

Continuous Exposure to House Dust Mite Elicits Chronic Airway Inflammation and Structural Remodeling

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It is now fully appreciated that asthma is a disease of a chronic nature resulting from intermittent or continued aeroallergen exposure leading to airway inflammation. To investigate responses to continuous antigen exposure, mice were exposed to either house dust mite extract (HDM) or ovalbumin intranasally for five consecutive days, followed by 2 days of rest, for up to seven consecutive weeks. Continuous exposure to HDM, unlike ovalbumin, elicited severe and persistent eosinophilic airway inflammation. Flow cytometric analysis demonstrated an accumulation of CD4⁺ lymphocytes in the lung with elevated expression of inducible costimulator a marker of T cell activation, and of T1/ST2, a marker of helper T Type 2 effector cells. We also detected increased and sustained production of helper T cell Type 2-associated cytokines by splenocytes of HDM-exposed mice on *in vitro* HDM recall. Histologic analysis of the lung showed evidence of airway remodeling in mice exposed to HDM, with goblet cell hyperplasia, collagen deposition, and peribronchial accumulation of contractile tissue. In addition, HDM-exposed mice demonstrated severe airway hyperreactivity to methacholine. Finally, these responses were studied for up to 9 weeks after cessation of HDM exposure. We observed that whereas airway inflammation resolved fully, the remodeling changes did not resolve and airway hyperreactivity resolved only partly.

Keywords: allergy and immunology; animal; asthma; models

Asthma is an airway inflammatory disease of a chronic nature (1, 2). As asthma is an allergen-induced, immune-driven inflammatory disease, "chronicity" can be understood as the consequence of recurrent episodes of airway inflammation resulting from intermittent allergen exposure or, alternatively, as persistent airway inflammation resulting from continuous allergen exposure. Exposure to seasonal allergens such as ragweed would exemplify the former, whereas exposure to perennial allergens such as house dust mite would be representative of the latter. Chronic allergen exposure likely triggers a distinct array of immunobiological and biochemical responses, the impact of which to the airway structure is thought to contribute significantly to clinical symptoms (3, 4). In humans, such changes include airway wall thickening, subepithelial fibrosis, collagen deposition, goblet

cell hyperplasia, myofibroblast hyperplasia, and epithelial cell hypertrophy (5, 6). Importantly, the molecular and biochemical processes underlying airway structural remodeling remain poorly understood.

Although studies examining the airways of humans with asthma are invaluable, they provide only a snapshot of what is surely a complex and dynamic process. Although murine models have been useful in understanding the basic cellular and molecular mechanisms of allergic sensitization and acute airway inflammation, they have generally been much less productive in advancing our understanding of the mechanisms and implications of chronic airway inflammation and remodeling. A central reason for this lies in the antigen generally used in these models, ovalbumin (OVA), because it has been shown that respiratory exposure to OVA does not lead to chronic airway inflammation, but instead results in inhalation tolerance, and continuous exposure to OVA in a sensitized animal leads to a diminution, in fact a complete abrogation, of airway inflammation (7). Our laboratory has developed novel mouse models of airway inflammation involving exposure to common environmental aeroallergens, such as ragweed (8) and house dust mite (9). Previous studies involved short-term exposure to aeroallergen extracts (7–10 days); in the present study we investigated the impact of prolonged exposure (5–7 weeks) to a house dust mite (HDM) extract on local and systemic immunoinflammatory responses, and on the functional and structural consequences of such exposure.

Our data show that chronic exposure to HDM leads to prominent and sustained airway eosinophilic inflammation, along with elevated serum levels of helper T cell Type 2 (Th2)-associated immunoglobulins and significantly increased production of Th2-associated cytokines by splenocytes *in vitro*. We have also identified, by flow cytometry, a persistent accumulation of activated/effector T cell subsets in the lung. Moreover, we have documented widespread and severe structural changes in HDM-exposed animals, with evidence of goblet cell hyperplasia and significant increases in subepithelial collagen deposition and contractile tissue. Finally, airway reactivity to methacholine was considerably increased after 5 weeks of HDM exposure. Although cessation of allergen exposure allowed for a rapid resolution of the immunoinflammatory response in the airway, persistent structural and functional abnormalities were observed even after 9 weeks of nonexposure.

Some of the results of these studies have been previously reported in the form of an abstract (10).

METHODS

Animals

Female BALB/c mice (6–8 weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed under specific pathogen-free conditions and a 12:12 hour light:dark cycle. All experiments described in this study were approved by the Animal Research Ethics Board of McMaster University (Hamilton, ON, Canada).

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Antigen Administration

Mice were exposed to either purified HDM extract (Greer Laboratories, Lenoir, NC) or OVA (Grade V; Sigma, Oakville, ON, Canada) intranasally (25 μ g of protein in 10 μ l of saline) for 5 days/week for up to seven consecutive weeks.

Collection and Measurement of Specimens

Blood and bronchoalveolar lavage fluid (BALF) were collected and evaluated as previously described (11).

Splenocyte Culture

Splenocytes were isolated and cultured, and supernatants were collected for ELISA analysis. *See* the online supplement for additional details on the methods used to make these measurements.

Quantification of Cytokines and Immunoglobulins

ELISA kits were purchased from R&D Systems (Minneapolis, MN). Total IgE and HDM-specific IgG1 levels in the serum were detected by antigen-capture ELISA. *See* the online supplement for additional details on the methods used to make these measurements.

Lung Cell Isolation and Flow Cytometric Analysis of Lung Cell Subsets

Lung mononuclear cells were isolated as previously described (12) and stained for flow cytometric analysis. Data were collected with a FACScan (BD Biosciences Immunocytometry Systems, Sunnyvale, CA) and were analyzed with WinMDI software (Scripps Research Institute, La Jolla, CA). *See* the online supplement for additional details on the methods used to make these measurements.

Measurement of Airway Hyperresponsiveness

Airway responsiveness was measured on the basis of the response of total respiratory system resistance to increasing intravenous doses of methacholine (MCh) as previously described (13). *See* the online supplement for additional details on the methods used to make these measurements.

Histology and Immunohistochemistry

Lungs were inflated with 10% formalin at a standard pressure of 20 cm H₂O. Tissues were then paraffin embedded and cut at a thickness of 3 μ m. Sections were stained with hematoxylin and eosin, Alcian blue (pH 2.5), and Picro Sirius red. To detect the presence of smooth muscle in the airway, immunohistochemistry was performed with anti- α smooth muscle actin (α -SMA), employing methods described in detail in the online supplement.

Morphometry

Images for morphometric analysis were captured with OpenLab version 3.0.3 (Improvision, Guelph, ON, Canada), via a Leica camera and microscope attached to a Macintosh computer (Mac OS 9 operating system). Analysis was performed on a custom computerized image analysis system (Northern Eclipse software, version 5 [Empix Imaging, Mississauga, ON, Canada] on a Pentium III computer [700-MHz processor, Windows 98 operating system]). Analysis of sections stained for α -SMA and Picro Sirius red was performed as previously described (14).

Data Analysis

Data were analyzed with SigmaStat version 2.03 (SPSS Inc., Chicago, IL), and are expressed as means $\pm$ SEM, unless otherwise indicated. Results were interpreted by analysis of variance followed by the Tukey *post hoc* test (when applicable). Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Cellular Profile of the BALF of Animals Continuously Exposed to HDM

BALB/c mice were chronically exposed to either OVA or HDM extract, without the provision of exogenous adjuvant, for up to

7 weeks. We observed robust eosinophilic airway inflammation, as evidenced by elevated total cells (Figure 1A) and eosinophils (Figure 1B) in BALF after 3, 5, and 7 weeks of continuous HDM exposure ($p < 0.05$ compared with naive animals). Eosinophils represented about 30% of total cells at these time points.

Flow Cytometric Analysis of Lung Mononuclear Cells during Continuous Exposure to HDM

Dendritic cells were identified as MHCII⁺/CD11c⁺, macrophages were identified as MHCII⁺/CD11b⁺, and B cells were identified as MHCII⁺/B220⁺. Table 1 demonstrates a rapid increase in the number of dendritic cells after only 1 week of antigen exposure, compared with naive animals, which remained elevated in the lungs of animals continuously exposed to HDM for 5 weeks. A similar trend was observed for macrophages, with higher expression in animals exposed to HDM for 5 weeks, compared with naive animals (Table 1). No changes in B cells were observed in animals exposed to HDM (Table 1). The populations of dendritic cells, macrophages, and B cells in OVA-exposed animals were similar to those observed in naive animals (data not shown).

We also evaluated the expansion of activated T lymphocytes (by inducible costimulator [ICOS] expression) and effector Th2 lymphocytes (by T1/ST2 expression) in the lungs. After 5 weeks of antigen exposure, we detected a substantial increase in the population of CD4⁺/ICOS⁺ cells (Figure 2A) in animals exposed to HDM compared with naive animals. Expression of the putative marker of Th2 cells, T1/ST2, on CD4⁺ cells (Figure 2B) was also increased in animals continuously exposed to HDM for 5 weeks compared with naive animals. The populations of CD4⁺/ICOS⁺ and CD4⁺/T1/ST2⁺ lymphocytes in OVA-exposed animals were similar to those observed in naive animals (data not shown).

In Vitro Cytokine Production by HDM-exposed Animals

In vitro recall of splenocytes challenged with HDM demonstrated considerable production of Th2-associated cytokines at every time point in the chronic allergen exposure protocol ($p < 0.05$). As seen in Figure 3, the production of interleukin (IL)-5 (Figure 3A) and IL-13 (Figure 3B) by cultured splenocytes was elevated in animals continuously exposed to HDM ($p < 0.05$). Similar increases in the *in vitro* production of IL-4 were also observed: 237, 227, 487, and 731 pg/ml at 1, 3, 5, and 7 weeks of HDM exposure, respectively. We observed no significant increases in the *in vitro* production of IFN- γ in HDM-exposed animals, and no significant changes in the *in vitro* production of IL-4, IL-5, IL-13, and IFN- γ in OVA-exposed mice (data not shown).

Impact of Continuous Exposure to HDM on Serum Immunoglobulin Production

Serum immunoglobulin levels of total IgE after 3 weeks of HDM exposure were significantly increased compared with naive animals ($p < 0.05$), and remained high in animals exposed to HDM for 5 and 7 weeks (Figure 3C). We also detected increased levels of HDM-specific IgG1 after 3 weeks of HDM exposure ($p < 0.05$), with similar levels seen after 5 and 7 weeks of exposure (Figure 3D).

BALF Cellular Profile and *In Vitro* Cytokine Production after Cessation of HDM Exposure and after *In Vivo* Allergen Recall

HDM administration was discontinued after 5 weeks of exposure. Total cells and eosinophils (Figures 4A and 4B) returned to baseline within 2 weeks of the final allergen exposure. However, splenocytes of these animals retained the ability to respond to HDM *in vitro*, as shown by elevated production of IL-5 and

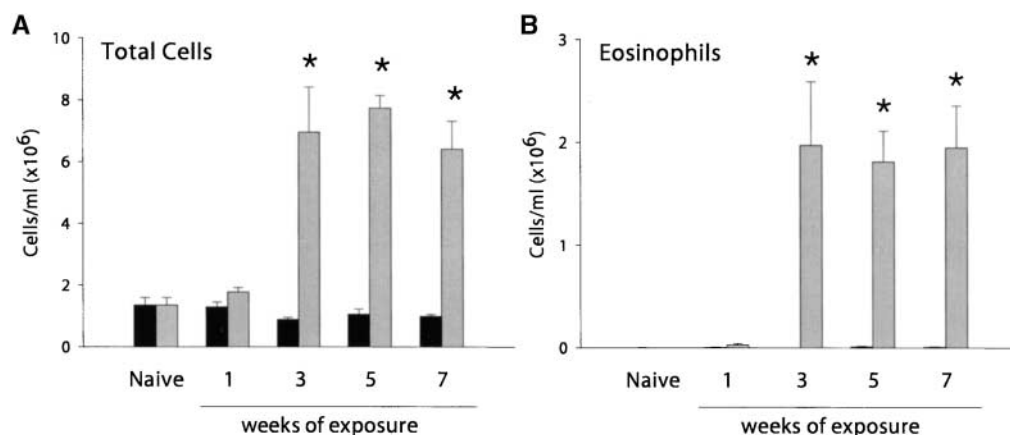


Figure 1. Inflammatory response observed after continuous exposure to ovalbumin (OVA) (black bars) or house dust mite (HDM) (gray bars). Total cells (A) and eosinophils (B) in bronchoalveolar lavage fluid (BALF) demonstrate abundant inflammation in the lungs of HDM-exposed animals, in comparison with naive and OVA-exposed animals. Animals were administered antigen intranasally (5 days/week) and inflammation was evaluated at the indicated time points. Data shown represent means $\pm$ SEM ($n = 8-12$), * $p < 0.05$ compared with naive animals. Statistical analysis was performed by one-way analysis of variance (ANOVA) with the Fisher least significant difference (LSD) *post hoc* test.

IL-13 up to 9 weeks after the last antigen exposure (Figures 4C and 4D), as well as after *in vivo* allergen recall (Table 2) ($p < 0.05$). Nine weeks after the last allergen exposure, animals were reexposed to HDM for three consecutive days, resulting in increased total cell and eosinophil numbers in the BALF (Table 2).

Histologic Analysis of Lung Inflammation and Goblet Cell Hyperplasia

Histologic evaluation of lung tissue in mice subjected to continuous antigen exposure corroborated our findings in the BALF. Low-magnification analysis demonstrated extensive peribronchial and perivascular airway inflammation in HDM-exposed animals (Figure 5B) compared with naive mice (Figure 5A). Higher magnification revealed marked eosinophilic infiltration into the airway parenchyma by 5 weeks of HDM exposure (Figures 5C and 5D). Histology of animals exposed to OVA was not different from that of naive animals (data not shown).

Continuous Exposure to HDM Is Associated with Structural Changes in the Airway

Marked morphologic changes to the airway epithelium and goblet cell hyperplasia were also apparent in animals continuously exposed to HDM for 5 weeks (Figure 6). After 5 weeks of HDM exposure, lung histology demonstrated increased numbers of mucin-producing cells and goblet cell hyperplasia (Figure 6B) and significant increases in peribronchial contractile elements (Figure 6D) and airway collagen deposition (Figure 6F), as com-

pared with naive animals (Figures 6A, 6C, and 6E, respectively). The observed increases in contractile elements and collagen accumulation in the airways were quantified morphometrically, as shown in Figure 7. After 3 weeks of HDM exposure, we detected a nonsignificant trend for increases in both collagen deposition (50%) and α -SMA staining (34%) when compared with naive animals (23 and 23%, respectively). However, after 5 weeks of exposure, these increases were significantly elevated above baseline (collagen, 55%; α -SMA, 53%). Whereas the observed increases in α -SMA staining (45%) appeared to have decreased somewhat 9 weeks after cessation of HDM exposure, we observed a trend for increased collagen staining (65%) at this time point (Figure 7). There were no increases in Picro Sirius red or α -SMA staining in OVA-exposed animals (data not shown).

Continuous Exposure to HDM Results in Severe Airway Hyperreactivity

The key indicators of airway responsiveness (as described in Leigh and coworkers [14]), namely, airway reactivity (the rate of increase in respiratory resistance for a given increase of dose of MCh), airway sensitivity (the lowest dose of MCh to produce bronchoconstriction), and maximal inducible bronchoconstriction (maximum respiratory resistance), were evaluated in naive animals, animals continuously exposed to HDM for 5 weeks, and animals exposed to HDM for 5 weeks followed by a resolution period. Compared with naive animals, 5-week-exposed animals demonstrated a significant and severe increase in airway reactivity as evidenced by the slope of the dose-response curve to MCh (Figure 8). Significantly higher maximal inducible bronchoconstriction was also observed in these animals (Figure 8) ($p < 0.05$). After cessation of HDM exposure, these indicators of airway responsiveness showed some improvement, but maximal inducible bronchoconstriction was still significantly elevated in these animals compared with naive animals (Figure 8) ($p < 0.05$). There was no evidence of changes in airway sensitivity in any group of animals, based on the calculated lowest dose of MCh required to increase respiratory resistance in naive animals. There were no changes observed in any indices of airway responsiveness in animals continuously exposed to OVA (data not shown).

DISCUSSION

It is now appreciated that allergic asthma is a disease of a chronic nature resulting from intermittent or continued aeroallergen

TABLE 1. FLOW CYTOMETRIC ANALYSIS OF ANTIGEN-PRESENTING CELLS IN ANIMALS EXPOSED TO HOUSE DUST MITE*

Cell Type	Percentage of Cells		
	Naive	1 week HDM (1 wk)	HDM (5 wk)
MHCII ⁺ CD11c ⁺	2.24	5.58	5.73
MHCII ⁺ CD11b ⁺	1.85	5.18	5.17
MHCII ⁺ B220 ⁺	9.13	8.90	7.25

Definition of abbreviation: HDM = house dust mite.

* Mice were exposed to HDM, 5 days/week, for 1 or 5 weeks. Nonexposed animals were used as naive controls. Mononuclear cells from four to six pooled lungs were collected and stained for MHCII, CD11c, CD11b, and B220 surface expression. Cells were analyzed by four-color flow cytometry.

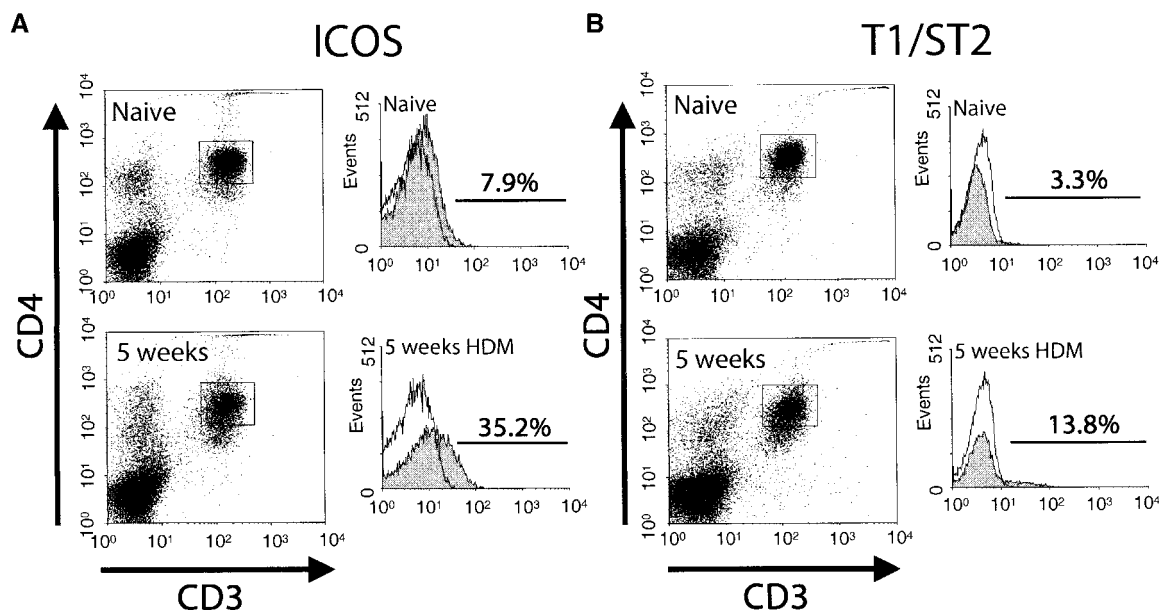


Figure 2. Flow cytometric analysis of CD3⁺/CD4⁺ cells in naive animals and in animals continuously exposed to HDM for 5 weeks. Animals received HDM intranasally, 5 days/week, for five consecutive weeks. Lung mononuclear cells were isolated and analyzed for ICOS and T1/ST2 expression on the CD3⁺/CD4⁺ population. HDM-exposed animals demonstrated a robust increase in inducible stimulator⁺ (A) and T1/ST2⁺ (B) CD3⁺/CD4⁺ lymphocytes in the lung, compared with naive animals.

exposure. In either case, the airway is ultimately subjected to a prolonged chronic inflammatory state that leads to a number of structural abnormalities collectively referred to as airway remodeling. These abnormalities affect both the epithelial and subepithelial compartments (14, 15), and are thought to have functional

consequences that contribute to clinical symptoms (15). Although our knowledge of aeroallergen-induced acute asthmatic inflammation has markedly increased, much less progress has been made toward understanding the mechanisms underlying chronic inflammation and tissue remodeling. Protocols utilizing

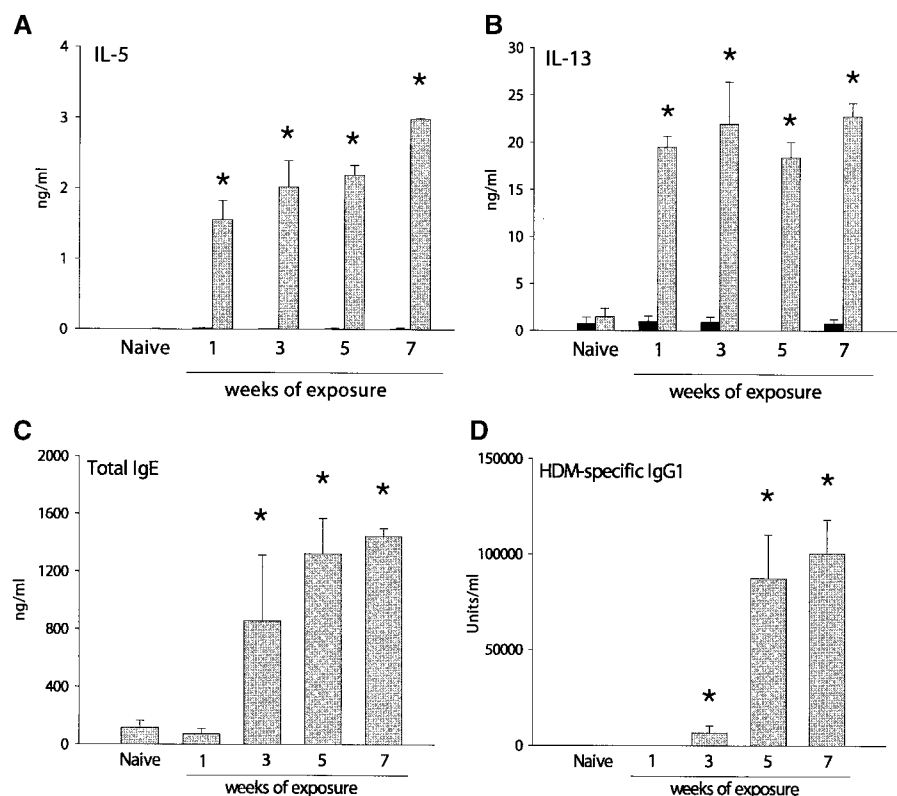


Figure 3. Effects of continuous antigen exposure on T helper type 2 (Th2)-associated humoral immune responses. *In vitro* expression of Th2-associated cytokines interleukin (IL)-5 (A) and IL-13 (B) was elevated in animals continuously exposed to HDM. Splenocytes were isolated at the indicated time points and cultured for 120 hours in medium alone (black bars) or medium supplemented with HDM (gray bars). Serum levels of total IgE (C) and HDM-specific IgG1 (D) in HDM-exposed animals were also significantly increased at the indicated time points, compared with naive animals. Data represent means $\pm$ SEM ($n = 4-12$), * $p < 0.05$ compared with naive animals. Statistical analysis was performed using one-way ANOVA with the Fisher LSD *post hoc* test.

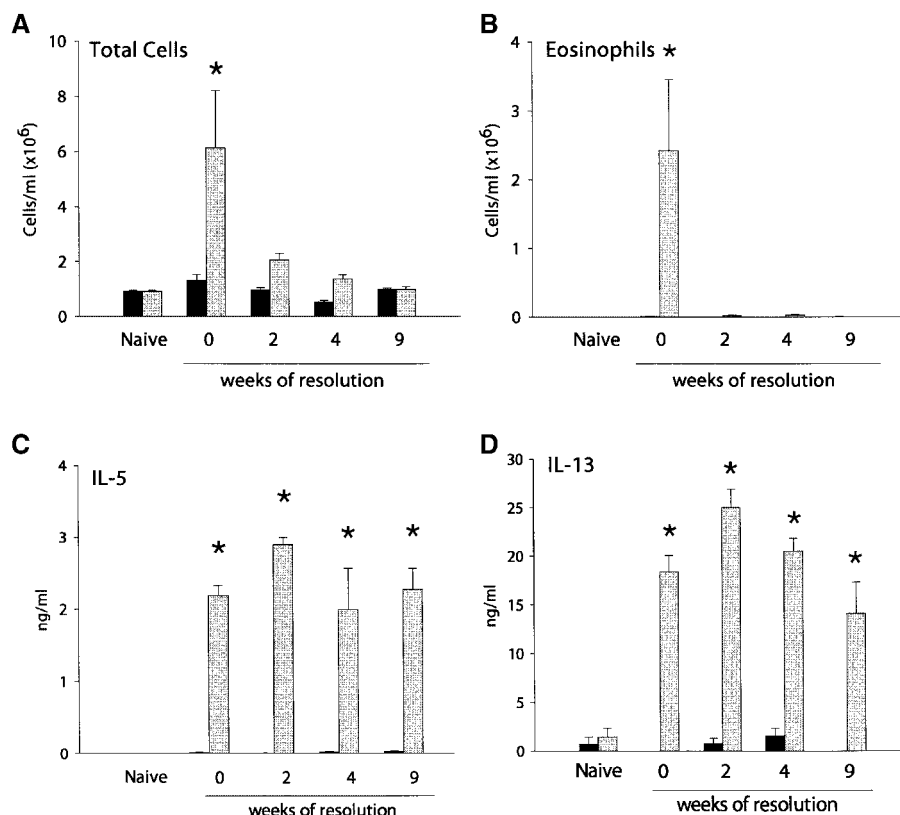


Figure 4. Effects of cessation of antigen exposure on cellular and humoral immune responses in animals continuously exposed to OVA (black bars) or HDM (gray bars). Total cells (A) and eosinophils (B) in the BALF demonstrate diminution of inflammation after cessation of antigen exposure. Animals were administered antigen intranasally, 5 days/week, for five consecutive weeks, at which point antigen administration was stopped. Inflammation in the lung was evaluated at the indicated time points. *In vitro* expression of Th2-associated cytokines IL-5 (C) and IL-13 (D) remained elevated in animals after cessation of HDM exposure. Splenocytes were isolated at the indicated time points and cultured for 120 hours in medium alone (black bars) or medium supplemented with HDM (gray bars). Data represent means $\pm$ SEM ($n = 4-8$), * $p < 0.05$ compared with naive animals. Statistical analysis was performed by one-way ANOVA with the Fisher LSD *post hoc* test.

surrogate allergens such as OVA have been unable to establish chronic airway eosinophilic inflammation and, therefore, are inadequate to mechanistically recapitulate some of the processes involved in airway remodeling.

In this study, we investigated the impact of continuous respiratory exposure to a common environmental aeroallergen, a purified HDM extract. House dust mites are a significant source of indoor allergens implicated in a variety of allergic symptoms in 10% of the population (9). In particular, *Dermatophagoides pteronyssinus* is the mite-related allergen most frequently associated with respiratory allergy (9, 16, 17). It is well known that the main antigens of house dust mite, *Der p* 1, 3, 5, and 9, are cysteine and/or serine proteases (18–20), and a number of *in vitro* studies have demonstrated that these antigens are able to stimulate airway epithelial cells to produce a number of cytokines, including granulocyte-macrophage colony-stimulating fac-

tor, likely through a protease-activated receptor 2-mediated mechanism (21, 22). *In vivo*, Gough and coworkers (23) have demonstrated that the proteolytic activity of *Der p* 1 enhances the allergenicity of this antigen in a conventional (i.e., peritoneal sensitization) acute murine model of airway inflammation. We have previously shown that intranasal exposure of mice to HDM for 10 days, without additional adjuvants, results in evident Th2 allergic airway eosinophilic inflammation that appears to be mediated by granulocyte-macrophage colony-stimulating factor (9). These studies impelled us to investigate the consequences of chronic, continuous exposure to this aeroallergen. The experiments presented here detail the immunoinflammatory events that occur in the effector organ (the airway/lung compartment) and in the periphery (serum and spleen), as well as the structural and functional impact of chronic aeroallergen exposure.

Our data show that continuous exposure to HDM leads to sustained airway eosinophilic inflammation, quantifiable in the BALF and clearly evident histopathologically (Figures 1, 5, and 6). Interestingly, this inflammatory response is not limitless; rather, it plateaus between 3 and 7 weeks of exposure. At the moment, the factors that limit the extent of the inflammatory response in the airway remain to be elucidated. As eosinophilia is a characteristic terminal event of Th2 responses, a distinct interaction between antigen-presenting cells and T cells is implicated in the initiation of this process (24). Thus, to extend our analysis of the immune response elicited by continuous HDM exposure, we performed flow cytometric analysis of lung mononuclear cells. This investigation demonstrated a considerable increase in the dendritic cell and macrophage populations (Table 1) and an expansion of the populations of both activated T cells and Th2 effector cells after 5 weeks of continuous HDM exposure (Figure 2).

Because allergic sensitization is a systemic process, it was of interest to investigate relevant markers of systemic Th2 immune

TABLE 2. BRONCHOALVEOLAR LAVAGE FLUID INFLAMMATION AND *IN VITRO* CYTOKINE PRODUCTION DURING *IN VIVO* ALLERGEN RECALL*

	<i>In Vivo</i> HDM Recall
Total cells	$3.8 \pm 0.7^\dagger$
Eosinophils	$6.1 \pm 2.7^\dagger$
IL-5	$3.2 \pm 0.8^\dagger$
IL-13	$59.6 \pm 6.1^\dagger$

* Mice were exposed to HDM, 5 days/week, for 5 weeks, at which point antigen exposure was stopped. Nine weeks after the last HDM administration, animals were readministered HDM for three consecutive days. Splenocytes were collected at sacrifice and cultured in medium alone or in medium supplemented with HDM for 120 hours. Data were measured as follows: total cells in the BALF, $\times 10^6$ /ml; eosinophils in the BALF, $\times 10^5$ /ml; IL-5, ng/ml; and IL-13, ng/ml.

$^\dagger p < 0.05$ when compared with naive animals; $n = 4$ per group.

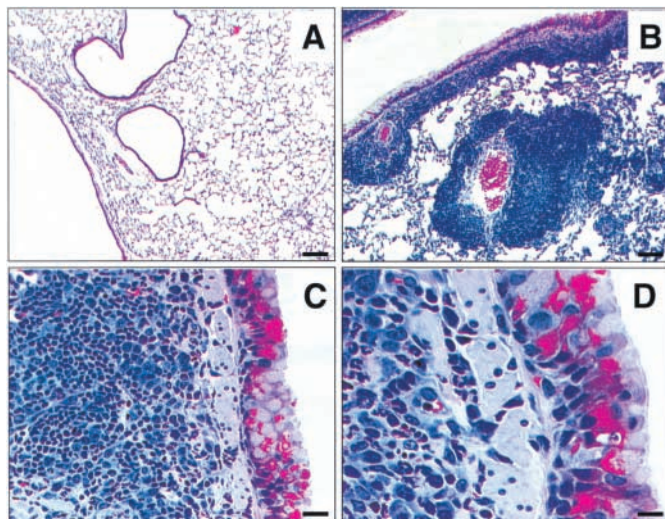


Figure 5. Histologic analysis of lung inflammation in HDM-exposed animals. Low-magnification analysis of lung tissue in naive mice (A) and in mice continuously exposed to HDM over a 5-week period (B) demonstrates severe peribronchial and perivascular inflammatory infiltrate. Higher magnification (C and D) shows eosinophilic infiltration of the lung parenchyma and airway wall. Naive animals were not manipulated whereas HDM-exposed animals were administered HDM at 5 days/week for five consecutive weeks. Lungs were dissected out at the time of sacrifice, fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. Scale bars: (A and B) 40 μ m; (C) 10 μ m; (D) 5 μ m.

activity. One such marker is *in vitro* splenocyte expression of Th2-associated cytokines, indicative of an antigen-specific memory response. Our data demonstrate elevated production of IL-4, IL-5, and IL-13 in mice chronically exposed to HDM *in vivo* (Figures 3A and 3B). These elevations were apparent after only 1 week of HDM exposure and were largely maintained over the ensuing 7 weeks. Furthermore, we provide evidence of a humoral response in HDM-exposed mice, as indicated by elevated serum levels of Th2-affiliated immunoglobulins, total IgE, and HDM-specific IgG1 (Figures 3C and 3D).

To examine the dependence of the immunoinflammatory response on the continuous presence of allergen, we ceased HDM exposure after 5 weeks and assessed immune and inflammatory parameters for an extended period of time. Our data show full resolution of airway eosinophilia in the BALF after 2 weeks, with a return to baseline total cell numbers after 9 weeks of nonexposure (Figures 4A and 4B). Whereas airway inflammation is clearly dependent on continued aeroallergen exposure, systemic markers of Th2 immunity are not. Indeed, even after 9 weeks of nonexposure, splenocytes have an unabated capacity to produce Th2-associated cytokines on HDM recall *in vitro* (Figures 4C and 4D; and Table 2). The maintenance of this memory capacity is manifested in the lung when mice are reexposed *in vivo* to HDM for 3 days, an exposure that, in a nonsensitized animal, would induce a negligible inflammatory response, but instead results in robust airway eosinophilic inflammation (Table 2).

The preceding findings afforded the opportunity to investigate the impact of chronic eosinophilic inflammation on the structure of the airway. In addition to goblet cell hyperplasia (Figure 6), generally observed after shorter exposure protocols, our data illustrate an increased expansion of peribronchial contractile elements, presumably myofibroblasts, as well as collagen

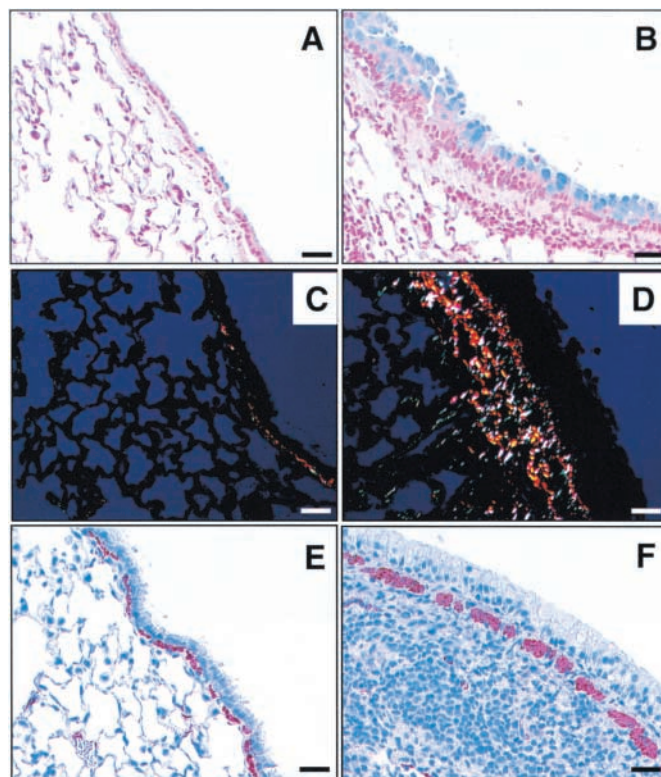


Figure 6. Histologic analysis of lung structural abnormalities in HDM-exposed animals. Alcian blue (pH 2.5) staining of lung histology demonstrates increased mucin production in epithelial goblet cells in HDM-exposed mice (B), compared with naive animals (A). Picro Sirius red staining visualized under polarized light was performed to demonstrate subepithelial accumulation of collagen in naive mice (C) and HDM-exposed mice (D). Immunohistochemistry for anti- α smooth muscle actin in naive mice (E) and HDM-exposed mice (F) indicates increased contractile elements in the airway wall in animals exposed to HDM. Naive animals were not manipulated whereas HDM-exposed animals were administered HDM at 5 days/week for five consecutive weeks. Scale bars: 20 μ m.

deposition in the subepithelial layer. These peribronchial and subepithelial changes, evident qualitatively by histology and immunohistochemistry (Figure 6), have been further quantified by morphometric analysis (Figure 7). Such abnormalities were incipient after 3 weeks of exposure and striking after 5 weeks. Repeated intermittent exposures to OVA in intraperitoneally sensitized mice have been shown to induce some of these structural abnormalities (14, 25, 26). That OVA and HDM are, from a biochemical standpoint, different entities would suggest that the molecular basis underlying these abnormalities is likely to be different. An observation of considerable interest, in our view, is that whereas airway inflammation fully resolves after a period of nonexposure to HDM, the airway remodeling changes do not. Studies involving lengthier exposure to HDM or later observations after the cessation of antigen exposure will be required to further assess the kinetics of the structural changes observed here.

Finally, continuous exposure to HDM has profound functional consequences. We show that animals chronically exposed for 5 weeks to HDM exhibit markedly increased airway hyper-reactivity to methacholine (Figure 8). Importantly, the airways of these mice remain hyperreactive, although less so, after 5 weeks of exposure to HDM followed by a period of nonexposure.

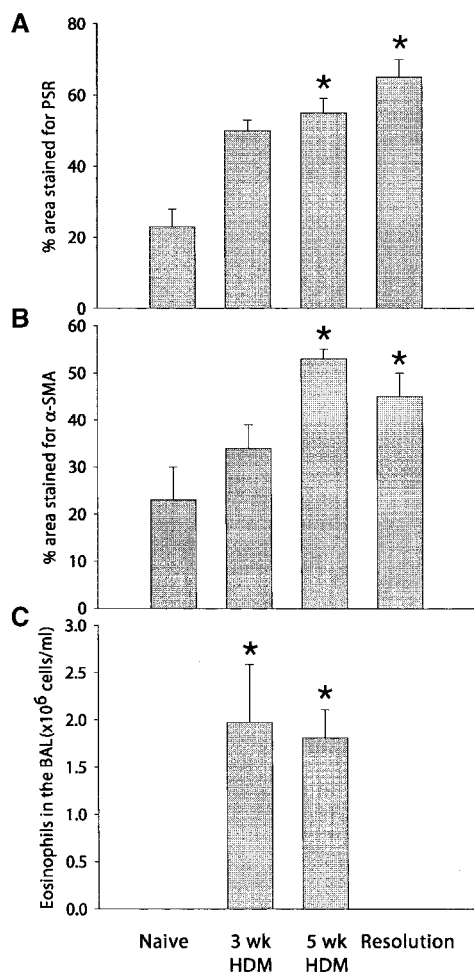


Figure 7. Morphometric analysis of lung histology. Structural changes were assessed in naive mice and in mice exposed to HDM at 5 days/week for 3 or 5 weeks, 9 weeks after the completion of 5 weeks of HDM administration. Data are expressed as a percentage of the area of interest stained with either Picro Sirius red (A) or α -SMA (B) at the indicated time points. For comparison, eosinophil numbers in the BALF are shown for each time point (C). Data represent means $\pm$ SEM ($n = 3-6$), * $p < 0.01$ compared with naive animals. Statistical analysis was performed by one-way ANOVA with the Fisher LSD *post hoc* test.

sure. Notably, airway inflammation is severe after 5 weeks of exposure and completely absent after a period of nonexposure. Presumably, then, whereas the airway hyperreactivity observed after this resolution period largely represents a remodeling-mediated dysfunction, that observed after 5 weeks of HDM exposure encompasses an additional inflammation-mediated component. Detailed further exploration of the evolution of structural and functional abnormalities in this model will likely improve our understanding of the various mechanisms contributing to airway hyperreactivity.

In summary, our data demonstrate that continuous exposure to HDM for up to 7 weeks results in sustained airway inflammation characterized by an accumulation of both Th2 effector cells and eosinophils that is dependent on continued exposure to the aeroallergen. We also show that continuous exposure to HDM results in persistent systemic Th2 immunity that is ultimately independent of aeroallergen exposure, as illustrated by splenocyte cytokine production and serum levels of immunoglobulins. This immunoinflammatory process is associated with profound

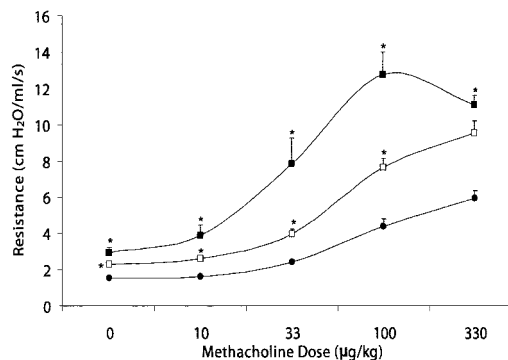


Figure 8. Analysis of airway hyperreactivity to methacholine in naive mice (closed circles) and animals exposed to 5 weeks of HDM (closed squares), and after cessation of antigen exposure (open squares). A dose-response relationship between respiratory resistance and increasing intravenous doses of methacholine is evident. HDM-exposed animals were administered HDM at 5 days/week for five consecutive weeks. Data represent means $\pm$ SEM ($n = 8-10$), * $p < 0.05$ compared with naive animals. Statistical analysis was by one-way ANOVA with the Fisher LSD *post hoc* test.

structural-functional abnormalities as demonstrated by progressive airway remodeling and severe bronchial hyperreactivity. While the airway inflammatory response resolves fully, the remodeling changes do not resolve at all and airway hyperreactivity resolves only partly.

Thus, we have introduced a novel model of chronic eosinophilic airway inflammation and tissue remodeling in response to continuous exposure to house dust extract. Whole mite extract is, from an immunologic perspective, a complex material, and further studies will be required to dissect the role of its various components in the outcomes that we have reported. However, this extract does mimic common environmental aeroallergen exposure, and the use of this material affords us the opportunity to elaborate a complete pathogenic sequence of events, from initiation to resolution. We surmise that the data presented here provide a foundation to explore the biochemical and molecular basis of aeroallergen-induced immunopathology, with the potential to identify novel therapeutic targets and to evaluate prospective new therapies, particularly in reference to tissue remodeling.

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