

Acute Allergen-Induced Airway Remodeling in Atopic Asthma

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Studies in animals and in human atopic skin suggest that allergen challenge may activate acute tissue remodeling changes via transforming growth factor- β pathways. We determined whether inhalational allergen challenge in subjects with mild asthma induces similar acute changes to the airway epithelial mesenchymal trophic unit (EMTU). Endobronchial mucosal biopsies obtained before and 24 h after challenge were examined by confocal microscopy for extracellular matrix deposition in the reticular basement membrane (RBM). Cells actively involved in extracellular matrix synthesis were identified as immunoreactive to heat shock protein 47, a chaperone of collagen synthesis. Interleukin-4/13 and transforming growth factor- β -activated cells were identified by specific antibodies to phosphorylated (phospho-) signal transducer and activator of transcription 6 and phospho-Smad2, respectively. After allergen challenge, there was a significant increase in the number of heat shock protein 47-positive airway fibroblasts ($P = 0.003$) and in the thickness of tenascin in the RBM ($P = 0.031$). There were also increases in the number of phospho-Smad2+ epithelial cells ($P = 0.04$) and nuclear phospho-Smad2+ fibroblasts ($P = 0.03$), as well as phospho-signal transducer and activator of transcription 6+ epithelial cells ($P = 0.03$), after allergen challenge. Thus, allergen challenge in patients with mild asthma induces activation of epithelial cells and fibroblasts in the EMTU as well as increased tenascin deposition within the RBM. Airway remodeling in asthma may, in part, result from repeated acute activation of the EMTU by allergen exposure.

Patients with asthma have accelerated loss of lung function over time, and some patients develop progressive fixed airflow obstruction. These features may reflect airway remodeling, which is a characteristic feature of asthma, with increased deposition of several extracellular matrix (ECM) proteins and collagens, both in the reticular basement membrane (RBM) and in the bronchial submucosa, recruitment and activation of fibroblasts and myofibroblasts (1), hyperplasia of airway smooth muscle (ASM) (2) and goblet cells (3), and angiogenesis (4). Thickening of the RBM is present in children with difficult asthma to the same extent as in adults (5), and there is increasing evidence that airway remodeling occurs early in childhood and that it may even predate the onset of symptoms in some cases (6).

The causes of airway remodeling in asthma and its relationship to airway inflammation remain the subject of debate. Key cytokines in these processes are transforming growth factor

(TGF)- β and interleukin (IL)-13. TGF- β exists as three isoforms (TGF- β 1, - β 2, and - β 3). TGF- β 1 is the most studied. They all regulate deposition of ECM proteins, such as collagen and fibronectin, and have other functions, including the inhibition of proliferation of many cell types. IL-13, on the other hand, activates TGF- β production and overexpression in the airway in mice induces many of the features of the remodeled asthmatic airway. TGF- β is produced by many cell types, including eosinophils (7). Our previous data, from *in vitro* coculture studies, allergen challenge in the skin, and by study of the effects of anti-IL-5 antibody (Ab) treatment of patients with asthma, suggest links between eosinophils, TGF- β 1, and remodeling (8–10). Specific reduction of airway eosinophils with a blocking monoclonal Ab to IL-5 was associated with reduced RBM deposition of tenascin, lumican, and procollagen III, together with reduced eosinophil TGF- β 1 expression (10). Studies in animal models of chronic airway challenge that recapitulate many of the features of airway remodeling in asthma also showed IL-5 and eosinophil dependence of ECM protein deposition within the RBM, and this in turn was related to the expression of TGF- β 1 (11).

Repair and remodeling events in the asthmatic airways, including deposition of collagen and other ECM proteins within the bronchial wall, are often regarded as chronic processes resulting from long-term tissue injury. However, Gizycki and colleagues showed an increase in myofibroblast formation 24 h after allergen challenge in subjects with atopy, suggesting that fibroblast activation can occur relatively rapidly after allergen exposure (1), and we have recently demonstrated infiltration of myofibroblasts, tenascin deposition, and eosinophil-derived TGF- β 1 expression 24 h after allergen challenge in the skin (8). Although increased expression of mRNA for TGF- β 1 may indicate increased production, and therefore activity of this cytokine, there is evidence that proteins of the TGF- β family are present in many tissues in an inactive form and that activity is posttranscriptionally regulated. Evidence of cellular activation through TGF- β can therefore be obtained by detection of phosphorylated downstream signaling molecules, such as Smad2. Such signaling has been demonstrated in allergen challenge models in mice, and phosphorylated (phospho-) Smad2 expression has also been increased in the airway epithelium of patients with asthma at baseline compared with nonatopic control subjects (12).

Airway remodeling in asthma has been suggested to involve reactivation of a development unit consisting of the airway epithelium, reticular BM, and associated attenuated fibroblast sheath. This has been termed the epithelial-mesenchymal trophic unit (EMTU) (13), and our hypothesis in this study was that allergen inhalation challenge in patients with asthma leads to acute activation of the EMTU with initiation of tissue remodeling.

Materials and Methods

Study Population and Design

Patients with asthma and control human volunteers without atopy were recruited by advertisement. Of the nine patients with asthma, seven were part of a study which has been described in detail elsewhere (14) and an additional two subjects the same allergen-challenge and

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Abbreviations: antibody, Ab; airway hyperreactivity, AHR; alkaline phosphatase-antialkaline phosphatase, APAAP; airway smooth muscle, ASM; 4',6-diamidino-2-phenylindole, DAPI; extracellular matrix, ECM; epithelial mesenchymal trophic unit, EMTU; eosinophil cationic protein, EPC; heat shock protein, HSP; interleukin, IL; late asthmatic reaction, LAR; phosphorylated, phospho-; reticular BM, RBM; signal transducer and activator of transcription 6, STAT6; transforming growth factor, TGF.

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bronchoscopy protocol was followed. Written informed consent was obtained from all subjects, and the study was approved by both of the London Chest Hospital and the Royal Brompton Hospital Ethics Committees.

Patients with asthma had a history of asthma together with documented reversible airways obstruction (12% or more), either spontaneously or after inhaled β -agonists. All subjects had an forced expiratory volume at 1 s $> 70\%$ predicted and required intermittent use of inhaled β_2 -agonists for control of symptoms. None had received either orally administered corticosteroids in the 6 mo or inhaled steroids in the 2 wk preceding the study. Patients with seasonal symptoms were studied outside the pollen season of the United Kingdom. None were smokers or had a history suggestive of a respiratory infection in the 3 wk before or during the study. Atopy was defined by positive skin prick test (wheal diameter of over 3 mm after subtraction of the negative control) to one or more aeroallergen extracts (house dust mite, cat dander, and Timothy grass pollen Soluprick [ALK, Hørsholm, Denmark]). Biopsies from normal subjects ($n = 7$) and patients with asthma ($n = 13$) were obtained as previously described.

Allergen Challenge

The allergen used for challenge was selected on the basis of the clinical history and a positive skin prick test. On the screening day, an allergen challenge protocol was standardized using a modification described by Chai and colleagues (15), as previously described (14). Patients were selected to continue the study if they had demonstrated an early AR of at least 20% (0–2 h after challenge) and a late asthmatic response (LAR) of at least 15% (2–8 h after challenge). The dose of allergen required to produce an LAR of 15% was predicted from the dose-response curve and magnitude of the LAR on the screening day. On the second challenge day, the cumulative dose was administered as a bolus, using the same nebulizer, dosimeter, and dosimeter settings.

Fiberoptic Bronchoscopy

Each subject received nebulized salbutamol (2.5 mg) and atropine (600 μ g) intravenously 15 min before fiberoptic bronchoscopy. Sedation was achieved with intravenously administered diazepam, and supplemental oxygen was given throughout the procedure. Patient oxygenation was monitored by pulse oximetry. Fiberoptic bronchoscopy was performed by the same operator for all subjects. After 1% lignocaine spray was applied to the nose and throat of the subject, the bronchoscope (Olympus Model OSE with a 2.2-mm-width biopsy channel; Olympus Corp., Lake Success, NY) was introduced. Local anesthesia of the larynx was produced with topical 4% lignocaine, and 2% lignocaine was used below the vocal cords. Endobronchial biopsies were then taken from either the right middle or lower lobe on segmental carinae. After the procedure, the patient was given an additional 2.5 mg of salbutamol and was observed on the ward for at least 4 h.

Biopsy Samples

Tissue biopsies were immediately fixed in 4% paraformaldehyde and washed in 15% phosphate-buffered saline–buffered sucrose (Sigma, Poole, UK), embedded in optimal cutting temperature, then snap-frozen in isopentane precooled in liquid nitrogen. Cryostat sections ($< 8 \mu$ m) were cut from biopsies, mounted onto Superfrost Plus slides, dried overnight at 37°C, wrapped in foil, and stored with silica gel at -80°C until use (all from VWR Scientific, Dagenham, UK).

Immunohistochemistry

Heat shock protein (HSP) 47 (clone M16.10A1, Stressgen) and phospho-signal transducer and activator of transcription (STAT) 6 (Tyr 641, clone 5A4, Cell Signaling Technology, Hitchin, UK) immunoreactivity were detected by the alkaline phosphatase–antialkaline phosphatase (APAAP) method as described previously (16). Briefly, tissue sections were pre-treated with phosphate-buffered saline containing 0.1% saponin for 30 min and incubated for 2 h in a humidity chamber with the relevant primary Ab at 1 μ g/ml or 1/100 dilution, respectively. Sections were then incubated with rabbit anti-mouse immunoglobulin G1 (1:30 for 30 min), followed by APAAP complexes (1:30 for 30 min). All sections were washed thoroughly (3 $\times$) between Ab incubations. Normal human serum was used to prevent nonspecific binding of the second- and third-layer antibodies. For detection of phospho-Smad2 (Upstate

Biotechnology, Milton Keynes, UK), sections were incubated overnight with an Ab that recognizes phospho-Smad2 (serine 465/467) at 1:100 dilution, followed by an alkaline phosphatase (AP)-conjugated goat anti-rabbit Ab. Abs to phospho-signal transducer and activator of transcription (STAT) 6 and -Smad2 do not detect the nonphosphorylated forms. The phosphatase substrate Fast red (Sigma) was used to develop the reaction-enabling signal visualization. The numbers of positively stained cells were counted in a zone 250 μ m deep as defined by a squared eyepiece graticule (Olympus Corp., Lake Success, NY) along the entire length of the epithelial BM of each section. Cell counts were expressed as number per unit length of BM (positive cells/mm BM). Fibroblasts were identified morphologically as being fusiform in shape, with elongated nuclei. Immunoreactive positive cells identified as leukocytes within this zone were not included in cell counts. All reagents used for immunohistochemistry were obtained from Dako (High Wycombe, UK) unless stated otherwise.

Double Immunohistofluorescence

Tenascin and phospho-Smad2. Sections were incubated overnight with tenascin Ab (Monosan, Uden, The Netherlands) (10) and phospho-Smad 2 Ab simultaneously, as described above. The tissue was then incubated for 30 min with a biotinylated goat anti-rabbit Ab (1:30), washed, and then incubated with 4 μ g/ml streptavidin-dichlorotriazinyl amino fluorescein (Jackson Laboratories, West Grove, PA) for 30 min, and 2 μ g/ml anti-mouse Alexa Flour 568 (Molecular Probes, Eugene, OR) simultaneously. All sections were washed thoroughly (3 $\times$) between Ab incubations. Sections were then incubated with 4',6-diamidino-2-phenylindole (DAPI) (Sigma) at 1 μ g/ml for 5 min, left to stand in water for 20 min, and mounted in fluorescent mounting medium.

HSP47 and phospho-Smad2. Sections were treated as described above for double tenascin and phospho-Smad2 staining, except that anti-HSP47 was applied for 2 h after overnight incubation with phospho-Smad2.

RBM: Tenascin, Procollagen III, and Lumican

Tissue sections were stained with a mouse Ab against tenascin or rabbit Ab against procollagen-III (Chemicon, Harrow, UK) or lumican (a generous gift of Dr. Peter Roughley, Shriners' Hospital for Crippled Children, McGill University, Canada), as described previously (10). Sections were then incubated with DAPI (to avoid measurement of intracellular protein) and mounted in fluorescent mounting medium. Appropriate isotype controls were included. Images were acquired using a Leica TCS SP confocal microscope (Leica, Heidelberg, Germany). The microscope settings were standardized to allow comparison of immunoreactivity intensity between different sections. Measurements were analyzed using Scion Image analysis software package (Scion Corporation, Frederick, Maryland). The thickness of immunoreactivity in the RBM area was calculated by taking multiple measurements over the length of the biopsy (> 100 measurements) at 10- μ m intervals. Briefly, at each measurement, a line was drawn perpendicular to, and across, the band of immunoreactivity in the RBM, and image analysis software was used to quantitate the length of the line (thickness). The values were averaged over the whole length of RBM to give mean thickness of immunoreactivity. As previously reported, the intraobserver error for analysis of BM proteins was $\pm 8.7\%$ (10), consistent with that reported by others (17, 18).

Eosinophil Accumulation

Eosinophils were detected using the monoclonal Ab EG2 (Pharmacia Upjohn, Milton Keynes, Buckinghamshire, UK), which recognizes the cleaved form of eosinophil cationic protein. Sections were counted "blind" by a single observer (per Ab). Positive cells stained red after developing with the alkaline phosphatase substrate Fast red. The numbers of positively stained cells were counted in the whole section and the size of the section determined with a squared eyepiece graticule. Cell counts were expressed as number per mm² (positive cells/mm²).

Statistical Analysis

Paired results are presented in addition to mean $\pm$ SEM. The paired Student *t* test was used to analyze changes in the numbers of positively immunoreactive cells/thickening of the RBM in response to allergen.

Data were analyzed using Graph Pad Prism (Graph Pad Software Inc., San Diego, CA). A P value of < 0.05 was accepted as significant.

Results

Allergen Challenge Is Associated with Increased Tenascin Expression within the RBM

Because we had previously shown increases in tenascin, lumican, and procollagen III in patients with asthma at baseline compared with normal subjects (10), we measured for deposition of these proteins in diluent and allergen challenge biopsies. There was a significant increase in tenascin ($P = 0.03$), but not in lumican or procollagen III thickness at the RBM (Figure 1). After allergen challenge there were also significant increases (medians and ranges) in the numbers of eosinophils ($P = 0.014$; before challenge: 33.8 [1.2–96] cells/mm²; after challenge: 65.3 [17.8–99] cells/mm²; $n = 8$), confirming that allergen challenge leads to increased eosinophilic inflammation in the patients with asthma.

Upregulation of HSP47 in the Airway EMTU in Asthma Compared with Control Subjects with Further Activation of Collagen Synthesis after Allergen Challenge

Figure 2 shows HSP47+ cells within the airway in a mucosal biopsy from an subject with asthma. A discrete layer of HSP47+ cells with fibroblast-like appearance was detected immediately beneath and parallel to the RBM, with positive cells also observed within the epithelium as well as deeper in the bronchial wall. The numbers of HSP47+ cells were significantly elevated in epithelial cells and fibroblasts from atopic patients with asthma compared with normal control subjects (Figure 3A). Allergen challenge of atopic patients with asthma resulted in a further significant increase as compared with diluent challenge in the numbers of HSP47+ fibroblasts and an increase in epithelial cells (Figure 3B). We also observed a number of HSP47 immunoreactive macrophages lying deeper within the mucosa. Examples of HSP47 immunoreactive-positive cells from diluent and allergen challenge sites are shown in Figures 3C and 3D, respectively.

Phospho-Smad2

Evidence for activation of epithelial cells and fibroblasts via TGF- β was obtained by measuring the numbers of phospho-Smad2+ cells. After allergen challenge, there was a significant increase in the total number of phospho-Smad2+ epithelial cells ($P = 0.04$) but not fibroblasts ($P = 0.20$) (Figure 4). However, analysis of phospho-Smad2 localization revealed increases in nuclear, but not cytoplasmic, phospho-Smad2+ cells in both epithelial cells ($P = 0.06$) and fibroblasts ($P = 0.03$). An example of phospho-Smad2+ cells above and below the RBM and tenascin immunoreactivity within the RBM is shown in Figure 5A. Nuclear versus cytoplasmic phospho-Smad2 positivity can be visualized by staining the tissue with the immunofluorescent nuclear (DNA) counterstain DAPI (Figure 5B). HSP47 (red

staining) and phospho-Smad (green staining) immunoreactivity could be colocalized (orange staining) to both epithelial cells and mesenchymal cells, such as fibroblasts, pericytes, and endothelial cells, as shown in Figure 5B.

Phospho-STAT6

There were increases in the total number of phospho-STAT6+ cells in both the epithelial cells ($P = 0.04$) and fibroblasts (diluent versus allergen challenge, $P = 0.06$) (Figure 6). The number of cytoplasmic phospho-STAT6+ cells was significantly increased after allergen challenge ($P = 0.04$), which almost reached significance in fibroblasts ($P = 0.06$). There was also an increase in nuclear phospho-STAT6 in epithelial cells ($P = 0.09$), but not in fibroblasts. An example of phospho-STAT6 staining with prominent nuclear and cytoplasmic phospho-STAT6 expression in the epithelial cell layer is shown in Figure 7.

Discussion

In this study, we show that allergen challenge of atopic patients with asthma led to acute upregulation of markers of tissue remodeling, with increased collagen processing and tenascin deposition within the RBM. This was associated with evidence of IL-4/IL-13 signaling in the airway epithelium and TGF- β signaling in airway fibroblasts.

Although airway remodeling in asthma is often considered a result of chronic inflammation, it is present early in the disease (5), and our previous studies of allergen challenge in the skin raised the possibility of acute tissue remodeling events in response to allergen. In wound healing, it is well established that normal repair in response to injury results in a sequential set of interacting events, including inflammation, mesenchymal activation, and re-epithelialization, with deposition of ECM proteins and subtle alterations in tissue architecture that occur in hours and days as opposed to months. Thus, airway remodeling in asthma may be an iterative process with repeated stepwise accretion to allergen-induced events. How many of the allergen-induced changes persist and how these changes are integrated over time will be important subjects for future study, as will their susceptibility to treatment, such as inhaled corticosteroids. It is of note that some studies do suggest that long-term steroid treatment may lead to partial reversal of remodeling changes (17), and this may account for the gradual but continuing improvement in airway hyperreactivity (AHR) with inhaled steroid treatment.

We were able to provide indirect evidence of collagen synthesis by the use of HSP47, which is a collagen-specific molecular chaperone thought to be essential for the proper processing and secretion of procollagen molecules. It was previously shown that TGF- β 1 activates heat shock transcription factor 1, which stimulates the transcription of HSP47 mRNA, resulting in increased expression of HSP47 (19). Our studies show that, compared with normal subjects, patients with asthma have elevated numbers

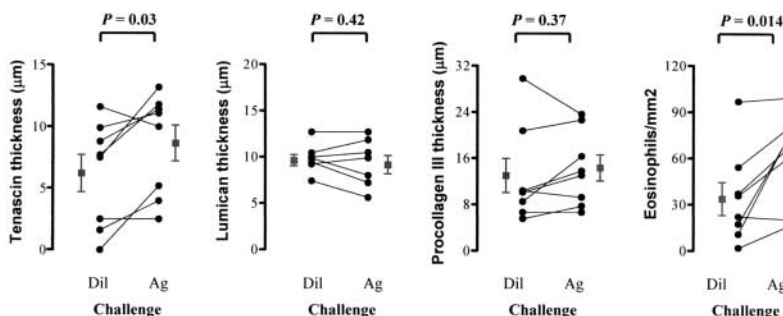


Figure 1. The effect of inhalational allergen challenge on the thickness of tenascin, lumican, and procollagen III, and the numbers of eosinophils, within the RBM. Error bars represent means $\pm$ SEM ($n = 7-8$).

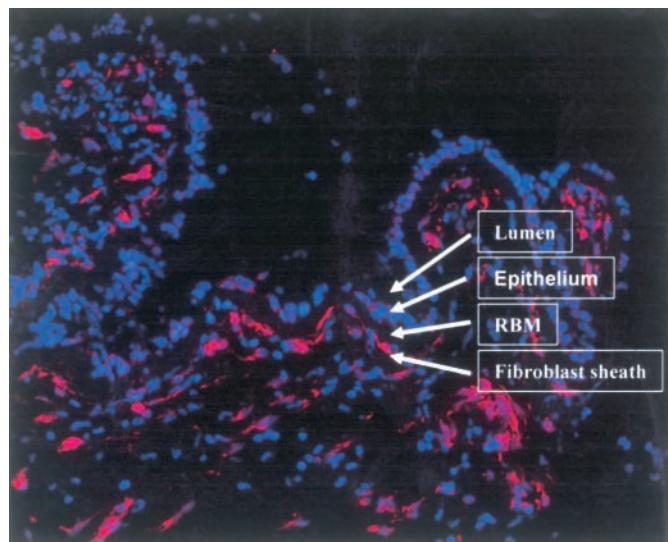


Figure 2. An immunofluorescent photomicrograph showing an example of HSP47 immunoreactivity in the airway mucosa of an patient with asthma (after challenge). Cells within the fibroblast sheath are *arrowed*.

of HSP47⁺ epithelial cells and fibroblasts beneath the RBM. Although there is no specific marker for airway fibroblasts, cell morphology suggested that there was an increase in both epithelial and fibroblast HSP47 immunoreactivity after allergen challenge.

Although allergen challenge was associated with increased expression of tenascin in the RBM at 24 h, we did not detect significant changes in lumican or procollagen III expression. This may reflect relatively small sample size or differential timecourse of activation of synthesis of these ECM proteins. It is of note that our previous study of anti-IL-5 treatment in asthma showed more pronounced effects on RBM tenascin deposition than on lumican or procollagen III, and it might be that tenascin is particularly labile within the RBM.

TGF- β in particular is a potent regulator of fibroblast/myofibroblast function and controls the production of several ECM proteins, including collagens, proteoglycans, and tenascin. Here we have shown acute upregulation of tenascin expression together with increased staining for HSP47 after allergen challenge in asthma. This was accompanied by evidence of TGF- β 1 signaling through increased nuclear localization of phospho-Smad2 in airway fibroblasts. TGF- β 1 activation was detected by nuclear localization of phospho-Smad2, which is suggestive of signaling via the active TGF- β 1 molecule in response to allergen challenge. This is in agreement with data from Rosendahl and colleagues, who found that a range of TGF- β family proteins (i.e., the structurally related activins and the bone morphogenic proteins) were upregulated in experimental allergic airway inflammation in mice (20, 21). These investigators also showed increased expression of phospho-Smads as evidence of TGF- β /activin signaling.

Evidence of active IL-13/IL-4 signaling within the EMTU was provided through the demonstration of phospho-STAT6. IL-13 activates epithelial cells and fibroblasts for latent TGF- β release (22). A direct effect of IL-13 on epithelial cells has been demonstrated through the selective expression of STAT6 under an epithelial-specific promoter in STAT6-null mice. Epithelial STAT6-positive mice showed significant increases in AHR and mucus overproduction after activation of an IL-13 transgene, but were unable to recapture airway inflammation and subepithelial fibrosis in response to IL-13 transgene gene activation (23, 24). Thus, competent IL-13 signaling via the epithelium alone was sufficient for the development of AHR and mucus production, suggesting that this direct effect of IL-13 on epithelial cells is associated with these two central features of asthma. However, this model may also suggest that activation of the epithelium through IL-13 is not critical for the induction of RBM thickening. In other animal models of allergic inflammation, IL-13 has been demonstrated to be a potent fibrotic agent, possibly through the upregulation of TGF- β 1. Alternatively, IL-13 has been demonstrated to enhance the effector functions of TGF- β 1 on fibroblasts (25). Thus, small increases in IL-13 signaling may greatly potentiate TGF- β 1-induced fibroblast ECM deposition.

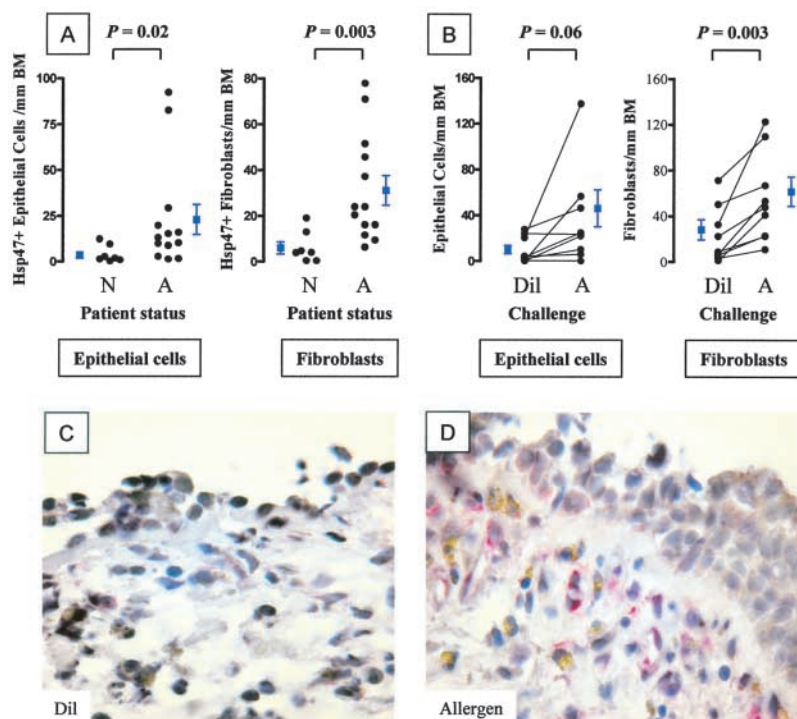


Figure 3. A comparison of the number of HSP47-positive cells in the epithelial or fibroblast layer (as shown in Figure 2) between (A) normal control subjects ($n = 7$) and individuals with asthma ($n = 13$), and (B) between diluent- and allergen-challenged subjects with asthma ($n = 9$). N denotes normal subjects, A denotes patients with asthma. Error bars represent the mean $\pm$ SEM. Representative photomicrographs of mucosal HSP47 immunoreactivity from one subject in response to diluent (C) or allergen (D) challenge are also shown.

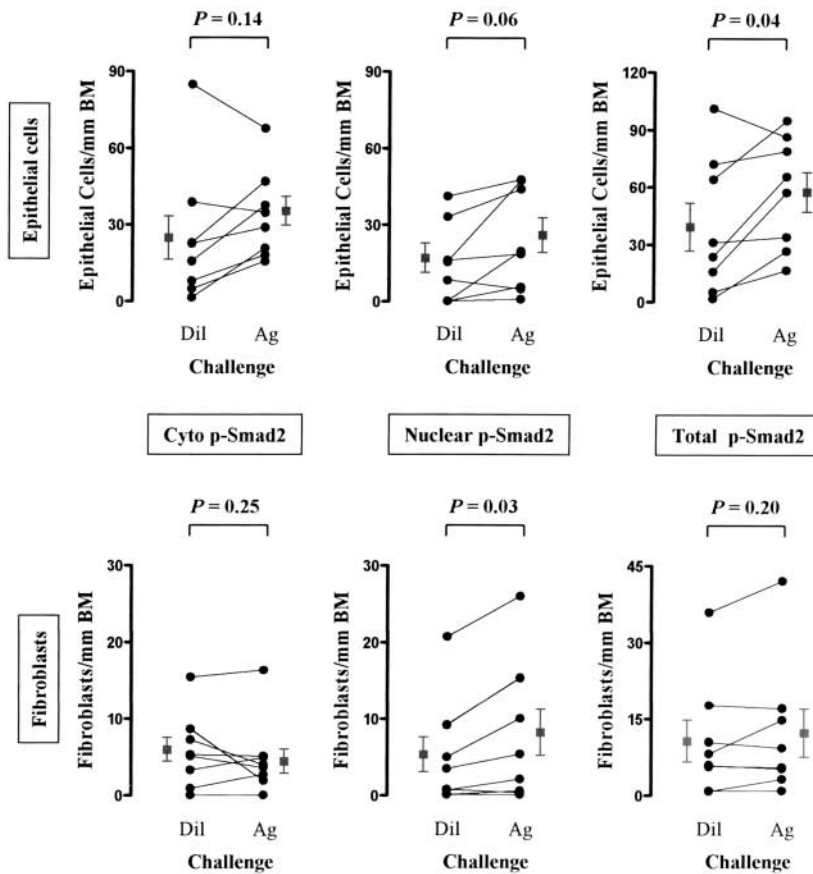


Figure 4. Effect of inhalational allergen challenge on the number of phospho-Smad2-positive cells in the epithelial or fibroblast layer (as illustrated in Figure 2). Phospho-Smad2 immunoreactivity was localized to either the cell cytoplasm or nucleus, and the combined counts expressed as total phospho-Smad2. Error bars represent the mean $\pm$ SEM ($n = 8$).

Unlike phospho-Smad2, increases in phospho-STAT6 were largely observed in the cytoplasm rather than the nucleus, both for epithelial cells and fibroblasts, which may suggest that translocation is slower for phospho-STAT6 than phospho-Smad2, or that phospho-Smad2 is more stable. Goumans and colleagues have demonstrated that Smad2 phosphorylation remains stable over time for at least 2 h (26). Alternatively, this observation may reflect rapid TGF- β 1 bioavailability, because the EMTU and eosinophils are a rich source of preformed TGF- β 1, and there is strong evidence for reservoirs of TGF- β 1 bound to extracellular components, such as decorin (27), whereas IL-13 is predominantly expressed by T cells and requires *de novo* translation.

There is continuing interest in the EMTU in asthma. It has been suggested that the remodeled phenotype observed in the asthmatic airway wall might be the product of an abnormal/dysfunctional epithelium with heightened susceptibility to injurious agents (6, 28). Dysregulation of normal repair processes could then promote chronic inflammation through the release of growth factors and chemokines by epithelial cells and fibroblasts (29). Alternatively, features of remodeling might be the result of chronic allergic inflammation leading to an overstimulated and persistent repair phenotype that recapitulates aspects of branching morphogenesis. Our data support the hypothesis that a repair phenotype is rapidly initiated in response to allergic insult, but do not support

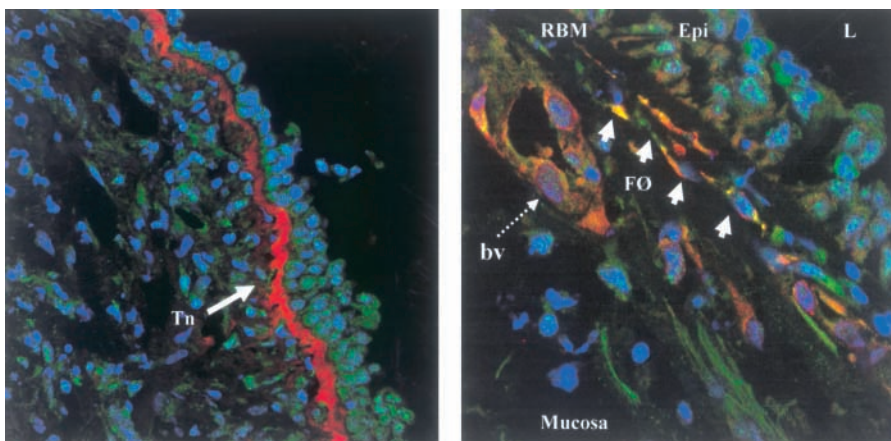


Figure 5. Immunofluorescent photomicrograph of tenascin (red) and phospho-Smad2 (green) immunoreactivity (left panel) and HSP47 (red) and phospho-Smad2 (green) immunoreactivity (right panel) in the airway mucosa. Colocalization of both proteins results in the appearance of a yellow-orange signal. Tn, tenascin; bv, blood vessel; L, lumen. Cell localization was on the basis of morphology: FO, fibroblast, Epi, epithelium.

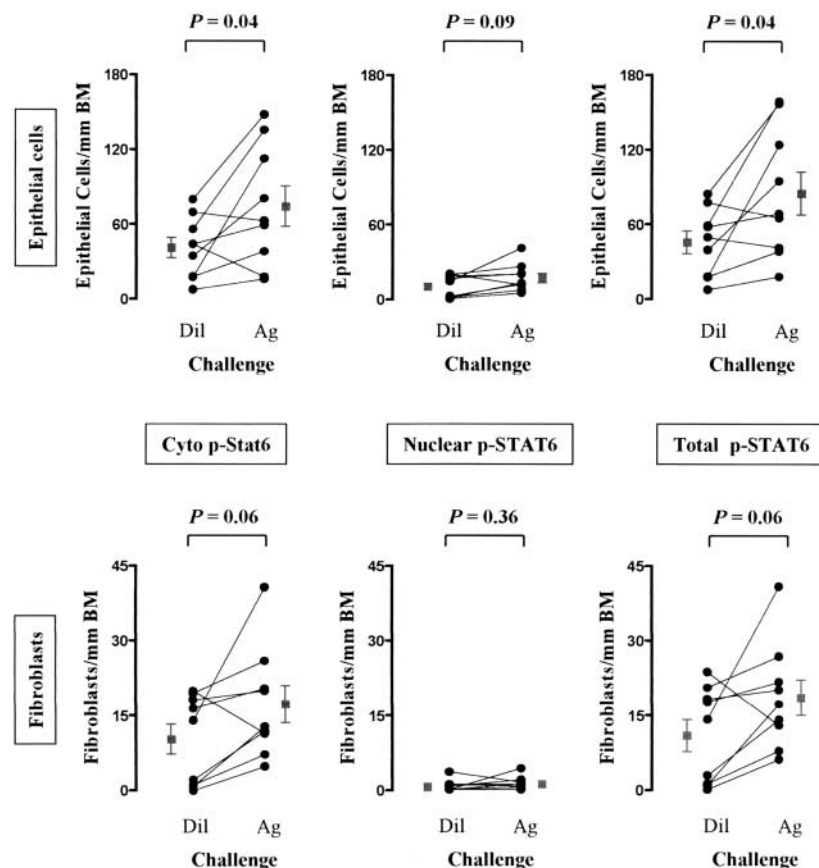


Figure 6. Effect of inhalational allergen challenge on the number of phospho-STAT6-positive cells in the epithelial or fibroblast layer (as illustrated in Figure 2). Phospho-STAT6 immunoreactivity was localized to either the cell cytoplasm or nucleus, and the combined counts expressed as total phospho-STAT6. Error bars represent the mean $\pm$ SEM ($n = 9$).

or confirm whether T helper type 2 cell-mediated allergic inflammation is superimposed on an already altered EMTU, or whether the inflammatory process is the initial trigger to generate the asthmatic phenotype, at least in individuals with atopy (28).

In conclusion, our studies indicate that allergen challenge induces acute activation of TGF- β and IL-4/IL-13 signaling—evidence of epithelial cell and fibroblast collagen synthesis, together with increased tenascin deposition within the asthmatic airway. This indicates that airway remodeling is a dynamic process that could result from repeated incremental activation of the EMTU.

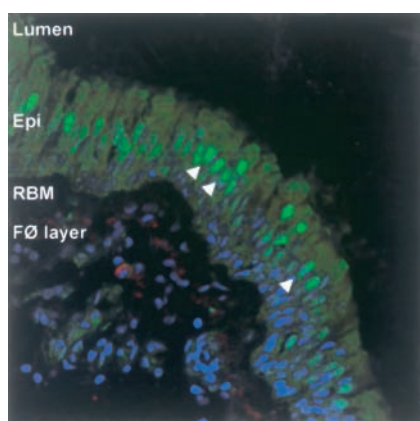


Figure 7. An immunofluorescent photomicrograph showing an example of phospho-STAT6 immunoreactivity in the airway epithelium of an individual with asthma (after challenge).

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