

Cigarette Smoke Differentially Affects Eosinophilia and Remodeling in a Model of House Dust Mite Asthma

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Although a similar prevalence of smoking is evident among patients with asthma and the general population, little is known about the impact of cigarette smoke on the immune inflammatory processes elicited by common environmental allergens. We investigated the impact of exposure to cigarette smoke on house dust mite (HDM)-induced allergic airway inflammation and its consequences for tissue remodeling and lung physiology in mice. BALB/c mice received intranasal HDMs daily, 5 days per week, for 3 weeks to establish chronic airway inflammation. Subsequently, mice were concurrently exposed to HDMs plus cigarette smoke, 5 days per week, for 2 weeks (HDMs + smoke). We observed significantly attenuated eosinophilia in the bronchoalveolar lavage of mice exposed to HDMs + smoke, compared with animals exposed only to HDMs. A similar activation of CD4 T cells and expression of IL-5, IL-13, and transforming growth factor- β was observed between HDM-treated and HDM + smoke-treated animals. Consistent with an effect on eosinophil trafficking, HDMs + smoke exposure attenuated the HDM-induced expression of eotaxin-1 and vascular cell adhesion molecule-1, whereas the survival of eosinophils and the numbers of blood eosinophils were not affected. Exposure to cigarette smoke also reduced the activation of B cells and the concentrations of serum IgE. Although the production of mucus decreased, collagen deposition significantly increased in animals exposed to HDMs + smoke, compared with animals exposed only to HDMs. Although airway resistance was unaffected, tissue resistance was significantly decreased in mice exposed to HDMs + smoke. Our findings demonstrate that cigarette smoke affects eosinophil migration without affecting airway resistance or modifying Th2 cell adaptive immunity in a murine model of HDM-induced asthma.

Keywords: cigarette smoke; allergy; house dust mite; inflammation; eosinophils

The adverse health effects of tobacco use are well-established (1). Cigarette smoking is the main cause of lung cancer (2) and a key risk factor in chronic obstructive pulmonary disease (COPD) (3). Increasing evidence suggests that cigarette smoking adversely affects immunity (4). Defects appear to be related to the seemingly contradictory nature of cigarette smoke as a proinflammatory but also immune-suppressive factor. The consequences of this altered immune status on immune and

inflammatory processes elicited by environmental agents, such as aeroallergens, are not well-understood, even though the prevalence of smoking among patients with asthma is comparable to that in the general population (5).

Extensive human and animal studies demonstrated a central role for Th2-polarized CD4⁺ T helper cells in allergic inflammation (6–12). Clinical and experimental evidence further suggests that chronic airway inflammation is associated with tissue remodeling (e.g., increased smooth muscle mass and the deposition of extracellular matrix) (13). Moreover, compelling evidence indicates that cigarette smoking is associated with worsening symptoms in patients with asthma, accelerated decline in lung function, and an impaired therapeutic response to corticosteroids (14). However, the consequences of smoking on the inflammatory processes elicited by environmental allergens and tissue remodeling remain poorly understood, both clinically and experimentally.

Numerous rodent models mimic the immunopathology of asthma (15). To date, most animal studies examining the impact of cigarette smoke on allergic airway inflammation used ovalbumin (OVA) (16–19), a surrogate allergen that unless introduced in the context of an adjuvant induces inhalation tolerance (20, 21). In contrast to OVA, the house dust mite (HDM), the most common environmental allergen, is intrinsically capable of inducing allergic sensitization (22). Moreover, continued exposure to HDMs is associated with persistent eosinophilic airway inflammation, tissue remodeling, and bronchial hyperresponsiveness (23), which are all hallmarks of allergic asthma.

This study investigated the impact of cigarette smoke on airway inflammation, tissue remodeling, and airway responsiveness, using a model of HDM-induced allergic airway inflammation, tissue remodeling, and airway hyperresponsiveness. We observed that although cigarette smoke generates a proinflammatory environment, it also attenuates HDM-induced eosinophilic airway inflammation. Mechanistically, we provide evidence that cigarette smoke does not affect CD4 T-cell activation and the expression of Th2 cytokines, but compromises eosinophil trafficking, likely through the decreased expression of eotaxin-1 and vascular cell adhesion molecule-1 (VCAM-1). Despite attenuated eosinophilia, mice exposed to smoke showed an increased deposition of collagen and unchanged airway resistance. Our results demonstrate the possible impact of cigarette smoke on the cellular trafficking of inflammatory cells in asthma, with differing effects on tissue remodeling and lung function.

MATERIALS AND METHODS

Animals

Female BALB/c mice (6–8 weeks old) were purchased from Charles River Laboratories (Montreal, PQ, Canada). Mice were maintained under specific pathogen-free conditions in an access-restricted area, on a 12-hour light–dark cycle, with food and water provided *ad libitum*. The Animal Research Ethics Board of McMaster University approved all experiments.

(Received in original form September 29, 2010 and in final form January 10, 2011)

This work was supported in part by the Ontario Thoracic Society, the Early Researcher Award Program of Ontario, and the Canadian Institutes of Health Research. A.L.-G. is supported by a doctoral scholarship from Fundación Caja (Madrid, Spain). C.M.T.B. holds a Canadian Thoracic Society Studentship. M.R.S. holds a Canadian Institutes of Health Research New Investigator Award.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 45, pp 753–760, 2011

Originally Published in Press as DOI: 10.1165/rncmb.2010-0404OC on February 11, 2011

Internet address: www.atsjournals.org

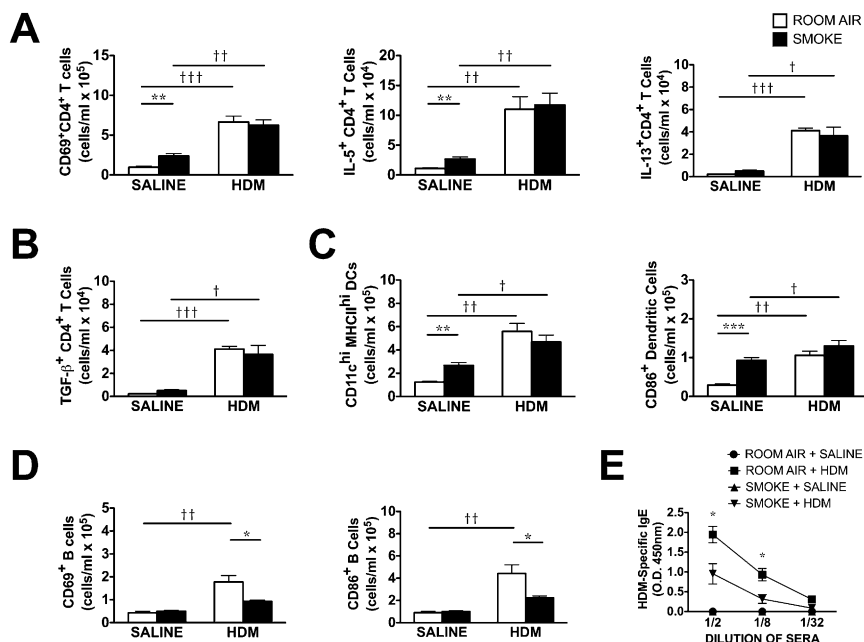


Figure 2. Effects of exposure to HDM and cigarette smoke on adaptive immunity. Mice were exposed to HDM and cigarette smoke, as described in Figure 1A, and flow cytometric analysis was performed on whole lungs to examine the frequency of (A) CD69⁺, IL-5⁺, and IL-13⁺ CD4⁺ (CD3⁺) T cells within the live cell gate (defined using side-scatter and forward-scatter parameters), (B) transforming growth factor (TGF)-β⁺ CD4⁺ (CD3⁺) T cells within the live cell gate, (C) CD11c^{hi} major histocompatibility complex (MHC) II^{hi} dendritic cells (DCs) in live cell gate (left) and percentage of CD86⁺ cells in the DC gate (right), and (D) CD69⁺ (left) and CD86⁺ (CD19⁺) B cells (right). (E) HDM-specific serum IgE concentrations. Statistical significance is shown for smoke + saline versus smoke + HDM ($P < 0.05$). Room air-exposed mice versus smoke-exposed mice, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Saline-treated mice versus HDM-inoculated mice, † $P < 0.05$, †† $P < 0.01$, and ††† $P < 0.001$. Results are presented as means ± SEMs of at least 5 mice per group, and the data represent at least two separate experiments.

Measurement of Lung Responsiveness

Airway resistance, tissue damping, and tissue elastance were determined as previously detailed (25), and as described in the online supplement.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics Software, version 18.0 (SPSS, Inc., Chicago, IL), and are expressed as means ± SEMs. Details of the statistical analysis are described in the online supplement. In all figures, symbols (* and †) are used to indicate significant differences observed between room air-treated versus cigarette smoke-treated groups, and saline-treated versus HDM-treated groups, respectively (i.e., * $P < 0.05$ and † $P < 0.05$, ** $P < 0.01$ and †† $P < 0.01$, and *** $P < 0.001$ and ††† $P < 0.001$).

RESULTS

Exposure to Cigarette Smoke Attenuates HDM-Induced Eosinophilic Inflammation

To investigate whether exposure to cigarette smoke affects HDM-induced eosinophilia, BALB/c mice were inoculated intranasally with 25 μg of HDM daily for 3 weeks to establish maximal eosinophilic airway inflammation, as previously described (22, 23). Subsequently, mice were concurrently exposed to HDM plus cigarette smoke for 2 weeks (HDM + smoke). An outline of the experimental protocol is presented in Figure 1A. Groups of animals were exposed to cigarette smoke either once or twice daily. As controls, we included room air-exposed and smoke only-exposed animals that were inoculated with vehicle only (saline), and room air-exposed animals that were inoculated with HDM. We observed significantly increased total cell and mononuclear cell numbers in the BAL after exposure to cigarette smoke once or twice daily, compared with room-air controls (Figure 1B). The effect of HDM + smoke on mononuclear cell numbers was marginal, and only reached significance under the conditions depicted in Figure 1B, but not in Figure 1C (or after 2 days of exposure to cigarette smoke, in Figure E1). In agreement with our previous observations, neutrophilia was only observed in saline-treated animals that were exposed to cigarette smoke twice daily (26). Inoculation with HDM for 5 weeks resulted in robust eosinophilia. The number (Figure 1B) and percentage (not

shown) of eosinophils were significantly decreased after either once-daily or twice-daily exposure to cigarette smoke. Similarly, concurrent exposure to HDM + smoke for 2 days significantly attenuated eosinophilia (Figure E1). We opted for twice-daily exposure to smoke for 2 weeks in all subsequent experiments, because the extent of attenuation was most robust under those experimental conditions.

We previously reported that the response elicited by HDMs was dose-dependent, and that a concentration of 1 μg of HDM elicited only minimal eosinophilia (25). We therefore investigated whether exposure to cigarette smoke would affect the inflammatory response elicited with a suboptimal concentration of HDM. To this end, BALB/c mice were inoculated with a low dose (1 μg) or 25 μg of HDM for 3 weeks. Subsequently, mice were concurrently exposed to HDM plus cigarette smoke for 2 weeks. Figure 1C shows robust eosinophilia in room air-exposed mice inoculated with a high dose of HDM. In agreement with the data shown in Figure 1B, cigarette smoke significantly attenuated the BAL total cell number and number of eosinophils (Figure 1C). Inhalation of the low dose of HDM elicited a small eosinophilic response that was not affected by concurrent exposure to cigarette smoke. Interestingly, low-dose and high-dose HDM significantly attenuated cigarette smoke-induced neutrophilia in animals exposed to cigarette smoke twice daily for 2 weeks. Similar numbers of neutrophils were evident in mice exposed to HDM + smoke for 2 days, compared with mice exposed only to HDM (Figure E1).

Impact of Cigarette Smoke on T Cells, Dendritic Cells, and B Cells

CD4⁺ T helper cells play a central role in allergic airway inflammation through the elaboration of Th2 cytokines (27). We therefore investigated whether attenuated eosinophilia was associated with decreased T-cell activation and expression of the Th2 cytokines IL-5 and IL-13. Flow cytometric analysis was used to examine T cells in lung-tissue digests. Whether alone or in combination with cigarette smoke, exposure to HDM resulted in similar increases of CD4⁺ T cells expressing the early activation marker CD69, and the cytokines IL-5 and IL-13 (Figure 2A). In line with our previous observations, exposure to cigarette smoke alone led to a small but significant increase in T cells expressing

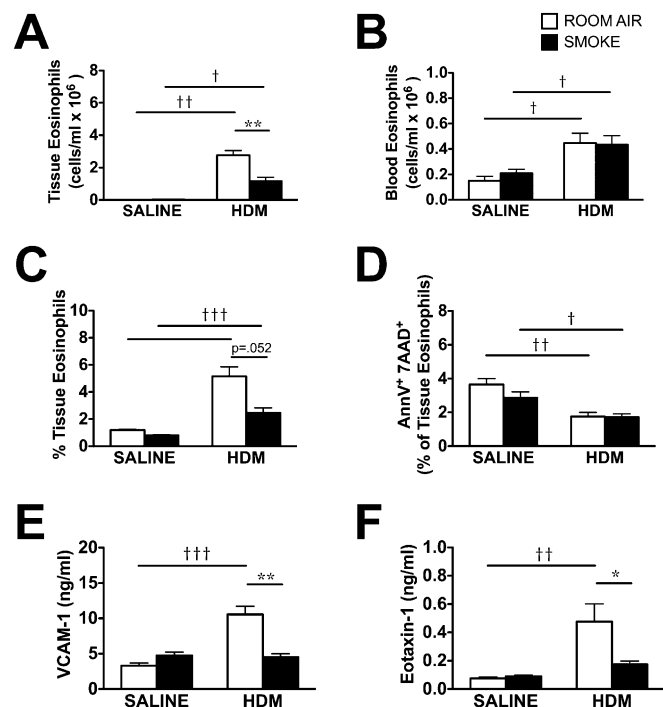
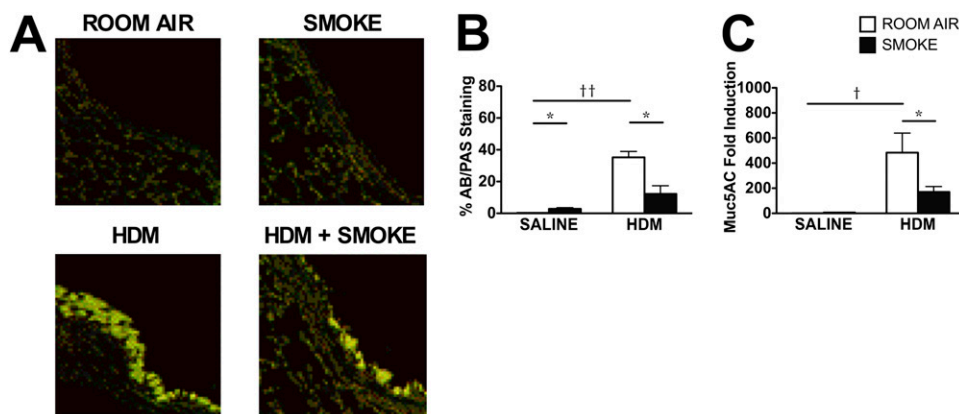


Figure 3. Recruitment and viability of eosinophils to the lungs during exposure to smoke and HDM. Mice were exposed to HDM or saline (control) for 5 weeks, with or without concurrent exposure to cigarette smoke during the final 2 weeks. Data show eosinophil counts in whole-lung tissue (A) or blood (B), as determined by Hema-3 staining and differential cell counts. (C) Percentage of tissue eosinophils, as assessed by flow cytometry (side-scatter high [SSC^{hi}]/Gr-1^{lo}). (D) Frequency of annexin V⁺ 7AAD⁺ (SSC^{hi}/Gr-1^{lo}) tissue eosinophils. AnnV, annexin V. (E) Vascular cell adhesion molecule-1 (VCAM-1) and (F) eotaxin-1 expression in whole-lung homogenates. Room air-exposed mice versus smoke-exposed mice, * $P < 0.05$ and ** $P < 0.01$. Saline-treated mice versus HDM-inoculated mice, † $P < 0.05$, †† $P < 0.01$, and ††† $P < 0.001$. Results are presented as means $\pm$ SEMs of at least 5 mice per group, and the data represent at least two separate experiments.

CD69 and IL-5 (24). Because transforming growth factor- β (TGF- β) was shown to regulate allergic responses (28), we also examined the number of TGF- β ⁺ (CD4⁺) T cells in the lungs (Figure 2B). Although TGF- β ⁺ (CD4⁺) T cells were increased after challenge with HDM, the numbers were similar between room air-exposed and cigarette smoke-exposed animals.



Hammad and colleagues clearly showed that dendritic cells (DCs) are necessary for eliciting an allergic response to HDM (29). Both exposure to cigarette smoke and HDM challenge alone led to a significant increase of CD11c^{hi} major histocompatibility complex Class II^{hi} lung DCs (Figure 2C). We observed a similar increase of DCs in mice exposed to HDM and HDM + smoke. The number of DCs expressing the costimulatory molecule CD86 also increased to a similar degree after exposure to cigarette smoke, HDM, and HDM + smoke.

Because B cells and IgE were shown to regulate allergic responses (30), we assessed the activation status of B cells and IgE production in mice exposed to HDM + smoke. Inoculation with HDM alone led to a significant increase in activated (CD69⁺ or CD86⁺) B cells (Figure 2D). Concurrent exposure to HDM and cigarette smoke significantly attenuated the activation of B cells. HDM-induced increases in HDM-specific serum IgE also declined after co-exposure to cigarette smoke (Figure 2E).

Cigarette Smoke Reduces HDM-Induced Airway Eosinophilia without Significantly Altering Eosinophil Survival in Lung Tissue

To explore further the mechanism of decreased eosinophilia in response to HDM + smoke, we studied whether the reduction in BAL eosinophils after exposure to HDM + smoke was also evident in lung tissue and blood. Lung eosinophils were identified in Hema-3-stained cytospins prepared from lung single-cell suspensions. Consistent with the BAL, the numbers (Figure 3A) and percentages (data not shown) of eosinophils decreased in the lung tissues of mice concurrently exposed to HDM + smoke, compared with those receiving HDM only. Importantly, we observed a similar increase in blood eosinophils in mice exposed to HDM alone or mice exposed to HDM + smoke (Figure 3B), suggesting that decreased numbers of eosinophils in the BAL and lung tissue may result from either increased cell death as a consequence of exposure to cigarette smoke, or altered eosinophil trafficking to the lungs. To that end, we assessed markers of eosinophil cell death by flow cytometry. Eosinophils were characterized as high side-scatter (SSC), Gr-1-low cells (SSC^{hi} Gr-1^{lo}), as reported previously (24, 31). In agreement with the data shown in Figure 3A, using standard hemocytologic procedures to classify eosinophils, we observed decreased percentages of eosinophils in the lungs of HDM + smoke-exposed mice compared with HDM only-exposed mice (Figure 3C). Despite differences in tissue eosinophils, markers of cell death (Annexin V⁺ 7-Aminoactinomycin D⁺) were decreased to a similar extent in the presence and absence of smoke (Figure

Figure 4. Expression of mucus in the lungs of HDM-exposed and cigarette smoke-exposed mice. (A) Formalin-fixed lung tissue samples of mice exposed to HDM and cigarette smoke (as outlined in Figure 1A) were stained with alcian blue/periodic acid-Schiff (AB/PAS) (magnification, $\times 100$). (B) Quantitative analysis of image signal intensity. (C) Quantitative real-time PCR analysis of Mucin5AC mRNA expression of whole lung from mice, treated as described in MATERIALS AND METHODS. Room air-exposed mice versus smoke-exposed mice, * $P < 0.05$. Saline-treated mice versus HDM-inoculated mice, † $P < 0.05$ and †† $P < 0.01$. Results are presented as means $\pm$ SEMs of at least 5 mice per group, and the data represent at least two separate experiments.

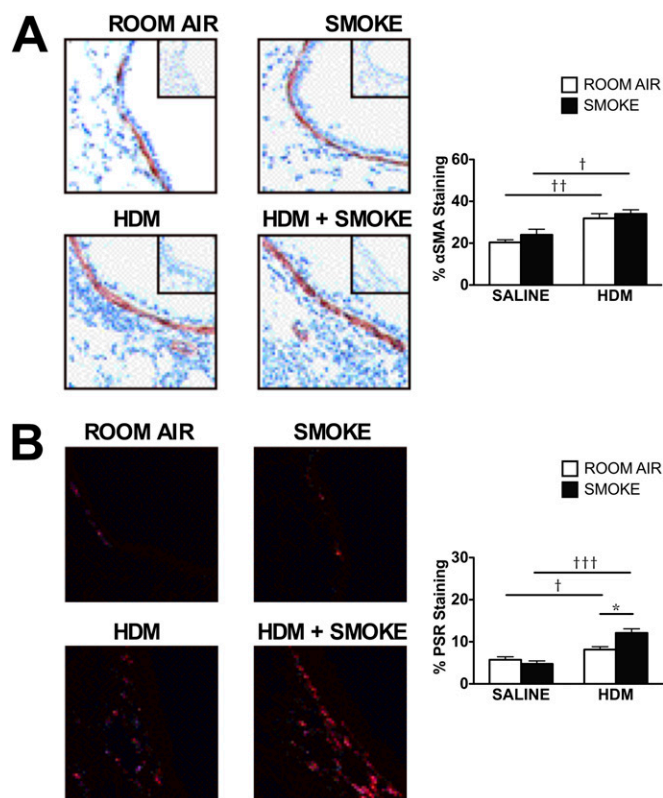


Figure 5. Smooth muscle actin and collagen expression in lungs of HDM-exposed and cigarette smoke-exposed mice. Formalin-fixed lung tissue samples of mice exposed to HDM or saline and cigarette smoke or room air were stained with (A) anti-smooth muscle actin (α SMA) or (B) Picro Sirius red (PSR) to detect α SMA and collagen, respectively (magnification, $\times 100$). Unstained samples depicted in insets in A. Left: Representative images. Right: Quantitative analysis of image signal intensity. Room air-exposed mice versus smoke-exposed mice, $*P < 0.05$. Saline-treated versus HDM-inoculated mice, $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, and $\dagger\dagger\dagger P < 0.001$. Results are presented as means $\pm$ SEMs of at least 5 mice per group, and the data represent at least two separate experiments.

3D). This suggests that the decreased eosinophilia in the HDM + smoke-exposed mice may not be explained by increased cell death, implicating the altered trafficking of eosinophils from the blood to the lung tissue in mice exposed to cigarette smoke.

Given the central role of the chemokine eotaxin-1 and the adhesion molecule VCAM-1 in eosinophil trafficking (32), we assessed the expression of these molecules in response to HDM and HDM + smoke in whole-lung cell homogenates. Notably, exposure to cigarette smoke significantly attenuated the expression of VCAM-1 and eotaxin-1 in the lungs of mice exposed to HDM (Figures 3E and 3F).

Cigarette Smoke Decreases the Production of Mucus while Increasing Collagen Deposition in Mice Exposed to HDM

In light of smoke's dampening effect on inflammation, we investigated whether cigarette smoke affects the production of mucus, smooth muscle mass, and the deposition of extracellular matrix. As shown in Figures 4A and 4B, exposure to HDM markedly increased the amount of alcian blue/periodic acid-Schiff staining in the airways, which was attenuated in mice concurrently exposed to HDM and cigarette smoke. Similarly, we observed a significantly decreased expression of Mucin5AC mRNA in HDM + smoke-exposed mice compared with HDM only-exposed mice (Figure 4C). Challenge with HDM led to

a modest increase in smooth muscle actin staining that was not affected by concurrent exposure to cigarette smoke (Figure 5A). In contrast, the combination of HDM + smoke led to a significant increase in subepithelial collagen deposition, compared with smoke-alone or HDM-alone controls (Figure 5B).

Cigarette Smoke Does Not Affect Airway Resistance but Attenuates Tissue Resistance and Elastance in HDM-Exposed Mice

Finally, we assessed the consequences of concurrent exposure to HDM and cigarette smoke in lung function. Mice were exposed to increasing doses of aerosolized methacholine (MCh), and parameters of respiratory mechanics were measured by flexi-Vent (SCIREQ Inc., Montreal, PQ, Canada). As shown in Figure 6A, neither exposure to cigarette smoke nor challenge with HDM changed baseline airway or tissue resistance. Notably, exposure to smoke attenuated baseline tissue elastance, a measure of lung stiffness. After MCh nebulization, mice exposed to HDM for 5 weeks showed a significant increase in airway resistance, tissue resistance, and tissue elastance, compared with saline-exposed animals (Figures 6B–6D). The increase in airway resistance was similar in mice concurrently exposed to HDM + smoke, indicating a similar narrowing of the central airways. In the lung parenchyma and small airways, we observed a significant increase of tissue resistance and elastance in HDM-challenged mice (Figures 6C and 6D). Mice exposed to HDM + smoke showed significantly decreased tissue resistance, whereas tissue elastance was similar to that in HDM-challenged mice. Thus, although we observed no significant difference in airway resistance or tissue elastance between HDM-treated and HDM + smoke-treated animals, cigarette smoke significantly attenuated tissue resistance in HDM-exposed mice.

DISCUSSION

Compelling evidence suggests that cigarette smoking adversely affects patients with asthma (14). We therefore sought to investigate the impact of cigarette smoke on immune inflammatory processes associated with asthma in a murine model using HDM, a common environmental allergen.

Mice were challenged with HDM for 3 weeks before concurrent exposure to HDM and cigarette smoke for 2 days or 2 weeks. We opted for 3 weeks of exposure to HDM because Johnson and colleagues reported maximal airway eosinophilic inflammation at that time point (23). This experimental protocol also mimics the clinical scenario in which the asthmatic phenotype would be expressed before the commencement of smoking. We used HDMs because they are the most common environmental allergen and they are clinically relevant. HDM, unlike the commonly used surrogate allergen, ovalbumin, is intrinsically capable of eliciting allergic sensitization via the respiratory mucosa. We included exposure to cigarette smoke for either 2 days or 2 weeks to account for the acute (2-day) and more prolonged effects of cigarette smoke on allergic airway inflammation.

Exposure to cigarette smoke significantly attenuated the eosinophilic response elicited by HDM. This finding was unexpected, because cigarette smoke is a known proinflammatory stimulus, generating a lung environment seemingly conditioned to exacerbate allergen-induced inflammatory processes. For example, exposure to smoke is associated with increased expression of granulocyte-macrophage colony stimulating factor (GM-CSF) (33), an inflammatory mediator that is known to facilitate allergic mucosal sensitization, and to exacerbate

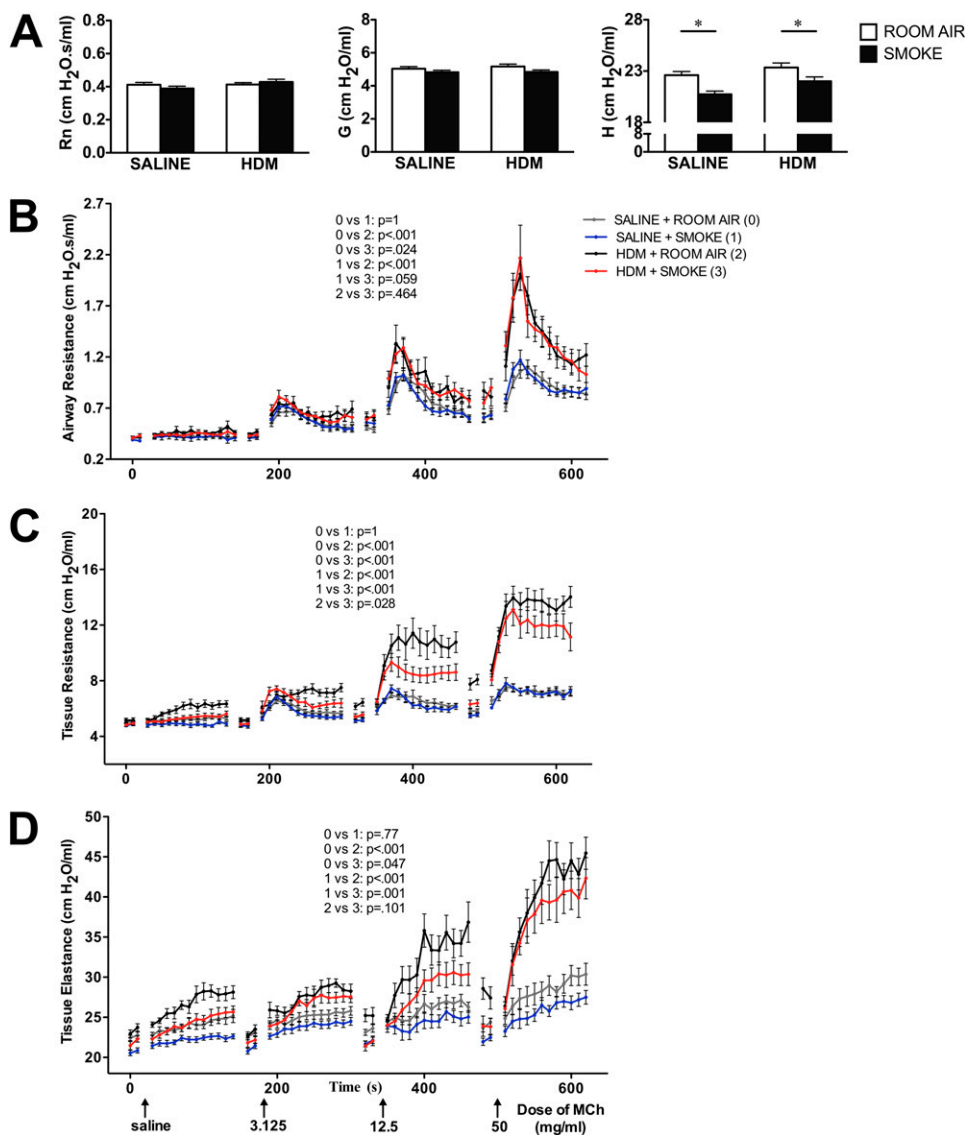


Figure 6. Baseline measurements and dose-response relationship of airway resistance, tissue resistance, and tissue elastance. Airway mechanics in mice exposed to HDM or saline for 5 weeks, with or without cigarette smoke in the final 2 weeks, were assessed using the flexiVent™ system. (A) Mean values of two measurements before methacholine (MCh) nebulization are depicted for each parameter (room air-exposed mice versus smoke-exposed mice, * $P < 0.05$). Rn, airway resistance; G, tissue damping; H, tissue elastance. The entire dose-response to MCh for (B) airway resistance, (C) tissue resistance, and (D) tissue elastance is shown as mean value per measurement. Results are presented as means $\pm$ SEMs of 19 or 20 mice per group, from two independent experiments. For statistical analyses shown in B–D, the treatment groups saline + room air, saline + smoke, HDM + room air, and HDM + smoke are represented as 0, 1, 2, and 3, respectively.

immune inflammatory processes (22, 34). However, our findings are consistent with previous reports of smoke's ability to attenuate eosinophilic inflammation, in both experimental models (16, 17, 33, 35) and patients with asthma (5). The presence of eosinophils after 3 days of rest at the end of the HDM–cigarette smoke protocol is consistent with the sustained presence of cytokines after the cessation of exposure to HDM, as previously described (23). Concurrent exposure to HDM and cigarette smoke also attenuated smoke-induced neutrophilia, and further investigations of the mechanism involved will be of interest. In contrast to these clinical and experimental observations, Moerloose and colleagues (18) reported that exposure to cigarette smoke exacerbated inflammatory processes in a model of OVA-induced allergic airway inflammation. Mechanisms contributing to this exacerbated response were not provided, making differences between the studies difficult to interpret.

Allergic inflammation is regulated by components of the adaptive immune system that include Th2-polarized CD4 T cells (36). We observed a similar expansion of T cells expressing the Th2 cytokines IL-5 and IL-13 in HDM-sensitized mice exposed to smoke or room air. Hence decreased eosinophilia was unlikely to be related to the attenuated Th2 cell function. Similar reductions in eosinophils occur when the environmental

allergen ragweed is combined with cigarette smoke, without any apparent change in Th2 cells (17). These findings are at variance with a report by Thatcher and colleagues (19), which showed a reduced expression of IL-4 and IL-5 in the lungs of mice exposed to cigarette smoke. Differences in the experimental approach, such as the use of an innocuous antigen versus a complex environmental allergen, the route of sensitization, and the timing of allergen and smoke exposure may in part account for the disparities between studies.

Immune mechanisms that mediate the suppression of eosinophilic inflammation were described in previous models of asthma. The production of both IL-10 and TGF- β by FoxP3⁺ regulatory T cells and the production of IL-10 by dendritic cells were shown to suppress allergic airway inflammation (37–39). Because we detected a similar increase in TGF- β ⁺ T cells and in FoxP3⁺ T cells (data not shown) in HDM-exposed and HDM + smoke-exposed mice, the attenuated eosinophilia was unlikely to be related to regulatory T cells, suggesting that responses to environmental allergens may be regulated by T cell-independent mechanisms.

The observation that cigarette smoke led to diminished lung eosinophilia without affecting blood eosinophil counts suggests that cigarette smoke affects eosinophil trafficking. In support of

this notion, cigarette smoke attenuated the HDM-induced expression of the eosinophil chemoattractant eotaxin-1 (40) and the cell adhesion molecule VCAM-1 (41), providing a molecular mechanism for compromised eosinophil trafficking. Lung epithelium and endothelium are the primary sources of eotaxin-1 and VCAM-1 expression, respectively (42, 43). Although the mechanisms of attenuated eotaxin-1 and VCAM-1 expression remain elusive, the compromising effects of cigarette smoke on the airway epithelium and endothelium are well established (44). The attenuated expression of VCAM-1 may also affect neutrophil recruitment, because previous reports documented a role of this adhesion molecule in neutrophil migration *in vitro*, and *in vivo* in a model of hepatic injury (45, 46).

Exposure to cigarette smoke also attenuated the HDM-induced activation of B cells and the production of IgE. Because B cells and IgE were shown to regulate eosinophilia in models of asthma (30, 47), we cannot rule out that attenuated eosinophilia in HDM + smoke-exposed mice may be, at least in part, related to the observed reduction in serum IgE and B-cell activity.

Airway collagen deposition is a characteristic feature of asthma. Although we observed decreased eosinophilia, HDM-induced collagen deposition was further increased in mice exposed to cigarette smoke. The mechanisms of increased collagen deposition, however, remain elusive, because concentrations of TGF- β , a critical tissue-remodeling factor, were similar between HDM-exposed and HDM + smoke-exposed animals. In contrast to collagen deposition, decreases in the production of mucus and goblet cells mirrored the decline in eosinophilic inflammation.

One of the most striking observations in this study involves HDM-induced airway hyperresponsiveness (as measured by airway resistance to nebulized MCh), which not altered by cigarette smoke, although eosinophilia was attenuated by more than 80%. In concordance with our findings, Fattouh and colleagues showed that eosinophils were nonessential for tissue remodeling and hyperresponsiveness in HDM models of asthma (48). Tissue remodeling and chronic inflammation are both believed to contribute significantly to lung dysfunction, although the relative contribution of each to the clinical manifestation of asthma remains controversial. Tissue remodeling was reported in murine models wherein eosinophils were depleted (49, 50). Interestingly, cigarette smoke in the context of exposure to HDM significantly decreased the tissue resistive properties of the lung. Both airway and tissue resistance before MCh nebulization were comparable among groups, but tissue elasticity, the degree of lung stiffness, was decreased by exposure to smoke (Figure 6A). These lower values for baseline tissue elasticity are likely attributable to smoke inducing permanent alterations in mechanical lung properties. In contrast, the changes in tissue elasticity attributable to HDM exposure were likely influenced by small airway narrowing. Collectively, these data suggest that the classic model of allergic asthma that begins with inflammation and leads to tissue remodeling, followed by lung dysfunction, is oversimplified. Instead, the interactions between allergic inflammation, tissue remodeling, and lung dysfunction are complex, and may even occur independently.

In conclusion, we show that exposure to cigarette smoke attenuates allergic inflammation in response to the environmental allergen HDM. The production of mucus decreased and collagen deposition increased, whereas tissue resistance, but not airway resistance, was reduced. Mechanistically, cigarette smoke down-regulated the expression of the HDM-induced eosinophil-recruiting factors, eotaxin-1 and VCAM-1, and reduced the activation of B cells and the production of IgE. Taken together, these data indicate that cigarette smoke regulates

inflammatory mechanisms of eosinophil migration, with varying effects on lung remodeling and physiology in allergic asthma.

Author Disclosure: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors gratefully acknowledge the expert technical support of Joanna Kasinska, Susanna Goncharova, Pamela Shen, Ashling Kelly, and Mary Jo Smith, the secretarial assistance of Marie Colbert, and expert advice on statistical analyses by Sonia Shukla. The authors also thank Cale Zavitz for a critical reading of the manuscript.

References

- Moritsugu KP. The 2006 report of the surgeon general: the health consequences of involuntary exposure to tobacco smoke. *Am J Prev Med* 2007;32:542–543.
- Loeb LA, Ernster VL, Warner KE, Abbotts J, Laszlo J. Smoking and lung cancer: an overview. *Cancer Res* 1984;44:5940–5958.
- van Eeden SF, Sin DD. Chronic obstructive pulmonary disease: a chronic systemic inflammatory disease. *Respir Int Rev Thorac Dis* 2008;75:224–238.
- Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. *Nat Rev Immunol* 2009;9:377–384.
- Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. *Chest* 2001;120:1917–1922.
- Bentley AM, Durham SR, Robinson DS, Menz G, Storz C, Cromwell O, Kay AB, Wardlaw AJ. Expression of endothelial and leukocyte adhesion molecules intercellular adhesion molecule-1, e-selectin, and vascular cell adhesion molecule-1 in the bronchial mucosa in steady-state and allergen-induced asthma. *J Allergy Clin Immunol* 1993;92:857–868.
- Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, Schwartz LB, Durham SR, Jeffery PK, Kay AB. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991;88:661–674.
- Robinson DS, Hamid Q, Ying S, Tsiocopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298–304.
- Jaffar Z, Roberts K, Pandit A, Linsley P, Djukanovic R, Holgate S. B7 costimulation is required for IL-5 and IL-13 secretion by bronchial biopsy tissue of atopic asthmatic subjects in response to allergen stimulation. *Am J Respir Cell Mol Biol* 1999;20:153–162.
- Gavett SH, Chen X, Finkelman F, Wills-Karp M. Depletion of murine CD4⁺ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 1994;10:587–593.
- Watanabe A, Mishima H, Renzi PM, Xu LJ, Hamid Q, Martin JG. Transfer of allergic airway responses with antigen-primed CD4⁺ but not CD8⁺ T cells in brown Norway rats. *J Clin Invest* 1995;96:1303–1310.
- Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. *J Clin Invest* 1999;103:175–183.
- Ramos-Barbón D, Ludwig MS, Martin JG. Airway remodeling: lessons from animal models. *Clin Rev Allergy Immunol* 2004;27:3–21.
- Thomson NC, Chaudhuri R. Asthma in smokers: challenges and opportunities. *Curr Opin Pulm Med* 2009;15:39–45.
- Bates JHT, Rincon M, Irvin CG. Animal models of asthma. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L401–L410.
- Melgert BN, Postma DS, Geerlings M, Luinge MA, Klok PA, van der Strate BWA, Kerstjens HAM, Timens W, Hylkema MN. Short-term smoke exposure attenuates ovalbumin-induced airway inflammation in allergic mice. *Am J Respir Cell Mol Biol* 2004;30:880–885.
- Robbins CS, Pouladi MA, Fattouh R, Dawe DE, Vujicic N, Richards CD, Jordana M, Inman MD, Stampfli MR. Mainstream cigarette smoke exposure attenuates airway immune inflammatory responses to surrogate and common environmental allergens in mice, despite evidence of increased systemic sensitization. *J Immunol* 2005;175:2834–2842.

18. Moerloose KB, Pauwels RA, Joos GF. Short-term cigarette smoke exposure enhances allergic airway inflammation in mice. *Am J Respir Crit Care Med* 2005;172:168–172.
19. Thatcher TH, Benson RP, Phipps RP, Sime PJ. High-dose but not low-dose mainstream cigarette smoke suppresses allergic airway inflammation by inhibiting T cell function. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L412–L421.
20. Holt PG, Batty JE, Turner KJ. Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunology* 1981;42:409–417.
21. Seymour BW, Gershwin LJ, Coffman RL. Aerosol-induced immunoglobulin (Ig)-E unresponsiveness to ovalbumin does not require CD8⁺ or T cell receptor (TCR)-gamma/delta⁺ T cells or interferon (IFN)-gamma in a murine model of allergen sensitization. *J Exp Med* 1998;187:721–731.
22. Cates EC, Fattouh R, Wattie J, Inman MD, Goncharova S, Coyle AJ, Gutierrez-Ramos J-C, Jordana M. Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. *J Immunol* 2004;173:6384–6392.
23. Johnson JR, Wiley RE, Fattouh R, Swirski FK, Gajewska BU, Coyle AJ, Gutierrez-Ramos J-C, Ellis R, Inman MD, Jordana M. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 2004;169:378–385.
24. Botelho FM, Gaschler GJ, Kianpour S, Zavitz CCJ, Trimble NJ, Nikota JK, Bauer CMT, Stämpfli MR. Innate immune processes are sufficient for driving cigarette smoke-induced inflammation in mice. *Am J Respir Cell Mol Biol* 2010;42:394–403.
25. Llop-Guevara A, Colangelo M, Chu DK, Moore CL, Stieber NA, Walker TD, Goncharova S, Coyle AJ, Lundblad LKA, O'Byrne PM, et al. *In vivo-to-in silico* iterations to investigate aeroallergen-host interactions. *PLoS ONE* 2008;3:e2426.
26. Gordon T, Bosland M. Strain-dependent differences in susceptibility to lung cancer in inbred mice exposed to mainstream cigarette smoke. *Cancer Lett* 2009;275:213–220.
27. Barrett NA, Austen KF. Innate cells and T helper 2 cell immunity in airway inflammation. *Immunity* 2009;31:425–437.
28. Fattouh R, Midence NG, Arias K, Johnson JR, Walker TD, Goncharova S, Souza KP, Gregory RC Jr, Lonning S, Gaudie J, et al. Transforming growth factor-beta regulates house dust mite-induced allergic airway inflammation but not airway remodeling. *Am J Respir Crit Care Med* 2008;177:593–603.
29. Hammad H, Plantinga M, Deswarte K, Pouliot P, Willart MAM, Kool M, Muskens F, Lambrecht BN. Inflammatory dendritic cells—not basophils—are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. *J Exp Med* 2010;207:2093–2111.
30. Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C. Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. *J Exp Med* 1996;183:1303–1310.
31. Al-Garawi AA, Fattouh R, Walker TD, Jamula EB, Botelho F, Goncharova S, Reed J, Stämpfli MR, O'Byrne PM, Coyle AJ, et al. Acute, but not resolved, influenza A infection enhances susceptibility to house dust mite-induced allergic disease. *J Immunol* 2009;182:3095–3104.
32. Rosenberg HF, Phipps S, Foster PS. Eosinophil trafficking in allergy and asthma. *J Allergy Clin Immunol* 2007;119:1303–1312.
33. Trimble NJ, Botelho FM, Bauer CMT, Fattouh R, Stämpfli MR. Adjuvant and anti-inflammatory properties of cigarette smoke in murine allergic airway inflammation. *Am J Respir Cell Mol Biol* 2009;40:38–46.
34. Stämpfli MR, Wiley RE, Neigh GS, Gajewska BU, Lei XF, Snider DP, Xing Z, Jordana M. GM-CSF transgene expression in the airway allows aerosolized ovalbumin to induce allergic sensitization in mice. *J Clin Invest* 1998;102:1704–1714.
35. Thatcher TH, McHugh NA, Egan RW, Chapman RW, Hey JA, Turner CK, Redonnet MR, Seweryniak KE, Sime PJ, Phipps RP. Role of CXCR2 in cigarette smoke-induced lung inflammation. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L322–L328.
36. Kim HY, Dekruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat Immunol* 2010;11:577–584.
37. Kearley J, Barker JE, Robinson DS, Lloyd CM. Resolution of airway inflammation and hyperreactivity after *in vivo* transfer of CD4⁺CD25⁺ regulatory T cells is interleukin 10 dependent. *J Exp Med* 2005;202:1539–1547.
38. Chen W, Jin W, Hardegen N, Lei K-J, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4⁺CD25⁺ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF-beta induction of transcription factor FOXP3. *J Exp Med* 2003;198:1875–1886.
39. Li X, Yang A, Huang H, Zhang X, Town J, Davis B, Cockcroft DW, Gordon JR. Induction of Th2 cell allergen tolerance by IL-10-differentiated regulatory dendritic cells. *Am J Respir Cell Mol Biol* 2009;42:190–199.
40. Broide D, Sriramaraio P. Eosinophil trafficking to sites of allergic inflammation. *Immunol Rev* 2001;179:163–172.
41. Foster PS, Mould AW, Yang M, Mackenzie J, Mattes J, Hogan SP, Mahalingam S, McKenzie AN, Rothenberg ME, Young IG, et al. Elemental signals regulating eosinophil accumulation in the lung. *Immunol Rev* 2001;179:173–181.
42. Conroy DM, Williams TJ. Eotaxin and the attraction of eosinophils to the asthmatic lung. *Respir Res* 2001;2:150–156.
43. Albelda SM. Endothelial and epithelial cell adhesion molecules. *Am J Respir Cell Mol Biol* 1991;4:195–203.
44. Thorley AJ, Tetley TD. Pulmonary epithelium, cigarette smoke, and chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2007;2:409–428.
45. Reinhardt PH, Elliott JF, Kubes P. Neutrophils can adhere via alpha4beta1-integrin under flow conditions. *Blood* 1997;89:3837–3846.
46. Essani NA, Bajt ML, Farhood A, Vonderfecht SL, Jaeschke H. Transcriptional activation of vascular cell adhesion molecule-1 gene *in vivo* and its role in the pathophysiology of neutrophil-induced liver injury in murine endotoxin shock. *J Immunol* 1997;158:5941–5948.
47. Lindell DM, Berlin AA, Schaller MA, Lukacs NW. B cell antigen presentation promotes Th2 responses and immunopathology during chronic allergic lung disease. *PLoS ONE* 2008;3:e3129.
48. Fattouh R, Al-Garawi A, Fattouh M, Arias K, Walker TD, Goncharova S, Coyle AJ, Humbles AA, Jordana M. Eosinophils are dispensable for allergic remodeling and immunity in a model of house dust mite-induced airway disease. *Am J Respir Crit Care Med* 2010;183:179–188.
49. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999;103:779–788.
50. Tournay KG, Kips JC, Schou C, Pauwels RA. Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. *Clin Exp Allergy* 2000;30:79–85.

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