

Components of airway hyperresponsiveness and their associations with inflammation and remodeling in mice

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Background: Pathologic changes, including inflammation and remodeling, occur in the asthmatic airway. However, their relative contribution to the components of airway hyperresponsiveness (AHR) remains unclear.

Objective: Attempting to delineate AHR into discrete immune-mediated and structural remodeling components, we performed a detailed time course of the development, progression, and persistence of maximal respiratory system resistance, airway reactivity, and airway sensitivity.

Methods: Mice exposed to increasing durations of persistent allergen were assessed for airway function, morphometry, and inflammation.

Results: Allergen exposure resulted in increases for all indices of AHR that persisted for at least 4 weeks after chronic allergen exposure ($P < .01$ for all values). Early increases in AHR were associated with increases in immune-mediated events, including airway eosinophils ($P < .01$), whereas sustained AHR was associated with structural remodeling events. Increased maximal respiratory system resistance, evident by 6 weeks postallergen and persisting for at least 4 weeks after 8 weeks of chronic exposure, was associated with an increase in collagen deposition ($P < .01$). Increased airway reactivity and sensitivity, each evident by 1 week after allergen and persisting for at least 4 weeks after 8 weeks of chronic exposure, were associated with an increase in airway smooth muscle area ($P < .01$).

Conclusion: Our novel observation of distinct temporal relationships in the development, progression, and persistence of the individual indices of AHR supports our hypothesis that multiple underlying factors contribute to airway dysfunction. **Clinical implications:** These findings illustrate the importance of clearly addressing specific components of airway dysfunction to provide greater insight into specific pathophysiologic

mechanisms in airway disease. (J Allergy Clin Immunol 2006;117:1212-1220.)

Key words: Asthma, pathophysiology, bronchial hyperreactivity, fibrosis, smooth muscle, time course

Asthma is a heterogeneous disease characterized by chronic airway inflammation, airway remodeling, and airway hyperresponsiveness (AHR). AHR, the tendency of the airways to exhibit excessive narrowing in response to various stimuli, consists of greater maximal bronchoconstriction, hyperreactivity (increased slope of the bronchoconstrictor dose-response curve), and hypersensitivity (leftward shift of the bronchoconstrictor dose-response curve).

Airway hyperresponsiveness is present in nearly all individuals with asthma and often relates to disease severity.¹ Indeed, clinical manifestations of asthma can be attributed to AHR.² Furthermore, the management of asthma can be substantially improved when treatment is guided by the degree of AHR.³

We have previously suggested that the mechanisms underlying AHR are complex and multifactorial.² Although these mechanisms are incompletely understood, inflammation and structural remodeling of the airway appear to be involved.^{2,4} Indeed, eosinophils, which commonly dominate the airways of patients with asthma,⁵ may play an important role in AHR, although results from animal studies are conflicting on the importance of eosinophils in AHR.^{6,7} Despite AHR being associated with airway inflammation, the observation that profound AHR is sustained in asthma irrespective of prolonged treatment with anti-inflammatory corticosteroids^{3,8} supports our hypothesis that other mechanisms likely account for a major component of AHR. Remodeling, including thickening of the airway wall, subepithelial fibrosis, hyperplasia and hypertrophy of smooth muscle cells, and hyperplasia of myofibroblasts and goblet cells⁹⁻¹¹ may be responsible, at least in part, for sustained AHR.^{4,12,13} Indeed, airway biopsies from patients with asthma suggest that both the degree of smooth muscle thickness and the extent of subepithelial fibrosis relate to disease severity¹⁴ and magnitude of AHR.^{12,15}

The relative contribution of airway inflammation and remodeling to the development, progression, and persistence of AHR is unclear. Although we and others have

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Abbreviations used

AHR: Airway hyperresponsiveness
 BAL: Bronchoalveolar lavage
 EC₅₀: Concentration of MCh resulting in 50% maximal effect
 HDM: House dust mite
 R_{RS}: Respiratory system resistance

observed progressive increases in remodeling indices with chronic ovalbumin exposure,^{16,17} this occurs over the same period as progressive decreases in inflammatory indices, attributed to the development of immunologic tolerance.¹⁸ This finding makes it impossible to attribute changes in airway function clearly to either remodeling or inflammatory processes. However, the finding that chronic exposure to house dust mite (HDM) is not associated with tolerance, but rather results in persistent inflammation, as well as remodeling and sustained dysfunction,¹⁹ provides us with an excellent opportunity to observe discrete pathophysiologic relationships during the course of allergen exposure.

The purpose of the current study was to observe whether there are temporal relationships between specific markers of inflammation or remodeling, and specific components of airway dysfunction. To achieve this, we (1) performed a detailed time course of the development, progression, and persistence of AHR; (2) divided AHR into its fundamental elements of maximal airway constriction, airway reactivity, and airway sensitivity; and (3) compared the time course of these functional indices with those of inflammation and aspects of airway remodeling. We hypothesized that the individual components of AHR would display distinct time courses, and that these would be made up of both early, transient, immune-mediated components and delayed, sustained, remodeling-mediated components.

METHODS

Animals

Female BALB/c mice, age 6 to 12 weeks ($n = 15/\text{group}$ [physiology]; $n = 10/\text{group}$ [bronchoalveolar lavage (BAL); morphometry]; $n = 6/\text{group}$ [ELISA]), were purchased from Charles River Laboratories (Saint-Constant, Quebec, Canada). Mice were housed in environmentally controlled specific pathogen-free conditions for the duration of the study. All procedures were reviewed and approved by the Animal Research Ethics Board at McMaster University (Hamilton, Ontario, Canada).

Allergen challenge

Mice were exposed to HDM *Dermatophagoides pteronyssinus* extract (Greer Laboratories, Lenoir, NC) intranasally (25 μg in 35 μL ; resulting in optimal distribution to the lower respiratory tract²⁰) for 5 days, followed by every other day exposures weekly for as long as 8 weeks (see this article's Fig E1 in the Online Repository at www.jacionline.org). Separate groups of mice were studied 24 hours after 1, 2, 3, 6, and 8 weeks of HDM, and 72 hours, 1, 2, and 4 weeks after 8 weeks of HDM. Control mice received intranasal saline challenges and were studied 24 hours after 1 or 8 weeks of HDM. Data

from control groups were combined because there were no significant differences between groups in any assessed measurement.

Airway responsiveness

Airway responsiveness was measured on the basis of the response of total respiratory system resistance (R_{RS}) to intravenous saline and increasing doses (10, 33, 100, and 330 $\mu\text{g}/\text{kg}$) of methacholine by using the flow interrupter technique described previously.^{16,21,22} Briefly, maximal R_{RS} was calculated by subtracting baseline R_{RS} to intravenous saline from the greatest methacholine-induced R_{RS} . Airway reactivity was calculated as the slope of the line for R_{RS} at 10, 33, and 100 $\mu\text{g}/\text{kg}$ methacholine. Airway sensitivity was calculated as the inflection point (concentration of MCh resulting in 50% maximal effect [EC₅₀]) of the methacholine sigmoidal dose-response curve with the following equation:

$$R_{RS} = \text{Minimum } R_{RS} + (\text{Maximum } R_{RS} - \text{Minimum } R_{RS}) \\ \div \left(1 + 10^{((\text{Log EC}_{50} - \text{Log methacholine Dose}) \times \text{HillSlope})} \right)$$

where HillSlope is the slope at the inflection point of the generated curve.

For data that did not exhibit a sigmoidal shape because of a drop in R_{RS} at high doses of methacholine, the curve was generated by constraining Maximum R_{RS} to the maximum observed R_{RS} in that mouse.

BAL

Bronchoalveolar lavage was performed as described previously (see this article's Online Repository at www.jacionline.org).²¹ Active TGF- β 1 levels in BAL fluid were assessed by ELISA (Quantikine; R&D Systems, Minneapolis, Minn) according to the manufacturer's recommendations.

Lung histology and morphometry

Lungs were dissected, processed, and stained as described previously (see this article's Online Repository at www.jacionline.org).^{16,23} Tissue sections were stained with hematoxylin and eosin, picrosirius red, and periodic acid-Schiff to quantify eosinophils, collagen, and mucin-containing goblet cells, respectively. Immunohistochemistry was performed using an antibody against α -smooth muscle actin (Clone 1A4, Dako Corp, Carpinteria, Calif), and an antibody against phospho-Smad2 (Cell Signaling Technology Inc, Danvers, Mass) to quantify contractile elements and TGF- β receptor-mediated cell signaling events,²⁴ respectively. Morphometric quantification of stained sections was performed as described previously (see this article's Online Repository at www.jacionline.org).^{16,23} Quantification of phospho-Smad2 staining was assessed in a 100- μm band extending beneath the luminal surface of the epithelium.

Statistical analysis

Reported values are expressed as means and SEMs. EC₅₀ data were log-transformed before statistical analysis because this has been shown to produce a normal distribution of airway responsiveness measurements²⁵; these data were therefore presented as geometric mean and %SEM. Data analysis was performed by using an independent Student *t* test because of heteroscedasticity of variances. To minimize the probability of type I errors, α was adjusted below 0.05 by using Bonferroni correction based on the number of relevant comparisons.

RESULTS

Airway inflammation

Chronic exposure to allergen resulted in a prolonged elevation in airway inflammation. Eosinophils in both lung

TABLE I. BAL fluid cellular contents after allergen exposure†

Time-point	Eosinophils	Neutrophils	Lymphocytes
Saline	0.01 (0.00)	0.16 (0.04)	0.01 (0.01)
1 wk HDM	1.29 (0.26)*	0.91 (0.16)*‡	0.26 (0.05)*‡
2 wk HDM	1.33 (0.11)*	0.87 (0.10)*‡	0.30 (0.05)*‡
3 wk HDM	1.95 (0.24)*‡	0.72 (0.14)*‡	0.26 (0.05)*‡
6 wk HDM	2.48 (0.27)*‡	0.78 (0.14)*‡	0.33 (0.06)*‡
8 wk HDM	2.43 (0.29)*‡	0.74 (0.11)*‡	0.31 (0.08)*‡
72 h after	2.86 (0.40)*	0.33 (0.04)*	0.47 (0.12)*
8 wk HDM			
1 wk after	2.79 (0.57)*	0.22 (0.08)	0.20 (0.07)
8 wk HDM			
2 wk after	0.30 (0.04)*	0.03 (0.01)	0.06 (0.02)
8 wk HDM			
4 wk after	0.02 (0.01)	0.14 (0.03)	0.08 (0.02)
8 wk HDM			

*Significantly different from saline ($P < .01$).

†Numbers in each column are means $\times 10^4$; (SEM) in parentheses.

‡Represents a plateau in cell enumeration such that there was no further significant change in cell numbers at subsequent time-points during the HDM exposure period (ie, not including post-HDM data).

tissue and BAL were elevated after 1, 2, 3, 6, or 8 weeks of HDM compared with control ($P < .01$). This increase in eosinophils plateaued by 3 weeks of HDM (Table I; see this article's Fig E2 in the Online Repository at www.jacionline.org). Similarly, BAL neutrophils and mononuclear cells were increased after 1, 2, 3, 6, or 8 weeks of HDM compared with control ($P < .01$ for both values). Neutrophils and mononuclear cells plateaued by 1 week of HDM. Allergen-induced airway inflammation was attenuated by 1 week after 8 weeks of HDM compared with control ($P < .01$ for both values) and resolved toward baseline by 2 weeks after 8 weeks of HDM. By 4 weeks after 8 weeks of HDM, there were no significant increases in inflammatory indices in BAL compared with control. Similarly, a marked decline was observed in tissue eosinophils after 8 weeks of HDM, although tissue eosinophils were still evident 4 weeks after the final HDM exposure (see this article's Fig E2 in the Online Repository at www.jacionline.org).

Airway morphometry

Chronic exposure to allergen resulted in airway remodeling (Fig 1).

Collagen deposition. There was an increase in collagen deposition following 6 and 8 but not 1, 2, or 3 weeks of HDM compared with control ($P < .01$). This increase in collagen persisted for at least 4 weeks after 8 weeks of HDM, with a further increase in collagen deposition 2 weeks after 8 weeks of HDM compared with 24 hours after 8 weeks of HDM ($P = .025$).

Area of smooth muscle. Smooth muscle area was elevated after 1, 2, 3, 6, and 8 weeks of HDM compared with control ($P < .01$). The elevation in smooth muscle area was not dependent on the duration of allergen exposure, because there was no significant difference in smooth muscle area among 1, 2, 3, 6, or 8 weeks HDM. The

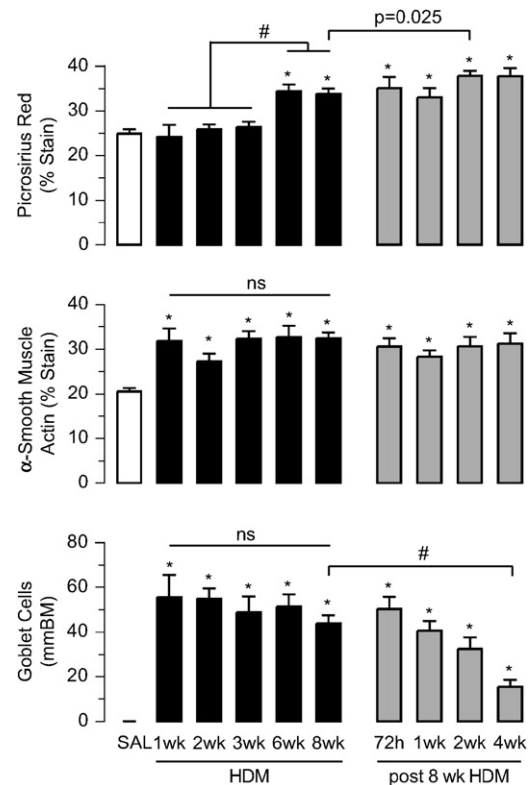


FIG 1. Time course of indices of airway remodeling after HDM exposure (means $\pm$ SEMs), including collagen deposition (% picrosirius red stained area), smooth muscle area (% α -smooth muscle actin stained area), and airway goblet cell numbers. * $P < .01$ compared with saline control; # $P < .0125$; ns, $P > .05$ compared with indicated groups. BM, Basement membrane.

significant increase in smooth muscle area did not appreciably change during the 4 weeks after 8 weeks of HDM.

Goblet cell hyperplasia. Mucin-containing goblet cells were elevated after 1, 2, 3, 6, and 8 weeks of HDM compared with control ($P < .01$). Similarly, the elevation in goblet cells was not dependent on the duration of allergen exposure, because there was no significant increase in the number of goblet cells among 1, 2, 3, 6, and 8 weeks of HDM. There was a progressive decline in the number of goblet cells from 72 hours through 1, 2, and 4 weeks after 8 weeks of HDM. By 4 weeks after 8 weeks of HDM, goblet cells were reduced compared with 24 hours after 8 weeks of HDM ($P < .0125$).

Airway physiology

Chronic exposure to allergen resulted in a progressive increase in airway dysfunction and the development of sustained airway dysfunction (Fig 2).

Maximal respiratory system resistance. Maximal methacholine-induced R_{RS} was increased after 2, 3, 6, and 8 weeks of HDM compared with control ($P < .01$; Fig 2). This increase in maximal R_{RS} reached an initial plateau between 2 and 3 weeks of HDM. Further allergen exposure resulted in an elevation in maximal R_{RS} after 6 or 8 weeks of HDM compared with the initial plateau at 2 and 3

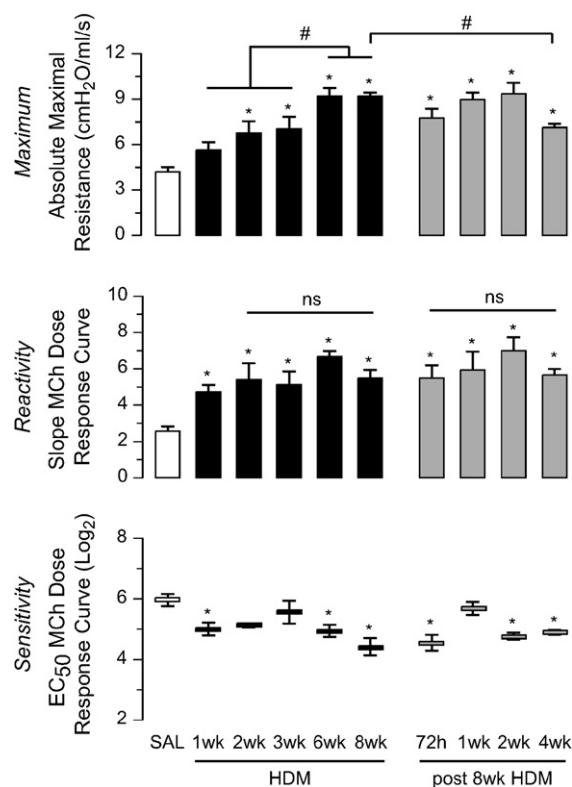


FIG 2. Time course of components of airway dysfunction, including maximal airway resistance, airway reactivity, and airway sensitivity, in response to increasing doses of intravenous methacholine (MCh) after HDM exposure (means $\pm$ SEMs). * $P < .01$ compared with saline (SAL) control; # $P < .0125$; ns, $P > .05$ compared with indicated groups.

weeks of HDM ($P < .0125$). This further increase in maximal R_{RS} subsequently plateaued between 6 and 8 weeks of HDM. The increase was sustained for at least 4 weeks after 8 weeks of HDM compared with control ($P < .01$). However, the sustained maximal R_{RS} was attenuated at 4 weeks compared with 24 hours after HDM, corresponding with a time when airway inflammation was resolved (Table I; see this article's Fig E2 in the Online Repository at www.jacionline.org).

Airway reactivity. Airway reactivity was increased after 1, 2, 3, 6, and 8 weeks of HDM compared with control ($P < .01$; Fig 2). The increase in airway reactivity plateaued by 2 weeks of HDM and persisted for the duration of allergen exposure. This increase in airway reactivity was sustained for at least 4 weeks after 8 weeks of HDM compared with control ($P < .01$).

Airway sensitivity. Airway sensitivity was increased (represented by a decrease in the EC₅₀ of the methacholine dose-response curve) after 1 week of HDM compared with control ($P < .01$; Fig 2). No further increases were observed beyond this initial change. With the unexplained exception of the 3 week HDM and 1 week after 8 weeks of HDM, the change in sensitivity was sustained for at least 4 weeks after 8 weeks of HDM compared with control ($P < .01$).

Baseline R_{RS} . Baseline R_{RS} increased by 6 weeks of HDM compared with control ($P < .01$), and with the

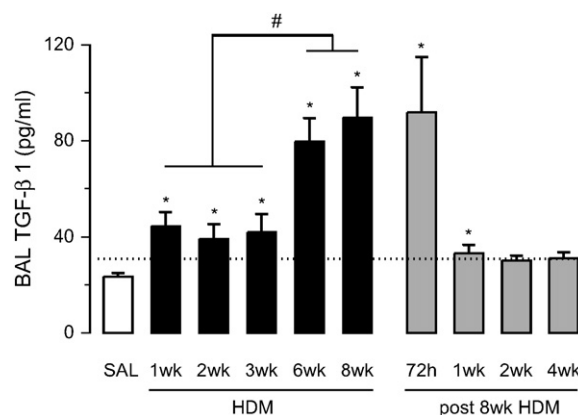


FIG 3. Time course of BAL TGF- β after HDM exposure (means $\pm$ SEMs). * $P < .01$ compared with saline (SAL) control; # $P < .0125$ compared with indicated groups. Dashed line represents lower limit of detection on the basis of the manufacturer's recommendations.

exception of a single measurement (1 week after allergen), persisted for at least 4 weeks beyond the final allergen exposure (see this article's Fig E3 in the Online Repository at www.jacionline.org). In fact, by 4 weeks after 8 weeks of HDM, baseline R_{RS} was further elevated compared with 24 hours after 8 weeks of HDM ($P < .0125$).

TGF- β cytokine and signaling

Chronic exposure to allergen resulted in a transient increase in BAL TGF- β 1 (Fig 3). TGF- β 1 levels were increased after 1, 2, 3, 6, and 8 weeks of HDM compared with control ($P < .01$). The increase in TGF- β 1 reached an initial plateau between 1, 2, and 3 weeks of HDM. Further allergen exposure resulted in an elevation in TGF- β 1 after 6 or 8 weeks of HDM compared to the initial plateau at 1, 2, and 3 weeks of HDM ($P < .0125$). This further increase in TGF- β 1 subsequently plateaued between 6 and 8 weeks of HDM. The increase in TGF- β 1 was transient, returning to baseline by 1 to 2 weeks after 8 weeks of HDM. In addition, chronic exposure to allergen resulted in an increase in TGF- β -mediated signaling events in the airway environment. Lung tissue sections stained for phospho-Smad2, an activated intracellular signaling molecule downstream of the TGF- β receptor, following 8 weeks of HDM, at a time when BAL TGF- β 1 is maximally elevated, were 3-fold higher (15.7% [1.7%] vs 5.6% [0.2%]) than control ($P < .05$; see this article's Fig E4 in the Online Repository at www.jacionline.org).

DISCUSSION

In this study, we extend previous findings made with chronic allergen exposure models^{16,17,19} and for the first time (1) show detailed kinetics of the development, progression, and persistence of AHR; (2) divide AHR into its fundamental elements of maximal airway constriction, airway reactivity, and airway sensitivity; and (3) begin to delineate AHR into associated discrete immune-mediate and structural remodeling components.

Our novel observation of distinct plateaus in the development, progression, and persistence of R_{RS} , airway reactivity, and airway sensitivity (Fig 2) supports and extends our overall hypothesis that multiple underlying factors, including immune-mediated (Table I; Fig 3; see this article's Fig E2 in the Online Repository at www.jacionline.org) and structural remodeling events (Fig 1), with distinct kinetics of expression, contribute to AHR. Our findings have afforded us greater insight into possible underlying mechanisms than has previously been available.

The first insight afforded by the delineation of AHR into discrete components is that the development of increased maximal R_{RS} is associated with airway inflammation, dominated by an eosinophilia, an increase in smooth muscle area, and goblet cell hyperplasia. Previously, we have observed the resolution of transient AHR in mice, 2 weeks after brief allergen exposure, at a time corresponding to the resolution of tissue eosinophils and BAL IL-13, despite persistent increased smooth muscle area and epithelial mucin content.¹⁶ Furthermore, we have reported that transient AHR can be inhibited by anti-immune interventions,^{16,26-28} suggesting that the early development of methacholine-induced maximal R_{RS} is likely a result of immune-mediated events mediated, at least in part, by airway eosinophils, as opposed to airway structural remodeling.

The progression of maximal R_{RS} exhibits a distinct increase by 6 weeks of chronic allergen exposure. Of the outcomes we measured, this increase in maximal R_{RS} is primarily associated with a discrete increase in picrosirius red stain, suggesting a principal role of collagen deposition in this progression of airway dysfunction. These findings are supported by Laprise et al,⁴ who observed a significant correlation between the extent of subepithelial fibrosis and AHR, and proposed that increased collagen deposition may potentiate airway narrowing. Clearly, increased extracellular matrix may decrease fluctuations in airway size during tidal breathing, which would influence the ability of airway smooth muscle to develop tension²⁹ and has been related to increased airway responsiveness in human beings.³⁰ Interestingly, others have suggested that matrix deposition may protect against airway narrowing.³¹ Although protective mechanisms may exist, our findings suggest that in mice, the overall effect of increased extracellular matrix is an increase in airway dysfunction. The observed increase in maximal R_{RS} may also be attributed, in part, to additional factors including changes in airway tethering to surrounding parenchyma; however, such outcomes were not assessed in this study.

The persistence of maximal R_{RS} lasts for at least 4 weeks after chronic allergen exposure. The temporal association of this change with the progressive increase in picrosirius red stain further supports a role for collagen deposition in airway dysfunction. Despite the increase in collagen during the 4-week period after chronic allergen exposure, there is an attenuation in maximal R_{RS} . On first impression, these findings may appear inconsistent with the hypothesis that collagen deposition plays a central role in maximal R_{RS} . However, this attenuation in maximal R_{RS} is also associated with the resolution of airway

inflammation and an attenuation in mucin-containing goblet cells, both of which likely play important but transient roles in airway dysfunction, as discussed previously. Thus, during and after chronic allergen exposure, we propose that maximal R_{RS} is composed of both a transient immune-mediated component and a sustained remodeling component. The near complete resolution of airway inflammation and attenuation of early remodeling events, such as goblet cell hyperplasia, corresponds to the resolution of transient maximal R_{RS} , whereas persistent remodeling events, such as collagen deposition, are associated with the underlying sustained maximal R_{RS} . These findings further strengthen the hypothesis that sustained AHR is the consequence of persistent airway remodeling, rather than ongoing cellular inflammation. Clearly, this is a complex event, and precisely ascribing airway dysfunction into discrete underlying causes, including specific indices of airway remodeling and residual airway inflammation, may not be entirely possible.

The early development of airway reactivity and sensitivity, like maximal R_{RS} , is related to immune-mediated events, dominated by eosinophils, as well as early remodeling events, including increases in smooth muscle area and goblet cell hyperplasia. However, unlike maximal R_{RS} , the progression of airway reactivity and sensitivity appears unchanged during allergen exposure. Therefore, airway reactivity and sensitivity do not appear to be associated with collagen deposition, because there was no increase in either measure of dysfunction corresponding with the prominent increase in collagen deposition by 6 weeks of allergen exposure. Furthermore, the persistence of airway reactivity and sensitivity appears to be independent of goblet cell hyperplasia, because this dysfunction persists despite the progressive resolution of goblet cells after chronic allergen exposure. Thus, it is tempting to attribute changes in airway reactivity and sensitivity to airway smooth muscle. This association is supported by Cox et al,³² who reported a significant improvement in airway responsiveness in patients with asthma that persisted for at least 2 years after the reduction of airway smooth muscle mass by bronchial thermoplasty. With increasing access to muscle samples from human biopsy, direct correlations between airway smooth muscle characteristics and AHR will allow greater insight into the association between airway smooth muscle and airway reactivity and sensitivity.

Interestingly, this is the first time we have reported changes in baseline resistance after chronic allergen exposure. These findings are consistent with previous reports of a significant association between the severity of AHR and a low prechallenge FEV₁ % predicted in patients with asthma and chronic obstructive pulmonary disease.³³ The persistence and progressive increase in baseline resistance beyond the near complete resolution of allergic inflammation support the hypothesis that structural remodeling events play a prominent role in airway dysfunction. Indeed, the profile of changes in baseline resistance is strikingly similar to those of collagen deposition (Fig 1; see this article's Fig E3 in the Online Repository at www.jacionline.org). Thus, it is tempting to draw a causal

relationship between increased baseline resistance and collagen. However, whether an increase in collagen deposition or other factors, including residual inflammation, is directly responsible for an increase in baseline resistance remains to be determined. It is well known that baseline airway function in patients with asthma will influence measures of airway responsiveness.³³ This is thought to be related to the influence of initial airway caliber on the degree of narrowing in response to agonist-induced constriction. Thus, it appears likely that in our model, part of the explanation for the sustained airway hyperreactivity may be found in the reduced baseline caliber, suggesting a common underlying mechanism. The increase in collagen deposition appeared to have a functional relevance given its association with maximal R_{RS} and baseline resistance.

TGF- β is a potent profibrotic mediator that is increased in the asthmatic airway and is implicated in airway remodeling.^{34,35} Therefore, we decided to explore a possible temporal association between TGF- β and the observed changes in collagen deposition. Interestingly, the increase in collagen deposition by 6 weeks corresponded to a discrete increase in BAL TGF- β 1 (Fig 3) and TGF- β 1-mediated intracellular signaling events (see this article's Fig E4 in the Online Repository at www.jacionline.org). These findings are supported by Kumar et al,³⁶ who similarly reported a relationship between TGF- β 1 accumulation and airway collagen deposition in an animal model of chronic allergen exposure. Additionally, Boulet et al¹⁵ have reported a positive correlation between PC₂₀ and the degree of subepithelial fibrosis. We therefore hypothesize that TGF- β 1 plays a critical role in airway remodeling and dysfunction. Indeed, McMillan et al³⁷ have reported that the inhibition of TGF- β in an animal model of chronic allergen exposure resulted in the reduction of allergen-induced fibrotic remodeling events. However, whether TGF- β inhibition prevents or reverses airway dysfunction has not yet been reported to our knowledge.

In addition, we have previously suggested a causal relationship between contractile tissue area and maximal bronchoconstriction.¹⁶ This is in agreement with mathematical modeling suggesting the contractile cell mass is the major determinant of airway narrowing in asthma.³⁸ However, our current findings that contractile tissue area increases early in response to allergen exposure and does not increase further with chronic allergen exposure despite significant increases in maximal bronchoconstriction are inconsistent with this hypothesis. This discrepancy may be a result of the magnitude of changes in smooth muscle mass, or the fact that assumptions on airway geometry used in mathematical modeling are not valid in this *in vivo* model of allergen-induced sustained AHR. Therefore, our findings are consistent with the hypothesis that factors other than the amount of contractile tissue appear to influence the degree of maximal airway narrowing.¹⁶ In this respect, we can not discount a critical role for changes in smooth muscle phenotype or function in the pathophysiology of AHR. Airway smooth muscle cells are phenotypically heterogeneous and exhibit functional plasticity.^{39,40} It has been suggested that airway

smooth muscle cell phenotype and function may be dependent on extracellular matrix.^{41,42} Whether phenotypic and functional changes in airway smooth muscle occur in our model that ultimately contribute to greater airway dysfunction is currently under investigation.

In this study, we used the flow interrupter technique for assessing total respiratory system resistance, which includes the contribution of the airways (of all sizes), tissue, and chest wall. Although we are unable to conclude definitively which of these components are the major contributors to the components of airway dysfunction measured here, evidence suggests that the peripheral airways and tissue are the major contributors to R_{RS} .⁴³ However, whether these regions uniquely contribute to the components of airway dysfunction is unknown.

Our observation that the distinct components of airway dysfunction have different time courses and pathological associations is attractive, given that these components of airway dysfunction may have clinical implications in the diagnosis and therapy of asthma and chronic obstructive pulmonary disease.⁴⁴ These findings illustrate the importance for research to address components of airway dysfunction clearly rather than use single markers of AHR. Indeed, measures of airway function such as maximal R_{RS} , airway reactivity, and airway sensitivity, as opposed to nonspecific dimensionless parameters with arguable validity,⁴⁵ may well help in preventing conflict and confusion and enable the elucidation of specific pathophysiologic mechanisms. In this study, we have begun to uncover relevant associations between inflammatory and structural events with specific changes in airway function. However, as with any model system, it is important to recognize the strengths, weaknesses, and limitations of the model. Thus, while we have begun to explore factors underlying sensitivity, reactivity, and maximum effect in our model, the observed changes in sensitivity are in fact quite small compared with the contrast between subjects with and without asthma. We propose that further understanding of relevant pathophysiological relationships will come with selective, individually targeted interventions, applied to mouse models of chronic allergen exposure. However, work is still needed in bridging the gap between model systems and human disease to improve on current unselective therapeutic approaches to disease management.

In summary, we have shown for the first time the development, progression, and persistence of the components of AHR. The development of airway dysfunction appears to be associated with immune-mediated events dominated by eosinophils. The progression and persistence of maximal R_{RS} appear to be associated with a delayed, progressive increase in collagen deposition, whereas airway reactivity and sensitivity appear to be associated with an early, sustained increase in airway smooth muscle area. Although we have begun to delineate AHR into associated discrete immune-mediated and structural remodeling components, the definitive mechanisms underlying the development, progression, and persistence of AHR remain unclear. However, the findings from this study are consistent with our underlying hypothesis that

AHR is a complex and multifactorial pathology composed of discrete processes that present as an early immune-mediated transient AHR followed by a persistent structural remodeling-mediated sustained AHR. In conclusion, the findings from this study illustrate the importance of research to clearly address specific components of airway dysfunction, to enable the elucidation of specific pathophysiologic mechanisms in airway diseases and ultimately the development of selective therapeutic interventions for improved disease management.

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