ORIGINAL ARTICLE

Effect of Bronchoconstriction on Airway Remodeling in Asthma

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ABSTRACT

BACKGROUND

Asthma is characterized pathologically by structural changes in the airway, termed airway remodeling. These changes are associated with worse long-term clinical outcomes and have been attributed to eosinophilic inflammation. In vitro studies indicate, however, that the compressive mechanical forces that arise during bronchoconstriction may induce remodeling independently of inflammation. We evaluated the influence of repeated experimentally induced bronchoconstriction on airway structural changes in patients with asthma.

METHODS

We randomly assigned 48 subjects with asthma to one of four inhalation challenge protocols involving a series of three challenges with one type of inhaled agent presented at 48-hour intervals. The two active challenges were with either a dust-mite allergen (which causes bronchoconstriction and eosinophilic inflammation) or methacholine (which causes bronchoconstriction without eosinophilic inflammation); the two control challenges (neither of which causes bronchoconstriction) were either saline alone or albuterol followed by methacholine (to control for nonbronchoconstrictor effects of methacholine). Bronchial-biopsy specimens were obtained before and 4 days after completion of the challenges.

RESULTS

Allergen and methacholine immediately induced similar levels of bronchoconstriction. Eosinophilic inflammation of the airways increased only in the allergen group, whereas both the allergen and the methacholine groups had significant airway remodeling not seen in the two control groups. Subepithelial collagen-band thickness increased by a median of 2.17 μm in the allergen group (interquartile range [IQR], 0.70 to 3.67) and 1.94 μm in the methacholine group (IQR, 0.37 to 3.24) ($P<0.001$ for the comparison of the two challenge groups with the two control groups); periodic acid–Schiff staining of epithelium (mucus glands) also increased, by a median of 2.17 percentage points in the allergen group (IQR, 1.03 to 4.77) and 2.13 percentage points in the methacholine group (IQR, 1.14 to 7.96) ($P=0.003$ for the comparison with controls). There were no significant differences between the allergen and methacholine groups.

CONCLUSIONS

Bronchoconstriction without additional inflammation induces airway remodeling in patients with asthma. These findings have potential implications for management.

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Asthma is a common chronic respiratory condition characterized clinically by an excessive tendency toward reversible airway narrowing. This may arise in response to everyday environmental exposure and is worsened both by intercurrent infection and, in sensitized persons, by allergen exposure. In pathological terms, asthma is characterized by airway inflammation and by structural changes in airway tissues, such as epithelial goblet-cell hyperplasia, subepithelial collagen deposition, and smooth-muscle hypertrophy — collectively referred to as airway remodeling.[1-3] Since an inhaled-allergen challenge in atopic asthma induces eosinophilic inflammation of the airway and changes in the extracellular matrix,[4] and since a reduction in airway eosinophils has been reported to reduce certain markers of airway remodeling,[5] such structural changes in the tissues have been considered a consequence of eosinophilic airway inflammation.[6] This paradigm, however, fails to account for the potential contribution of airway narrowing to airway remodeling. Bronchoconstriction generates excessive mechanical forces within the airways that distort tissue cells,[7,8] and mechanical forces within other organs are known to induce tissue remodeling.[9-11] In vitro studies in a variety of models have shown that ex vivo compression of the airway epithelium results in changes that mimic those identified and associated with remodeling in vivo.[12-15] We therefore hypothesized that the airway narrowing induced by allergen exposure in vivo in patients with asthma may in itself be a sufficient stimulus for the development of airway remodeling and that such remodeling is not solely dependent on induced recruitment of airway eosinophils.

To test this hypothesis, we performed repeated challenges with exposure to either allergen (to induce bronchoconstriction with airway eosinophil recruitment) or methacholine (to induce bronchoconstriction alone) in volunteers who had mild atopic asthma. Two additional groups of volunteers with asthma served as controls, undergoing repeated challenges with either saline placebo (to control for the challenge procedures) or methacholine after they had received albuterol to prevent bronchoconstriction (to control for any additional nonbronchodilator, receptor-mediated actions of methacholine within the airways). The effect of these challenges on the airway was evaluated by assessing changes in markers of airway remodeling in endobronchial tissue obtained by fiberoptic bronchoscopy before and after the challenge.

Methods

Study Subjects

We recruited adults with asthma who met the following criteria: a positive skin-prick test for allergen extract from the house dust mite *Dermatophagoides pteronyssinus*, abnormal airway reactivity (defined by a provocative concentration of methacholine required to reduce the forced expiratory volume in 1 second [FEV_1] by 20% [PC_{20}] of less than 8 mg per milliliter), no history of smoking, and treatment with a short-acting beta-agonist only as required. The study was approved by the local research ethics committee, and all subjects gave written informed consent.

Protocol and Clinical Measurements

The study timelines are shown in Figure 1. Initial assessments included spirometry, skin-prick testing, and measurement of bronchial reactivity, followed after a minimum of 14 days by bronchoscopy. The subjects were then assigned in equal numbers to one of four challenge groups — allergen, methacholine, saline, or methacholine preceded by albuterol — according to permuted-block randomization in a parallel-group study design. At least 14 days after the initial bronchoscopic examination, subjects underwent three consecutive inhalation challenges at 48-hour intervals, followed by a second bronchoscopic examination 4 days after the final challenge. Subjects recorded symptom scores daily in a validated asthma-control diary[16] for the week preceding and the week of the repeated challenges.

The challenges with allergen and methacholine were adjusted to cause an immediate reduction in FEV_1 of at least 15%. The allergen challenges were performed with the use of an APS Pro nebulizer (Jaeger), as previously described,[17] and the methacholine and saline challenges were performed as recommended.[18] The albuterol–methacholine challenge initially involved inhaling nebulized albuterol (5 mg) followed by double the concentration of methacholine that had been determined at the screening visit to induce a 20% fall in FEV_1. On each challenge day, FEV_1 was measured at 20, 30, 45, and 60 minutes and thereafter at 30-minute intervals until 10 hours after completion of the challenge procedures. In order to accurately assess

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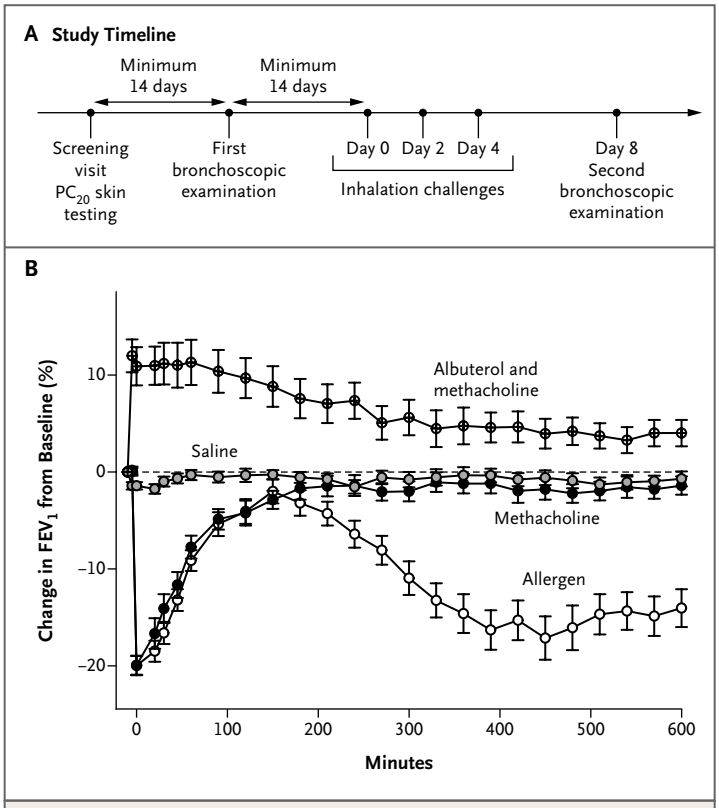

Figure 1. Study Timeline and Changes in Forced Expiratory Volume in 1 Second (FEV_1) after Inhalation Challenges in Subjects with Asthma.

Panel A shows the study timeline. Baseline screening, which included spirometry, allergen skin-prick testing, and measurement of bronchial reactivity to an initial methacholine challenge (PC_{20}), was followed by a washout period of at least 14 days, the first bronchoscopic examination, and another minimum 14-day washout period. The four groups of subjects with asthma (12 in each group) who had been randomly assigned to allergen, methacholine, saline, or albuterol followed by methacholine then underwent three inhalation challenges on days 0, 2, and 4. The final bronchoscopic examination took place on day 8. Panel B shows the mean (±SE) changes in FEV_1 after the repeated challenges in the four groups.

the natural history of the airway response to the challenges over this time period, no bronchodilator medication was given.

Spirometry, skin-prick testing, and fiberoptic bronchoscopy were performed as standard assessments and in accordance with established guidelines.[19] All bronchoscopic examinations were performed without complications. The study was performed in compliance with the protocol, which is available with the full text of this article at NEJM.org. (Additional methodologic details are provided in the Supplementary Appendix at NEJM.org.)

ANALYSIS OF BRONCHOSCOPIC SAMPLES

Bronchoalveolar-lavage fluid was processed to obtain cytospin preparations for differential cell counts and supernatant samples. Biopsy specimens were processed for histochemical staining, as previously described.[20] Two sections (2-μm thick), spatially separated by at least 30 μm to avoid repeat analysis of the same cell, from each randomly oriented biopsy specimen were stained and analyzed by two observers, each unaware of the subject's exposure group. Mean data were used for statistical analysis, and all staining was quantified by computerized-image analysis, as previously described.[21]

EOSINOPHILIC SPECIFICITY OF CHALLENGES

Without knowledge of the challenge medium, we counted 400 cells from each bronchoalveolar-lavage fluid sample on coded cytospin slides to determine the percentage of eosinophils on differential cell count. We measured eosinophil cationic protein concentrations in the fluid supernatant, using a commercial enzyme-linked immunosorbent assay kit (MBL International), according to the manufacturer's instructions. Biopsy specimens were stained for eosinophil cationic protein (EG2, Diagnostics Development) to enumerate eosinophils (cells per square millimeter) within the airway mucosa, as previously described.[20]

EPITHELIAL REPAIR AND AIRWAY REMODELING

Biopsy specimens were stained immunohistochemically with the use of a transforming growth factor β (TGF-β) polyclonal antibody (ab50716, Abcam) and monoclonal antibodies against collagen type III (IE7-D7, Chemicon) and Ki67 (MIB1, Dako). Periodic acid–Schiff (PAS) staining was used to detect goblet cells.[22] The percentage of epithelial expression of TGF-β and PAS staining and the thickness of the lamina reticularis delineated by collagen type III immunoreactivity were measured in or under all sections of intact, longitudinally oriented epithelium. Nucleated cells staining for Ki67 were counted within the epithelium and the lamina propria as separate compartments (expressed as cells per millimeter for epithelium and cells per square millimeter for lamina propria).

STATISTICAL ANALYSIS

Characteristics of the subjects and spirometric results were summarized with the use of descriptive statistics, and between-group differences were assessed by means of one-way analysis of variance. Methacholine provocative concentrations, reported as geometric means and ranges, were analyzed with the use of the Kruskal–Wallis test. All other

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data, which were nonparametrically distributed and are expressed as median values with interquartile ranges, were analyzed with the use of the Kruskal–Wallis test for between-group comparisons, as well as the Mann–Whitney test for comparisons between pairs of groups when appropriate. Paired testing of data obtained within groups before and after challenge was performed with the use of the Wilcoxon test.

No corrections for multiple comparisons were made with respect to the four tissue-remodeling outcome measures, although Bonferroni's correction was applied to the post hoc analysis of the between-group or within-group comparisons to allow for the number of comparisons performed (six comparisons for each variable).[23] A P value less than or equal to 0.05 was considered to indicate statistical significance. All tests were two-tailed.

RESULTS

CHARACTERISTICS OF SUBJECTS

Of the 84 volunteers screened, 52 were eligible for enrollment; 48 of the 52 subjects were then randomly assigned to the four challenge groups (12 in each group) and completed the study. Baseline lung function, atopic status, and the percentage of eosinophils and eosinophil cationic protein concentrations in bronchoalveolar-lavage fluid did not differ significantly among the four groups (Table 1).

LUNG FUNCTION AND SYMPTOM SCORES

In the groups challenged with allergen or methacholine, there were consistent and similar immediate mean (±SE) reductions in FEV_1 of 21.2±7.6% and 22.4±7.4%, respectively. Saline challenge was not associated with significant changes in FEV_1, whereas albuterol pretreatment before methacholine challenge significantly increased baseline FEV_1 ($P<0.001$) and prevented the acute bronchoconstrictor response to the methacholine challenge (Fig. 1). There was no significant difference between the immediate changes in FEV_1 after the allergen and methacholine challenges ($P=0.42$), and results in both these groups differed significantly from those in the saline and albuterol–methacholine challenge control groups (Table 2). Allergen but not methacholine challenge was associated with a significant late allergic response ($P<0.001$) (Fig. 1). There were no significant between-group differences in symptom scores during the 2-week study (Table 1 in the Supplementary Appendix).

AIRWAY EOSINOPHIL RECRUITMENT

Four days after the last challenge, there were increases in the percentage of eosinophils and in eosinophil cationic protein in the samples of bronchoalveolar-lavage fluid from the allergen group but not in the samples from the methacholine, saline, and albuterol–methacholine groups, with significant differences between the allergen-challenge group and the other three groups ($P=0.004$ and $P=0.001$, respectively), as shown in Table 2 and Figure 2. Within the allergen-challenge group, the increases in these two variables after the challenge were significant ($P=0.04$ for eosinophils and $P=0.008$ for eosinophil cationic protein). The median number of endobronchial mucosal eosinophils also increased after the allergen challenge, from 3.25 cells per square millimeter (range, 0.63 to 6.63) to 11.00 cells per square millimeter (range, 1.38 to 14.38). No significant changes were evident in the other groups with respect to tissue eosinophils. There were no significant differences between groups in any other bronchoalveolar-lavage fluid or tissue cell types examined (Tables 2 and 3 in the Supplementary Appendix).

EPITHELIAL REPAIR AND STRUCTURAL REMODELING

Before the challenges, there were no significant differences among the four groups with respect to any of the histochemical measurements obtained (Table 1). After the repeated challenges, there was increased epithelial immunoexpression of TGF-β in the allergen and methacholine groups but not in the saline or albuterol–methacholine groups, with a significant difference between the challenge groups ($P=0.01$) (Table 2). Within the allergen and methacholine groups, the increases after challenge were significant ($P=0.04$ and 0.01, respectively), but there was no significant difference between the changes in these two groups ($P=0.6$) (Fig. 2). Epithelial Ki67 immunoexpression was increased after the challenges, with a significant difference between the challenge groups and the control groups ($P=0.001$). The within-group increases after repeated allergen and methacholine challenges were significant ($P=0.04$ for each comparison); the increases did not differ significantly between these challenge groups. No significant challenge-induced changes were evident with the saline or albuterol–methacholine challenge. The change in the percentage of epithelium that was positive for PAS staining differed significantly between groups after the challenges ($P=0.003$). The

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Table 1. Baseline Characteristics of the Subjects.*

Characteristic	Study Group				P Value†
	Allergen (N=12)	Methacholine (N=12)	Saline (N=12)	Albuterol–Methacholine (N=12)	
Sex (no.)					0.69
Male	3	4	4	2	
Female	9	8	8	10	
Age (yr)	23±3	25±10	21±4	21±4	0.26
FEV_1 (% of predicted value)	89±13	94±16.3	93±14	89±14	0.77
FVC (% of predicted value)	101±10	106±17	105±12	108±11	0.52
PC_{20} for methacholine (mg/ml)					0.64
Geometric mean	0.9	1.0	1.4	1.5	
Range	0.07–6.7	0.06–5.3	0.12–7.9	0.17–5.9	
Wheal diameter on allergen skin-prick test (mm)‡	7.3±1.5	6.5±3.3	7.4±3.6	7.4±2.4	0.85
Eosinophils in BAL (% of differential count)					0.50
Median	1.3	1.3	0.8	1.8	
Interquartile range	1.0–5.8	0.8–2.3	0.3–1.9	0.3–6.0	
Eosinophil cationic protein in BAL (ng/ml)					0.20
Median	1.5	0.8	0.4	2.1	
Interquartile range	0.5–4.5	0.3–3.7	0.1–3.2	1.3–3.9	
Epithelial immunostaining for TGF-β (%)					0.76
Median	0.98	2.00	1.74	1.80	
Interquartile range	0.90–3.25	1.13–3.26	0.98–2.48	0.67–2.32	
Ki67-positive cells in epithelium (no. of cells/mm of epithelial length)					0.09
Median	1.97	0.83	2.32	3.76	
Interquartile range	0.25–4.85	0.00–3.21	0.34–5.91	2.55–8.38	
Epithelium positive for PAS staining (%)					0.15
Median	2.60	1.75	5.08	3.72	
Interquartile range	0.33–5.10	0.97–2.96	2.24–7.67	0.66–5.44	
Collagen-band thickness (μm)					0.14
Median	7.47	7.96	7.48	10.76	
Interquartile range	7.01–8.81	6.59–9.28	6.12–9.29	7.78–13.51	
Ki67–positive cells in submucosal tissue (no. of cells/mm^2)					0.32
Median	7.10	3.18	6.19	5.80	
Interquartile range	2.58–12.53	1.71–7.01	3.12–11.00	1.81–21.61	

* Plus–minus values are means ±SE. BAL denotes bronchoalveolar-lavage fluid, FEV_1 forced expiratory volume in 1 second, FVC forced vital capacity, PAS periodic acid–Schiff, PC_{20} provocative concentration of methacholine required to induce a 20% reduction in FEV_1, and TGF-β transforming growth factor β.

† P values are for the comparison of the active-challenge groups with the control groups and were calculated with the use of one-way analysis of variance for normally distributed clinical characteristics, Fisher's exact test for differences in sex proportions, and the Kruskal–Wallis test for all other variables.

‡ The allergen was the house dust mite *Dermatophagoides pteronyssinus*.

allergen-induced and methacholine-induced changes were significantly greater than the changes induced by saline (P=0.04 and P=0.01, respectively). The photomicrographs in Figure 3 are representative examples of these changes.

After repeated challenges, the thickness of the sub–basement membrane collagen within the submucosa was increased in the allergen group (P=0.04) and in the methacholine group (P=0.02), with a significant difference between the chal-

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Table 2. Changes in Clinical, Bronchoalveolar-Lavage Fluid, and Immunohistochemical Variables after Repeated Inhalation Challenges with Allergen, Methacholine, Saline, or Albuterol–Methacholine.*

Variable	Study Group				P Value†
	Allergen (N=12)	Methacholine (N=12)	Saline (N=12)	Albuterol–Methacholine (N=12)	
Maximal change in FEV_1 from baseline during early asthmatic reaction (%)‡	−21.2±7.6	−22.4±7.4	−4.0±2.7	8.3±11.3	<0.001
Change in eosinophils in BAL (percentage points)					0.004
Median	3.1	−0.3	−0.3	−0.3	
Interquartile range	1.1 to 10.1	−1.1 to 0.5	−1.4 to 0.2	−1.4 to 0.2	
Change in eosinophil cationic protein in BAL (ng/ml)					0.001
Median	9.1	0.5	−0.2	1.5	
Interquartile range	3.4 to 31.5	−0.3 to 3.1	−1.5 to 0.6	−1.4 to 3.6	
Change in epithelial immunostaining for TGF-β (percentage points)					0.01
Median	0.85	2.15	0.42	0.04	
Interquartile range	0.25 to 1.85	0.69 to 4.62	−1.10 to 1.58	−0.63 to 0.75	
Change in Ki67-positive cells (no. of cells/mm of epithelial length)					0.001
Median	17.53	6.27	0.00	−0.18	
Interquartile range	2.31 to 24.73	2.01 to 12.31	−4.59 to 1.70	−2.18 to 1.78	
Change in positive epithelial PAS staining (percentage points)					0.003
Median	2.17	2.13	−1.82	−0.18	
Interquartile range	1.03 to 4.77	1.14 to 7.96	−4.48 to 0.04	−3.08 to 0.97	
Change in collagen-band thickness (μm)					<0.001
Median	2.17	1.94	−0.77	0.20	
Interquartile range	0.70 to 3.67	0.37 to 3.24	−1.23 to 0.17	−0.86 to 0.87	
Change in Ki67-positive cells in submucosal tissue (no. of cells/mm^2)					<0.001
Median	15.58	11.88	0.35	−0.48	
Interquartile range	8.94 to 29.36	4.27 to 18.81	−6.50 to 2.80	−5.85 to 5.76	

* Plus–minus values are means ±SE. BAL denotes bronchoalveolar-lavage fluid, FEV_1 forced expiratory volume in 1 second, PAS periodic acid–Schiff, and TGF-β transforming growth factor β.
† P values are for the comparison of the active-challenge groups with the control groups and were calculated with the use of one-way analysis of variance for the change in FEV_1 and with the use of the Kruskal–Wallis test for all other variables.
‡ The early asthmatic reaction occurs from 0 to 120 minutes after the inhalation challenge.

lenge groups and the control groups (P<0.001). No significant changes were evident in the saline or albuterol–methacholine group (Fig. 2 and Table 2). There was no significant difference in the increase in thickness between the allergen and methacholine groups (P=0.76). Submucosal changes in Ki67 immunoreactivity were also evident after the challenges, with a significant difference between the challenge groups and the control groups (P<0.001). Individual data for the study participants are shown for each variable in Figures 1 through 6 in the Supplementary Appendix.

DISCUSSION

This study shows that repeated bronchoconstriction in asthma promotes airway remodeling. The changes were evident 4 days after repeated airway challenges and were independent of the stimulus causing the bronchoconstriction. Furthermore, they appear to be independent of eosinophil recruitment into the airways, since, on the basis of the specific markers we chose, the remodeling changes evident after the allergen challenge (which induced airway eosinophil recruitment) were sim-

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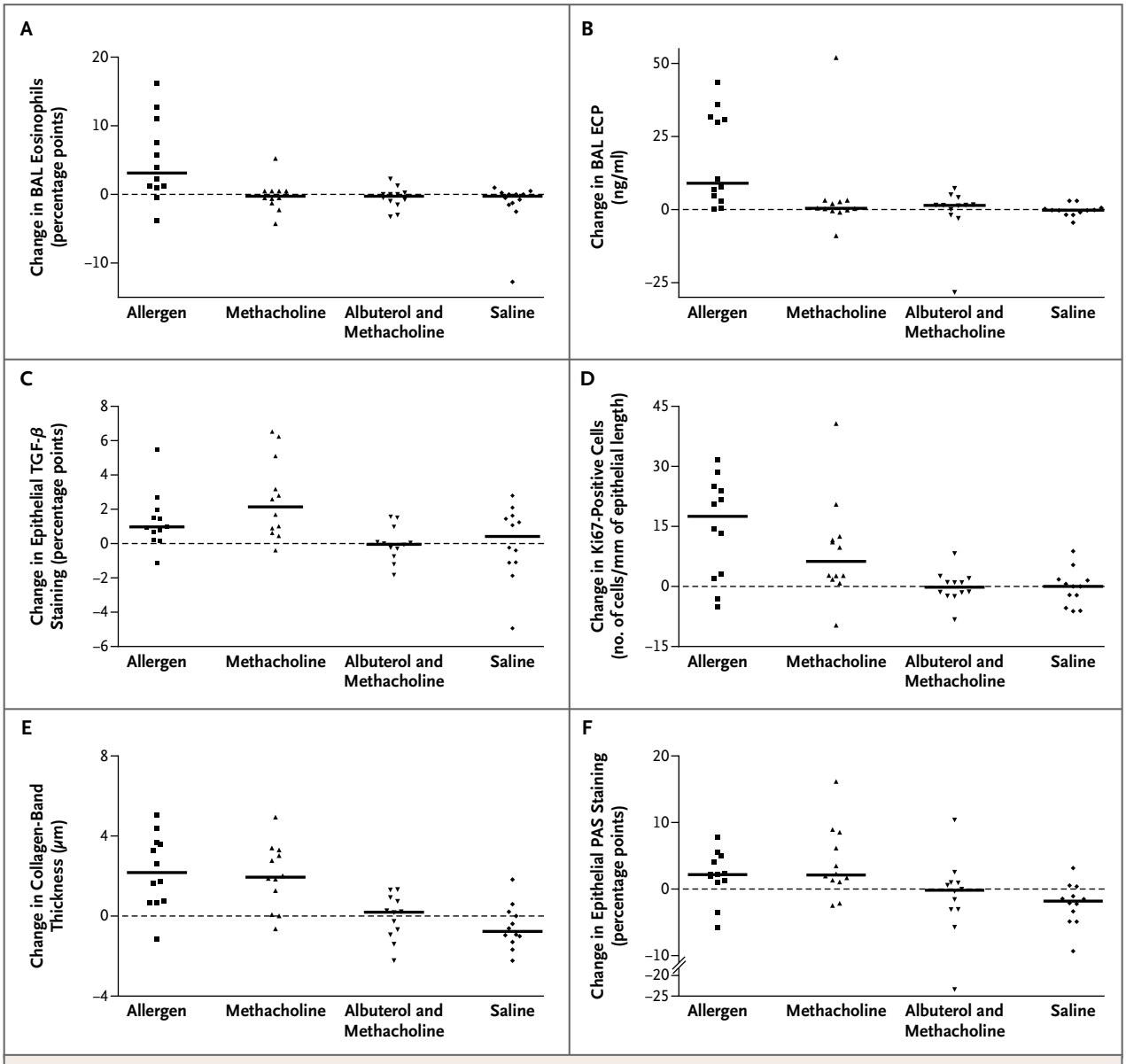

Figure 2. Changes in Markers of Eosinophilic Inflammation and Airway Remodeling after Repeated Inhalation Challenges.

All markers were measured before and after repeated challenges with dust-mite allergen (*Dermatophagoides pteronyssinus*), methacholine, albuterol followed by methacholine, or saline. The horizontal bars represent median values for the 12 subjects in each of the four groups. Panel A shows changes in eosinophils as a percentage of the differential cell count in samples of bronchoalveolar-lavage fluid (BAL). Panel B shows changes in the eosinophil cationic protein (ECP) concentration in BAL. Panel C shows changes in epithelial immunoexpression of transforming growth factor β (TGF-β) as a percentage of the total epithelial area. Panel D shows changes in the number of Ki67-positive cells per millimeter of epithelial length. Panel E shows changes in the thickness of the endobronchial sub-basement membrane collagen layer. Panel F shows changes in the percentage of the total epithelial area that was positive for periodic acid–Schiff (PAS) staining. Values in Panels A, C, and F are expressed as percentage points.

ilar to those seen after the methacholine challenge (which did not induce such recruitment). These findings have implications for the management of asthma, since airway remodeling has been linked to a decline in lung function and the loss of bronchodilator reversibility.[24] Currently, the primary aim of asthma management is to reduce symptoms and control the disease by targeting airway inflammation. The results of this study suggest that an additional target should be

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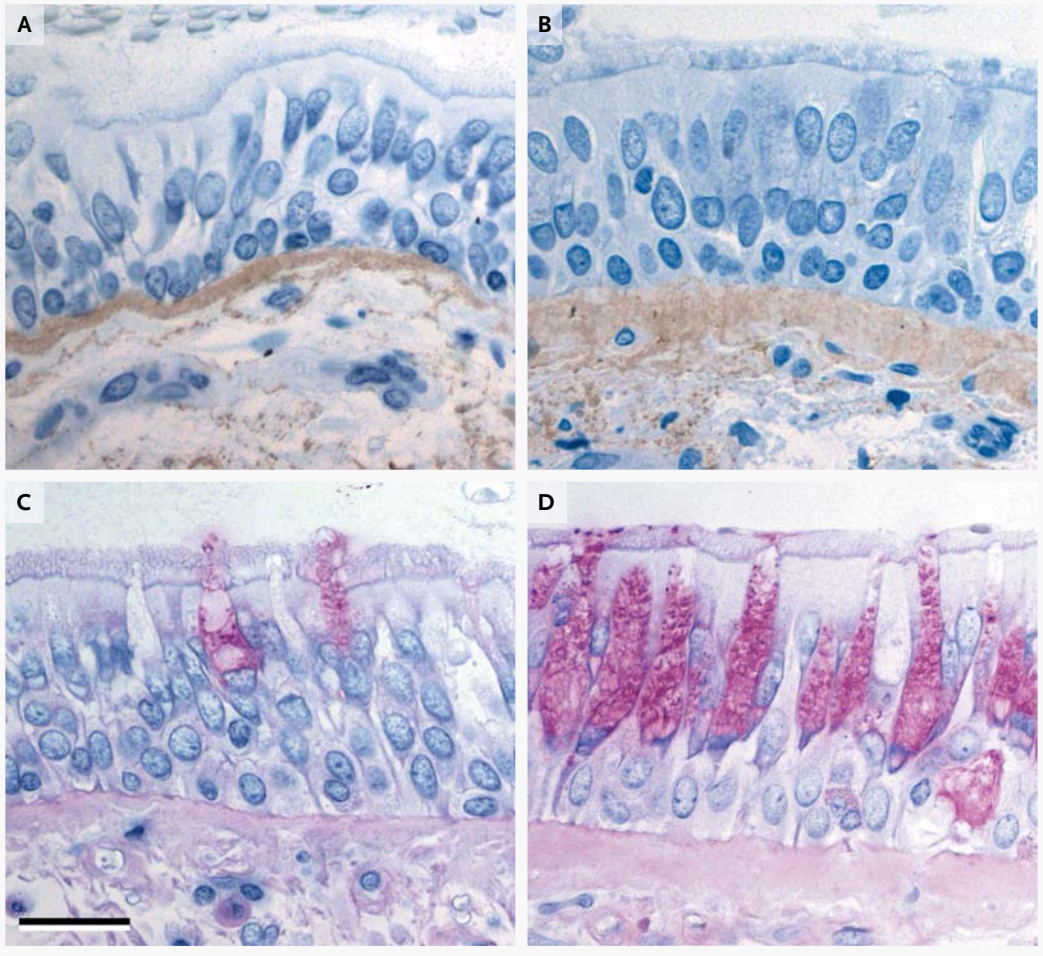

Figure 3. Representative Photomicrographs of Respiratory Epithelium from Bronchial-Biopsy Specimens before and after Repeated Inhaled Methacholine Challenge.

Panels A and C show the respiratory epithelium before the challenges, and Panels B and D show the epithelium after the challenges. Biopsy specimens were immunostained with an antibody to collagen type III (in Panels A and B) and with periodic acid–Schiff (in Panels C and D). The horizontal bar represents 30 μm.

to stabilize airway caliber and prevent bronchoconstriction.

An emerging concept in asthma is that of the epithelial–mesenchymal trophic unit through which genetic and environmental interactions influence the expression of asthma.[25] This concept identifies the epithelium as a key structural tissue. We contend that the cells of the epithelium, when activated, promote not only airway cell recruitment but also mesenchymal signaling, which induces myofibroblast transformation and initiates a wound-repair response as a key asthmatic event.[26] Epithelium-generated TGF-β is a crucial growth factor in this respect, since it induces myofibroblast transformation and stimulates collagen synthesis. In this study, we found that bronchoconstriction induced by either allergen or methacholine increases TGF-β immunoreactivity within the airway epithelium. Our study also provides evidence that repeated bronchoconstriction with either stimulus increases the thickness of the subepithelial collagen layer, which is indicative of an acute alteration in airway collagen dynamics and is consistent with the influence of epithelial mesenchymal signaling. These results translate the in vitro evidence of the relevance of mechanical forces to airway remodeling[12,15] to the in vivo situation in asthma. In vitro studies have also suggested that

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compressive forces can induce goblet-cell hyperplasia within the epithelium — an action attributable to TGF-β.[14] Our study clearly showed that bronchoconstriction after either the allergen or the methacholine challenge led to an increase in the percentage of epithelium staining for mucus-secreting cells that was not evident in the saline-challenge group. These changes thus identify the relevance of bronchoconstriction in asthma as a stimulus leading to excessive mucus production that may further contribute to occlusion of the airways.

The protein Ki67 regulates cell proliferation. The increased immunoexpression of Ki67 identified within both the epithelium and the submucosa 4 days after cessation of the repeated bronchoconstriction with allergen or methacholine is suggestive of compression-induced epithelial damage and ongoing proliferative repair responses within the epithelial mesenchymal trophic unit. The absence of an effect when methacholine inhalation was preceded by albuterol (to prevent bronchoconstriction) is consistent with the hypothesis that the effects of methacholine are not molecule-specific but related to induced bronchoconstriction (Section 3 in the Supplementary Appendix). This has implications not only for the understanding of the pathogenesis of asthma but also for the design of studies of airway remodeling that use bronchoprovocation. Although methacholine and allergen induced similar acute early bronchoconstrictor responses, only in the allergen-challenge group was there a late allergic reaction associated with a progressive fall in FEV_1. This was not associated with enhanced airway-remodeling changes. This absence of an additional effect may reflect different mechanisms of early and late bronchoconstrictor responses or the achievement of a threshold response with the immediate bronchoconstriction that limits the influence of repeated acute mechanical stimulation, as suggested by in vitro studies.[14]

We selected subjects for the study who were sensitive to house-dust mites, since challenge with these allergens favors airway eosinophil recruitment.[27] We have previously found that as few as six subjects with asthma are required to show significant eosinophil recruitment during the late reaction,[27] and other investigators have reported that significant airway remodeling is evident after allergen challenge in as few as nine volunteers with asthma.[4] For these reasons, it was feasible to select 12 subjects per challenge group for our complex study. Repeated challenges were undertaken to reproduce dynamic airway changes reflective of uncontrolled asthma and to translate findings from animal models, in which repeated allergen challenges have been shown to promote sustained airway eosinophil recruitment and airway remodeling.[28,29] We were able to reproduce these findings in our subjects with asthma 4 days after they were subjected to three separate challenges. In contrast to the findings with the allergen challenge, no eosinophil recruitment was evident with repeated methacholine-induced bronchoconstriction. Previously, airway structural changes after an allergen challenge were attributed to eosinophilic inflammation.[30] Since the same degree of allergen-induced or methacholine-induced acute bronchoconstriction produced indistinguishable remodeling changes in the present study, our data suggest that the allergen-induced eosinophil influx in the airway is not crucial for these changes to occur. However, because eosinophils were evident within the airways at baseline, this study cannot exclude the possibility that an eosinophil–epithelium interaction influenced the airway response to compression.

This study thus provides evidence that bronchoconstriction induces epithelial stress and initiates a tissue response that leads to structural airway changes. This finding not only has relevance for asthma but may also provide an explanation for the remodeling described in patients with chronic cough.[31] Since repeated epithelial stress may lead to remodeling, the prevention of bronchoconstriction itself should be an important aim of asthma management. The lack of focus on controlling airway caliber may explain why daily inhaled glucocorticoid therapy has not been shown to modify the natural history of lung-function changes in preschool-age and school-age children in long-term, prospective interventional studies.[32,33] Although treating the inflammatory component of asthma is the first-line approach to controlling the disease, bronchial hyperresponsiveness is frequently not normalized by inhaled glucocorticoid therapy, particularly in patients with more severe asthma, and additional therapy is required. We speculate that sustained and safe bronchoprotection in addition to adequate control of inflammation should be the aim in such patients in order to prevent the long-term adverse consequences of airway remodeling.

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REFERENCES

1. Fahy JV. Remodeling of the airway epithelium in asthma. Am J Respir Crit Care Med 2001;164:S46-S51.
2. Hashimoto M, Tanaka H, Abe S. Quantitative analysis of bronchial wall vascularity in the medium and small airways of patients with asthma and COPD. Chest 2005;127:965-72.
3. Beckett PA, Howarth PH. Pharmacotherapy and airway remodelling in asthma? Thorax 2003;58:163-74.
4. Kariyawasam HH, Aizen M, Barkans J, Robinson DS, Kay AB. Remodeling and airway hyperresponsiveness but not cellular inflammation persist after allergen challenge in asthma. Am J Respir Crit Care Med 2007;175:896-904.
5. Flood-Page P, Menzies-Gow A, Phipps S, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. J Clin Invest 2003; 112:1029-36.
6. Humbles AA, Lloyd CM, McMillan SJ, et al. A critical role for eosinophils in allergic airways remodeling. Science 2004; 305:1776-9.
7. Wiggs BR, Hrousis CA, Drazen JM, Kamm RD. On the mechanism of mucosal folding in normal and asthmatic airways. J Appl Physiol 1997;83:1814-21.
8. Tschumperlin DJ, Drazen JM. Mechanical stimuli to airway remodeling. Am J Respir Crit Care Med 2001;164:S90-S94.
9. Nishimura Y, Inoue T, Morooka T, Node K. Mechanical stretch and angiotensin II increase interleukin-13 production and interleukin-13 receptor alpha2 expression in rat neonatal cardiomyocytes. Circ J 2008;72:647-53.
10. Huang C-YC, Reuben PM, Cheung HS. Temporal expression patterns and corresponding protein inductions of early responsive genes in rabbit bone marrow-derived mesenchymal stem cells under cyclic compressive loading. Stem Cells 2005;23:1113-21.
11. Miyagawa A, Chiba M, Hayashi H, Igarashi K. Compressive force induces VEGF production in periodontal tissues. J Dent Res 2009;88:752-6.
12. Tschumperlin DJ, Shively JD, Kikuchi T, Drazen JM. Mechanical stress triggers selective release of fibrotic mediators from bronchial epithelium. Am J Respir Cell Mol Biol 2003;28:142-9.
13. Choe MM, Sporn PHS, Swartz MA. Extracellular matrix remodeling by dynamic strain in a three-dimensional tissue-engineered human airway wall model. Am J Respir Cell Mol Biol 2006;35: 306-13.
14. Park JA, Tschumperlin DJ. Chronic intermittent mechanical stress increases MUC5AC protein expression. Am J Respir Cell Mol Biol 2009;41:459-66.
15. Park JA, Drazen JM, Tschumperlin DJ. The chitinase-like protein YKL-40 is secreted by airway epithelial cells at baseline and in response to compressive mechanical stress. J Biol Chem 2010;285: 29817-25.
16. Juniper EF, O'Byrne PM, Ferrie PJ, King DR, Roberts JN. Measuring asthma control: clinic questionnaire or daily diary? Am J Respir Crit Care Med 2000;162: 1330-4.
17. Grainge C, Howarth PH. Repeated high-dose inhalation allergen challenge in asthma. Clin Respir J 2010 June 7 (Epub ahead of print).
18. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing — 1999: this official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 2000;161:309-29.
19. British Thoracic Society Bronchoscopy Guidelines Committee. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. Thorax 2001;56: Suppl 1:i1-i21.
20. Britten KM, Howarth PH, Roche WR. Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosal biopsies. Biotech Histochem 1993;68:271-80.
21. Puddicombe SM, Polosa R, Richter A, et al. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. FASEB J 2000;14:1362-74.
22. McManus JF. Histological demonstration of mucin after periodic acid. Nature 1946;158:202.
23. Proschan MA, Waclawiw MA. Practical guidelines for multiplicity adjustment in clinical trials. Control Clin Trials 2000; 21:527-39.
24. Pascual RM, Peters SP. The irreversible component of persistent asthma. J Allergy Clin Immunol 2009;124:883-90.
25. Holgate ST, Roberts G, Arshad HS, Howarth PH, Davies DE. The role of the airway epithelium and its interaction with environmental factors in asthma pathogenesis. Proc Am Thorac Soc 2009;6:655-9.
26. Zhang S, Smartt H, Holgate ST, Roche WR. Growth factors secreted by bronchial epithelial cells control myofibroblast proliferation: an in vitro co-culture model of airway remodeling in asthma. Lab Invest 1999;79:395-405.
27. Hatzivlassiou M, Grainge C, Kehagia V, Lau L, Howarth PH. The allergen specificity of the late asthmatic reaction. Allergy 2010;65:355-8.
28. Tamaoka M, Hassan M, McGovern T, et al. The epidermal growth factor receptor mediates allergic airway remodelling in the rat. Eur Respir J 2008;32:1213-23.
29. Labonté I, Hassan M, Risse PA, et al. The effects of repeated allergen challenge on airway smooth muscle structural and molecular remodeling in a rat model of allergic asthma. Am J Physiol Lung Cell Mol Physiol 2009;297:L698-L705.
30. Kelly MM, O'Connor TM, Leigh R, et al. Effects of budesonide and formoterol on allergen-induced airway responses, inflammation, and airway remodeling in asthma. J Allergy Clin Immunol 2010; 125(2):349.e13-356.e13.
31. Niimi A, Torrego A, Nicholson AG, Cosio BG, Oates TB, Chung KF. Nature of airway inflammation and remodelling in chronic cough. J Allergy Clin Immunol 2005;116:565-70.
32. Guilbert TW, Morgan WJ, Zeiger RS, et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med 2006;354:1985-97.
33. The Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. N Engl J Med 2000;343: 1054-63.

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