through a nose cone, tracheostomy was performed using a 20-gauge, stainless steel, four-way catheter, and an abdominal incision was made to expose the inferior vena cava (IVC). Mice were then injected intravenously with 50 mg/kg ketamine. This dose of ketamine was previously determined to be sufficient for anesthesia during the 15-minute protocol. Mice were mechanically ventilated at a constant tidal volume (0.008 ml/g body weight) and frequency (120 breaths/min) through two ports in the tracheal catheter. Airway pressure was measured by a pressure transducer at a third port of the catheter, and was recorded on a strip chart recorder. Acetylcholine (50 µg/kg) was injected into the IVC using a Hamilton (Reno, Nev., USA) gas tight syringe (50 µl) and 33-gauge needle. The injections were maintained at a constant rate (over 1 s) by a pneumatic injector. Airway reactivity to acetylcholine was defined as the integrated change in inspiratory airway pressure time index (APTI, cm H₂O × s).

Bronchoalveolar Lavage Fluid (BALF)

Immediately after measurement of airway reactivity, BALF was obtained by following procedures described previously [10]. Briefly,

the right lung of each mouse was lavaged in situ four times with Hanks' balanced salt solution (HBSS, 35 ml/kg, pH 7.2–7.4) and the recovered BALF was immediately cooled to 4°C. For each mouse, BALF returns were centrifuged (500 g at 4°C), and the supernatant from the first BALF return was decanted for determination of total protein (an indicator of lung permeability). Protein concentration was measured following the method of Bradford as indicated in the manufacturer's procedure (BioRad, Hercules, Calif., USA). The cell pellets from all four lavages were resuspended in 1 ml of HBSS and pooled, and the numbers of cells (per ml total BALF return) were counted with a hemocytometer. An aliquot (10 µl) of BALF cell suspension was cytocentrifuged (Shandon Southern Products, Pittsburgh, Pa., USA), and stained with Wright-Giemsa stain (Diff-Quik; Baxter Scientific Products, McGraw Park, Ill., USA) for differential cell analysis. Differential counts for macrophages, lymphocytes, epithelial cells, eosinophils and neutrophils were done by identifying 300 cells according to standard cytological techniques [10]. Epithelial cells in particular were identified by the presence of cilia. It is therefore likely that epithelial cell loss after exposure is underestimated as non-ciliated cells were not counted.

r Bla g 2 and r Der f 1 Allergens

The r Bla g 2 and r Der f 1 allergens were purchased from Indoor Biotechnologies (Charlottesville, Va., USA). The allergenic content of r Bla g 2 was 1 mg/ml and that of r Der f 1 was 0.9 mg/ml. The extract was stored at –20°C throughout the experiment.

Experimental Protocol

Mice (n = 4–15/group) were immunized intraperitoneally with saline (0.2 ml), 1 mg of 0.2 ml aluminum hydroxide [Al(OH)₃, alum], and r Bla g 2 (0.01, 0.1, 1 and 10 µg/mouse) adsorbed to alum. The procedure was repeated seven days later. Fourteen days after initial immunization, all mice were challenged oro-tracheally with r Bla g 2 (50 µg/mouse). Airway reactivity was assessed on day 17. Immediately after assessment of airway reactivity, mice were lavaged for evaluation of inflammation. The identical immunization and challenge procedure was used for r Der f 1 allergen. For the cockroach and dust mite combined group, the sensitizing dose was r Bla g 2 (0.05 µg) and r Der f 1 (0.05 µg) and a combined challenge dose of r Bla g 2 (25 µg) and r Der f 1 (25 µg).

Lung Tissue Preparation for Histopathology

Left lung lobes from mice treated with vehicle control, r Bla g 2, r Der f 1, and combined r Der f 1 and r Bla g 2 were fixed with zinc formalin, and sectioned 5 µm. Tissue sections were stained with hematoxylin and eosin for morphological comparison of airway epithelial damage, peribronchovascular cell infiltrate and interstitial alveolar cell infiltrate between animal groups.

Statistics

The effects of sensitization (saline, alum, alum + antigen) and allergen dose (0.01, 0.1, 1, 10 µg) on dependent variables were assessed by 2-way analysis of variance (ANOVA). The dependent variables were indicators of inflammatory response (lavageable cells and total protein) and airway reactivity (APTI). All ANOVA were done using Super ANOVA software package (Abacus Concepts, Inc., Berkeley, Calif., USA). Cell (group) sizes were 4–15. Fisher's protected LSD (least significant difference) analyses were used for a posteriori comparisons of means. Statistical significance was accepted at $p < 0.05$.

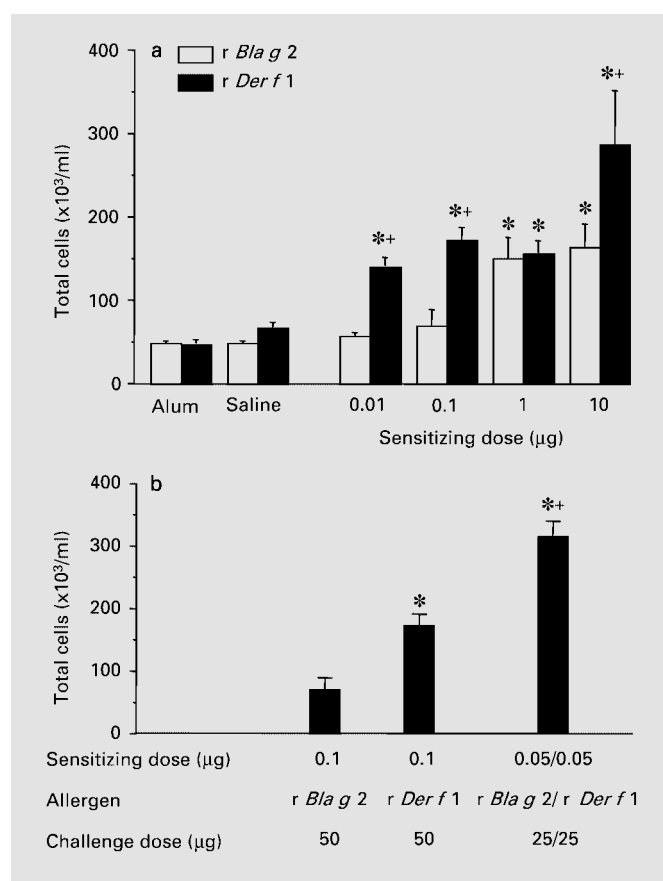


Fig. 1. Number of total cells in bronchoalveolar lavage fluid (n = 4–15 mice per group). Values are mean $\pm$ SE. **a** Recovered from r Bla g 2 or r Der f 1 exposed groups of mice. * Significantly different from vehicle control groups, $p < 0.05$. + Significantly different from r Bla g 2 exposed mice, $p < 0.05$. **b** Recovered from r Bla g 2 and r Der f 1 treated groups of mice with control groups. * Significantly different from r Bla g 2 exposed groups, $p < 0.05$. + Significantly different from r Der f 1 exposed mice, $p < 0.05$.

Results

Cellular Response

Response to r Bla g 2 Allergen. Challenge with r Bla g 2 allergen elicited significantly greater numbers of total BALF cells in mice immunized with 1.0 and 10.0 µg of r Bla g 2 compared with vehicle control mice (alum and saline) (fig. 1a). Significantly greater numbers of BALF cells were found in mice immunized with 1.0 and 10.0 µg of r Bla g 2 than mice immunized with 0.01 and 0.1 µg of r Bla g 2. Mean total BALF cell numbers in antigen-challenged alum and saline control mice were not different from those in mice immunized with 0.01 and 0.1 µg of r Bla g 2. Significantly greater mean numbers of eosinophils

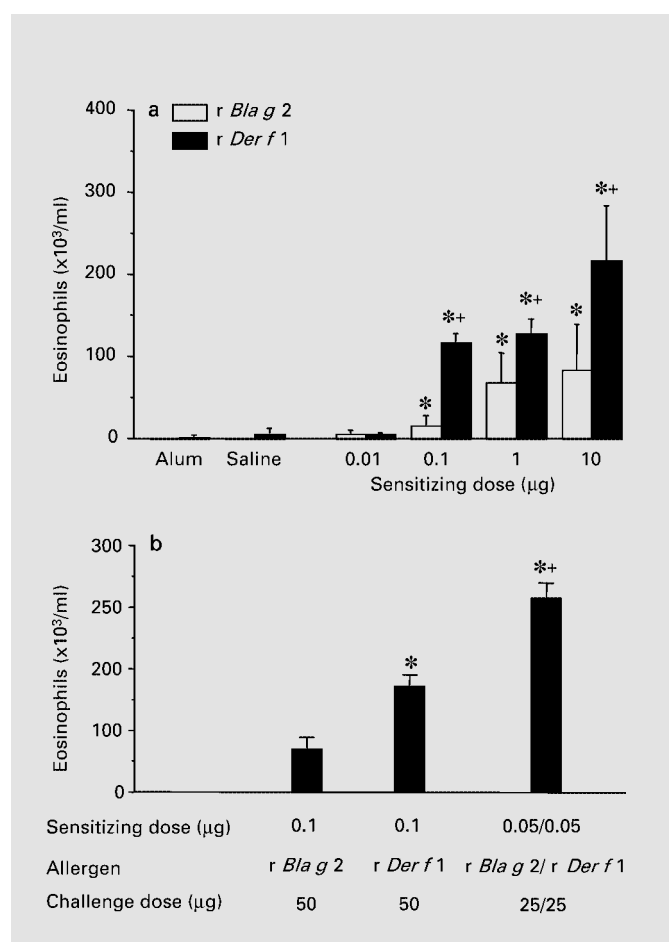


Fig. 2. Number of eosinophils in bronchoalveolar lavage fluid (n = 4–15 mice per group). Values are mean $\pm$ SE. **a** Recovered from r Bla g 2 or r Der f 1 exposed groups of mice. * Significantly different from vehicle control groups, $p < 0.05$. + Significantly different from r Bla g 2 exposed mice, $p < 0.05$. **b** Recovered from r Bla g 2 and r Der f 1 treated group of mice with control groups. * Significantly different from r Bla g 2 exposed group, $p < 0.05$. + Significantly different from r Der f 1 exposed mice, $p < 0.05$.

were found in mice immunized with 0.1, 1.0, and 10.0 μ g r Bla g 2 compared to those immunized with 0.01 μ g (fig. 2a). No significant difference in the BALF eosinophils were found in the 0.01 μ g r Bla g 2 groups compared to the control mice (alum and saline). The mean numbers of BALF epithelial cells were significantly increased in mice immunized with 10.0 μ g r Bla g 2 groups compared to mice immunized with 0.01 and 0.1 μ g r Bla g 2 (fig. 3a). No significant differences were found between the mean numbers of epithelial cells found in mice immunized with 0.01, 0.1, and 1.0 μ g r Bla g 2 compared to the control groups. The other cell types (macrophages, neutrophils

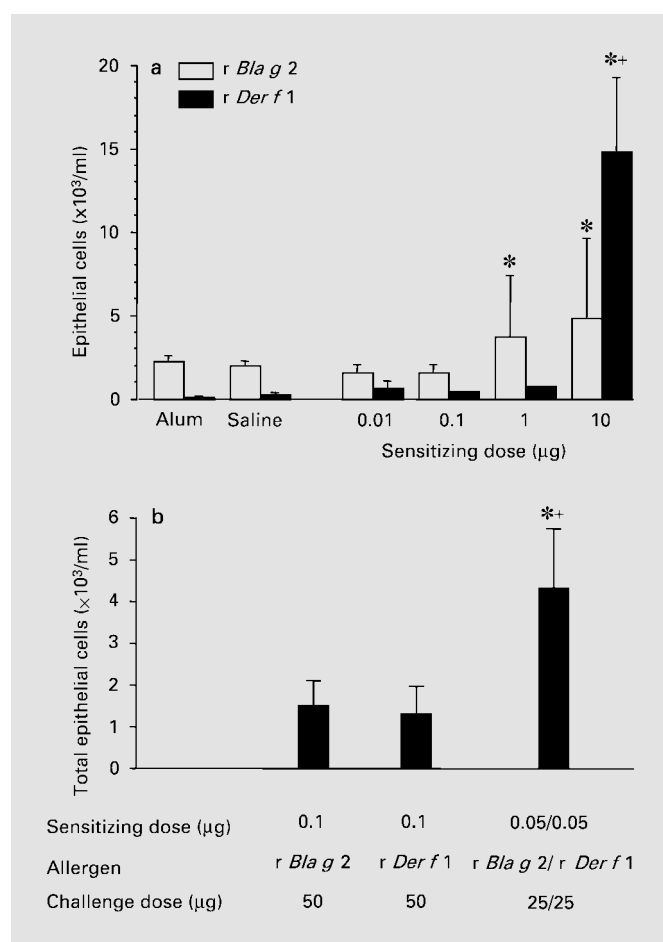


Fig. 3. Number of epithelial cells in bronchoalveolar lavage fluid (n = 4–15 mice per group). Values are mean $\pm$ SE. **a** Recovered from r Bla g 2 or r Der f 1 exposed groups of mice. * Significantly different from vehicle control groups, $p < 0.05$. + Significantly different from r Bla g 2 exposed mice, $p < 0.05$. **b** Recovered from r Bla g 2 and r Der f 1 treated group of mice with control groups. * Significantly different from r Bla g 2 group of mice, $p < 0.05$. + Significantly different from r Der f 1 exposed mice, $p < 0.05$.

and lymphocytes) are illustrated in table 1. Compared to the vehicle control groups, only the 1.0 and 10 μ g r Bla g 2 groups resulted in significant increase in macrophages, neutrophils and lymphocytes.

Response to r Der f 1 Allergen. Challenge with r Der f 1 allergen elicited significantly greater numbers of BALF cells in mice immunized with all doses (0.01, 0.1, 1.0, and 10.0 μ g) of r Der f 1 compared to alum and saline control animals (fig. 1a). At all doses except 1 μ g, significantly greater numbers of total BALF cells were found in mice immunized with r Der f 1 allergen compared to mice immunized with r Bla g 2. Significantly greater numbers

Table 1. Distribution of cells in BALF of the treatment groups (r Bla g 2)

Treatment groups	Macrophages $\times 10^3$	Neutrophils $\times 10^3$	Lymphocytes $\times 10^3$
Alum, vehicle control	42.3 $\pm$ 2.2	1.0 $\pm$ 0.2	0.6 $\pm$ 0.1
Saline, vehicle control	44.7 $\pm$ 2.0	1.1 $\pm$ 0.2	0.7 $\pm$ 0.1
0.01 μ g r Bla g 2	47.5 $\pm$ 4.2	1.2 $\pm$ 0.4	1.1 $\pm$ 0.1
0.1 μ g r Bla g 2	45.5 $\pm$ 7.5	1.4 $\pm$ 0.6	1.1 $\pm$ 0.4
1 μ g r Bla g 2	77.5 $\pm$ 15.5*	1.6 $\pm$ 0.5	1.7 $\pm$ 0.6*
10 μ g r Bla g 2	49.4 $\pm$ 8.3	2.1 $\pm$ 0.5*	2.7 $\pm$ 0.7*

Figures represent mean and SE. n = 6–12 mice/group.

* Significant difference ($p < 0.05$) control and specific immunizing dose group.

Table 2. Distribution of cells in BALF of the treatment groups (r Der f 1)

Treatment groups	Macrophages $\times 10^3$	Neutrophils $\times 10^3$	Lymphocytes $\times 10^3$
Alum, vehicle control	44.2 $\pm$ 4.3	0.6 $\pm$ 0.6	0.9 $\pm$ 0.5
Saline, vehicle control	60.2 $\pm$ 2.9	1.4 $\pm$ 0.9	1.1 $\pm$ 0.6
0.01 μ g r Der f 1	129.9 $\pm$ 11.2*	0.3 $\pm$ 0.3	3.8 $\pm$ 2.3
0.1 μ g r Der f 1	51.7 $\pm$ 9.1	1.2 $\pm$ 0.0	2.3 $\pm$ 0.8
1 μ g r Der f 1	26.3 $\pm$ 4.7	0.0 $\pm$ 0.0	1.2 $\pm$ 0.4
10 μ g r Der f 1	50.1 $\pm$ 5.7	0.0 $\pm$ 0.0	4.1 $\pm$ 2.1*

Figures represent mean and SE. n = 6–12 mice/group.

* Significant difference ($p < 0.05$) control and specific immunizing dose group.

of eosinophils were found in mice immunized with 0.1, 1.0, and 10.0 μ g r Der f 1 compared to mice immunized with 0.01 μ g r Der f 1 (fig. 2a). No differences were found between the mean numbers of BALF eosinophils in mice immunized with 0.01 μ g r Der f 1 compared to those in the control mice (alum and saline). The mean numbers of BALF eosinophils were significantly greater in r Der f 1 sensitized mice compared to r Bla g at all sensitizing doses except 0.01 μ g. Mean numbers of BALF epithelial cells were not significantly different between controls and mice immunized with 0.01, 0.1, or 1.0 μ g of r Der f 1 (fig. 3a). However, BALF epithelial cells numbers were significantly increased in the 10.0 r Der f 1 group compared to all other groups. Further, the numbers of BALF epithelial cells in mice immunized with 10.0 μ g of r Der f 1 were significantly higher than in the mice immunized with 10.0 μ g of r Bla g 2. The other cell types are illustrated in table 2. In none of the doses except 0.01 μ g, there were significantly greater numbers of mean macrophages in mice immunized with r Der f 1 allergen compared to vehicle control mice. The BALF neutrophils showed no difference in the vehicle control groups and the allergen immunized groups. Compared to the vehicle control groups,

only the 10 μ g r Der f 1 group resulted in significant increase in lymphocytes.

Response to Combined r Bla g 2 and r Der f 1. To determine whether combined r Bla g 2 and r Der f 1 has additive or synergistic pulmonary effects with intraperitoneal immunization and aspiration challenge, mice were immunized concurrently with intraperitoneal doses of r Bla g 2 (0.05 μ g) and r Der f 1 (0.05 μ g) on day 0 and 7 and then challenged by aspiration on day 14 together with r Bla g 2 (25 μ g/mice) and r Der f 1 (25 μ g/mice). To control for the total dose of antigen, two groups of mice were immunized with r Bla g 2 (0.1 μ g/mice) and r Der f 1 (0.1 μ g/mice), respectively. These groups of mice were then challenged with 50 (μ g/mice) of r Bla g 2 and r Der f 1 respectively. As expected (see above), significantly greater numbers of total BALF cells and eosinophils were found in mice immunized with r Der f 1 compared to mice immunized with r Bla g 2. Interestingly, significantly greater mean numbers of total BALF cells and eosinophils were found in mice with combined r Bla g 2 and r Der f 1 immunization compared with those in mice immunized with either r Bla g 2 or r Der f 1 alone (fig. 1b, 2b). Mean BALF epithelial cell numbers were significantly increased in the

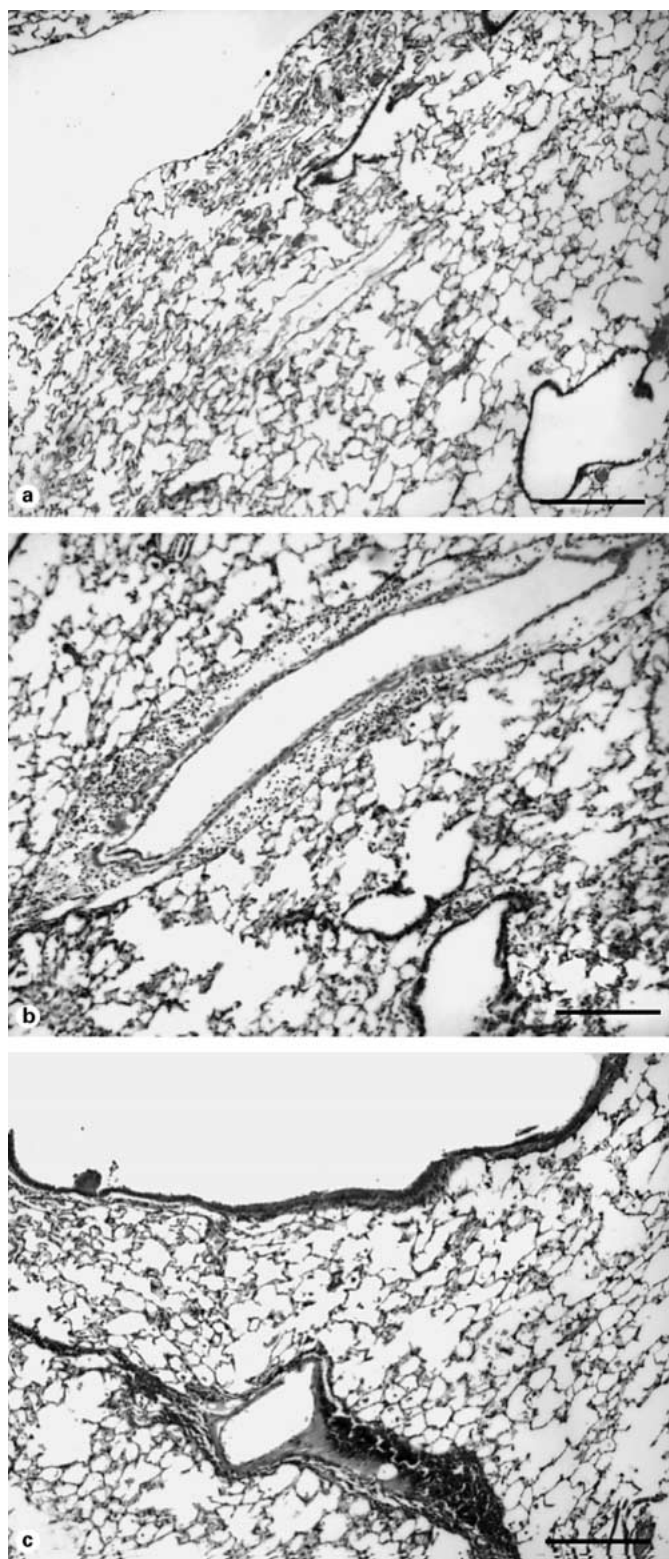


Fig. 4. Light photomicrographs of lungs from **a** vehicle control, **b** r Bla g 2, and **c** combined r Bla g 2 and r Der f 1 mice. Tissue sections were stained with hematoxylin and eosin.

combined r Bla g 2 and r Der f 1 group compared to the r Bla g 2 group of mice (fig. 3b). However, the mean BALF epithelial cells in the combined r Bla g 2 and r Der f 1 group was significantly different from epithelial cells recovered from the r Der f 1 group of mice.

Lung Histology

The lung histology are illustrated in figure 4a–c. In the vehicle control mice, the airway and peribronchovascular space were essentially normal. In contrast the r Bla g 2 group of mice had significant influx of inflammatory cells. The combined r Bla g 2 and r Der f 1 group had exaggerated airway epithelial damage, peribronchovascular cell infiltrate and interstitial alveolar cell infiltrate compared to the vehicle and r Bla g 2 groups.

Total BALF Protein

Response to r Bla g 2 Allergen. Relative to the control groups, none of the r Bla g 2 immunizing doses caused significant increases in the BALF protein concentrations (fig. 5a).

Response to r Der f 1 Allergen. The mean BALF protein concentrations in mice immunized with 0.01, 0.1, or 1.0 μ g r Der f 1 were not significantly different from each other or from controls (fig. 5a). However, the BALF protein response in the group of mice immunized with 10 μ g r Der f 1 was significantly increased compared to controls and the other inoculation groups.

Response to r Bla g 2 and r Der f 1. The mean BALF protein concentration in the combined antigen group was significantly greater than the mean protein concentration of BALF in the r Bla g 2 group and marginally different from the r Der f 1 group ($p = 0.059$) (fig. 5b).

Airway Reactivity

Response to r Bla g 2 Allergen. The mean APTI responses to ACh in mice immunized with 0.1 and 10.0 μ g r Bla g 2 allergen were significantly increased compared with the control mice (fig. 6a). The mean APTI responses in mice immunized with the lower concentrations (0.01 and 0.1 μ g) were not significantly different from the control vehicle mice with the exception of the mice in the 0.1- μ g group which was significantly higher than the saline control. The ACh-induced changes in the mean peak airway pressure changes were consistent with the changes in APTI (data not shown).

Response to r Der f 1 Allergen. Neither the APTI nor the mean peak pressure responses to ACh in mice inoculated with r Der f 1 were significantly increased compared to the ACh responses in control mice (fig. 6b).

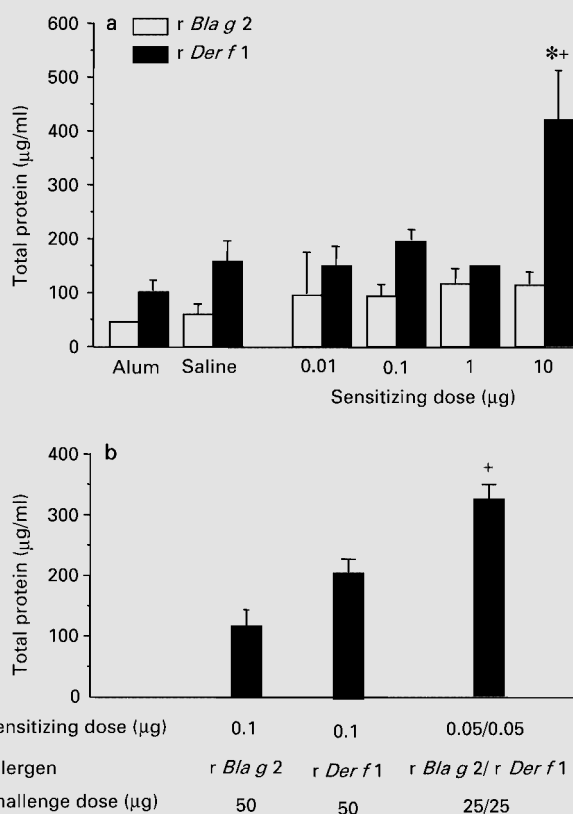
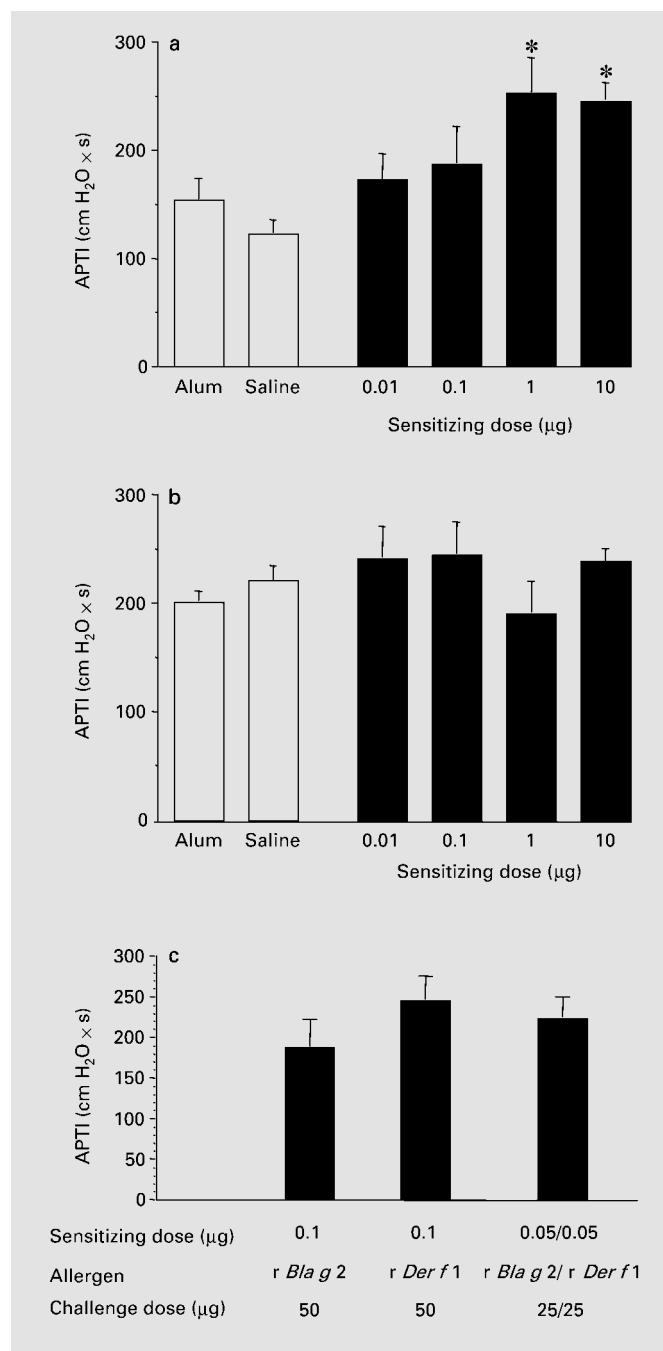


Fig. 5. Concentration of total protein in bronchoalveolar lavage fluid ($n = 4-15$ mice per group). Values are mean $\pm$ SE. **a** Recovered from r Bla g 2 or r Der f 1 exposed groups of mice. * Significantly different from vehicle control groups, $p < 0.05$. * Significantly different from r Bla g 2 exposed mice, $p < 0.05$. **b** Recovered from r Bla g 2 and r Der f 1 treated group of mice with control groups. * Significantly different from r Bla g 2 exposed group, $p < 0.05$. * Significantly different from r Der f 1 exposed mice, $p < 0.05$.

Fig. 6. Airway Pressure time Index (APTI) response to Ach (50 µg) in r Bla g 2 (**a**), r Der f 1 (**b**), and combined r Der f 1 and r Bla g 2 mice treated groups (**c**). Values are mean $\pm$ SE; $n = 4-15$ mice per group. * Significantly different from vehicle control groups, $p < 0.05$.

Response to r Bla g 2 and r Der f 1 Allergens. The mean peak airway pressure was not significantly increased in the combined r Bla g 2 and r Der f 1 group compared to mean peak airway pressure in mice immunized to either r Bla g 2 or r Der f 1 (fig. 6c).



Discussion

In this study we present a novel mouse model of experimental asthma with the demonstration of allergen specific dose responsiveness on airway inflammation with recombinant cockroach (r Bla g 2) and dust mite (r Der f 1) allergens. We showed that, at low immunizing doses to

both allergens, there was enhanced effect of the pulmonary inflammatory responses upon airway allergen exposure. This underlies the crucial role of both allergens for the development of allergic asthma in the 'inner city environment' as published previously [1, 3, 6].

Our results show that r Bla g 2 can induce dose-dependent airway inflammation and hyperreactivity in A/J mice. In case of r Der f 1 allergen, there was demonstration of only dose-dependent airway inflammation but not airway reactivity. Moreover, exposure to both allergens showed *enhanced* airway inflammation. These results support previously published data that while airway hyperresponsiveness is associated with inflammation in murine asthma, inflammation can develop without the allergen dose-dependent effect of hyperreactivity [9, 11]. Because there is an apparent dissociation between inflammatory cell infiltration and hyperreactivity in r Der f 1 as compared to r Bla g 2 in this model, we hypothesize that differences in allergenic potency may determine the hyperreactivity response in this model. Zhang et al. [20] reported that after OVA sensitization and aerosol exposure, BALB/c and C57BL/6 mice developed virtually identical degree of airway eosinophilia, but only BALB/c mice developed enhanced airway responsiveness. Together, these findings suggest that allergen-induced murine asthma may be allergen and strain specific [21–23].

Another important facet of this study is the dose of r Bla g 2 or r Der f 1 allergen used to induce the pulmonary response in the inbred mice. The dose of allergen is assumed to be one of the important factors in the polarized development of helper T cell subsets [21, 22]. Clarke et al. [23] demonstrated that C57BL/6 mice sensitized and challenged with as little as 1 µg of Der p 1 produced an extensive pulmonary eosinophilic inflammation which shared many of the features of the inflammation found in murine asthma. Zhou et al. [24] also demonstrated a dose response relationship between exposure to cockroach allergen and induction of sensitization in an experimental asthma in Hartley guinea pigs. In our model we showed that even at the 0.1-µg immunizing dose to the allergen there was a significant pulmonary inflammatory response on airway challenge especially with the r Der f 1 allergen.

The cellular airway inflammation observed in the combined r Bla g 2 and r Der f 1 allergen group permit inferences about the nature of the enhanced pulmonary response. The combined r Bla g 2 and r Der f 1 group compared to the r Bla g 2 or r Der f 1 alone groups caused a significant increase in BALF total cells and eosinophils. A comparative study on interactions between allergen exposure and allergic sensitization showed that subjects

exposed to elevated concentrations of more than one allergen had an up to seven-fold increase in the risk of respiratory symptoms, compared to non-exposed subjects [25]. Moreover, sensitized subjects exposed to elevated domestic concentrations of Der f 1 were found to have the highest risk of asthma attacks and respiratory symptoms. Based on these observations, it is tempting to speculate that the *enhanced* effect of airway inflammation by cockroach and dust mite allergens may be due to the difference in potency of the allergen which together may render the individual to be more susceptible [17, 26].

In our present study, r Der f 1 induced a significant increase in epithelial cells in the BALF. Most mite allergens are enzymes and several potential mechanisms whereby mite protease allergens could enhance allergic airway inflammation have been described [18, 26, 27]. For example proteolytically active Der p 1 is reported to significantly enhance allergic airway response in mice compared with enzymatically inactive allergen [28]. However, Bla g 2 lack enzymatic activity, yet a potent allergen, inducing allergic airway inflammation at low exposure levels [17, 29]. Although enzyme function per se is not required for proteins to elicit allergic airway responses, our data do not rule out the possibility that enzymes inhaled into the respiratory tract could contribute to the airway inflammation. This could occur if particles that are inhaled into the lung carry both allergens and enzymes simultaneously, and clearly both characteristics may coexist in our present study with combined r Bla g 2 and r Der f 1 allergens.

To our knowledge this is the first study that has developed experimental asthma using both recombinant cockroach and dust mite allergens in inbred mice. Differential exposure to domestic cockroach and dust mite allergens usually occur together in the inner city environment [5]. The degree to which individual susceptibility to develop sensitization to these allergens in the context of different exposures is yet to be determined. This model closely resembles the physiologic phenomena seen in human subjects with allergen-induced asthma [4]. Studies are underway in our laboratory to determine the molecular basis of this newly generated mouse models of human asthma and the mechanism(s) underlying the enhanced airway inflammatory responses.

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