

# Putting the Squeeze on Airway Epithelia

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Asthma is characterized by chronic inflammation, airway hyperresponsiveness, and progressive airway remodeling. The airway epithelium is known to play a critical role in the initiation and perpetuation of these processes. Here, we review how excessive epithelial stress generated by bronchoconstriction is sufficient to induce airway remodeling, even in the absence of inflammatory cells.

With every breath, the lung becomes exposed to an external environment that often contains allergens, bacteria, viruses, or environmental pollutants (23, 60). These irritants provoke inflammation and activate signaling cascades that protect lung function ordinarily but can disrupt lung function when they become dysregulated. In addition to these external exposures, the lung has internal challenges. With each respiratory cycle, for example, lung size changes appreciably (24, 59, 88), and the associated physical distortion is linked to the distortion of the lung's multiple cellular constituents (26, 27, 130). Resulting mechanical stresses acting on each of these cellular constituents has the capacity to modify the cellular microenvironment and activate cell signaling (27, 64, 70).

It is well known that mechanical stress is critical to lung development in utero; mechanical stress guides branching morphogenesis and alveolar growth and thus results in the attainment of normal lung function in adult life (81, 115). During lung development, mechanical stresses promote cell proliferation and differentiation through the activation of signaling pathways that affect the production of extracellular matrix molecules and the expression of specific genes (57). Unlike these ordinary stresses, excessive mechanical stresses generated under certain pathological conditions not only impair protective functions but also lead to injury and aberrant repair (88). Perhaps the most well known of these effects is ventilator-induced lung injury, which is associated with the application of mechanical ventilation for life support (88); in patients with this condition, excessive mechanical stresses are transmitted across the pleural surfaces and alveolar walls to constituent cells (83, 108). A rather different example, and the one that comprises the focus of this review, is the effect of mechanical stresses imposed by constricted airway smooth muscle during asthmatic exacerbations. Here, we address the biological and physical impact of these mechanical stresses on airway epithelial cells and the subsequent impact on progressive airway remodeling in asthma.

## Airway Remodeling in Asthma

Asthma is a common clinical syndrome that accounts for substantial morbidity and affects 5–10% of the population in developed countries. It is associated with a global economic burden of billions of dollars per year (31, 123). Asthma is characterized by chronic airway inflammation and intermittent episodic bronchoconstriction, both of which are associated with the clinical manifestations that characterize this condition (6, 8). In patients with chronic persistent asthma, the airway progressively undergoes structural changes that are collectively termed airway remodeling (Table 1); these changes include goblet-cell hyperplasia, thickening of the subepithelium with collagen deposition, angiogenesis of the subepithelial vascular plexi, and hypertrophy and hyperplasia of smooth-muscle cells (18, 41, 43, 56, 75). Airway remodeling is thought to contribute to the decline in lung function that occurs in some patients with asthma (6, 8). Although the precise cause of airway remodeling remains unknown, it is thought to derive from the inflammatory microenvironment of the asthmatic airway wall (18, 41, 43, 56, 75). Thus most theories of airway remodeling have attributed the observed changes to the effects of mediators and cytokines derived from inflammatory cells, with little or no attention paid to the impact of bronchoconstriction itself. In this article, we review evidence that bronchoconstriction itself, even in the absence of inflammation, can induce airway remodeling.

## Magnitude of Compressive Stress During Bronchoconstriction

The airways of all vertebrate species are lined with epithelial cells that form the air-tissue interface (23). During normal respiration, the magnitude of transmural and transepithelial stresses is low and on the order of transpulmonary pressure (62). However, during bronchoconstriction, the associated mechanical stress causes the airway wall to buckle, leading to the formation of rosette patterns [as seen on cross-section images in the article by

**Table 1. The role of bronchoconstriction in airway remodeling**

Feature of Airway Remodeling	Recapitulated by Experiments	
	In Vitro Compressive System (FIGURES 1 AND 2)	Brochoconstriction in Humans (FIGURE 3)
Inflammation	Possible (11, 109)	Not detected (34)
Subepithelial collagen deposition	Collagen type III (93)	Collagen type III (34)
Goblet cell hyperplasia	MUC5AC positive cells (69)	PAS staining (34)
Airway smooth muscle proliferation and contraction	Not determined	Not determined
Airway angiogenesis	Not determined	Not determined

Yager et al. (129)]. This occurs because the basement membrane has the mechanical characteristics of a bicycle chain, stiff in extension but floppy in compression (129). The precise patterns of collapse vary depending on the mechanical properties of the elements of the airway wall; in patients with asthma, in whom the airway wall is thickened (17, 41), numerous epithelial cells are apposed to each other and squeezed, and thus subjected to appreciable mechanical compressive stress (55, 125).

To estimate the magnitude of the stress imposed on the cells, Wiggs et al. (125) used finite-element methods to analyze the rosette patterns of deformation that are formed during bronchoconstriction (129). They estimated the mechanical properties of the two layers that were used to model the airway wall and estimate the hoop stress that airway smooth muscle would exert during maximal bronchoconstriction. On the basis of these assumptions, they found that, during maximal bronchoconstriction, airway epithelial cells are subjected to compressive stress at a magnitude of ~30 cmH<sub>2</sub>O, which is at least an order of magnitude greater than the magnitude of transepithelial stress on airway epithelial cells during normal breathing.

### Biological Effects of Compressive Stress on Airway Epithelial Cells

To determine whether stress of this magnitude has a biological impact on airway epithelial cells, Ressler et al. (73) used an in vitro model. Rat tracheal epithelial cells were grown in air-liquid interface (ALI) culture, and mechanical stress mimicking the stress generated during bronchoconstriction was modeled through the application of transepithelial air-pressure gradients (FIGURE 1). The study shows that airway epithelial cells respond rapidly and robustly to compressive stress (73). Ressler et al. specifically used the expression of genes known to be mechanically sensitive in other systems as markers of the biological

effects of compressive stress. They found that such stress induces the expression of RNA encoding early growth response 1 (Egr-1), endothelin 1, and transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1). They observed that the magnitude of the response is both pressure-dependent and time-dependent. In addition, they found that physiological pressure gradients at a magnitude of 3 cmH<sub>2</sub>O have no impact on gene expression, whereas pressure gradients at a magnitude of 30 cmH<sub>2</sub>O result in substantial expression of the sentinel transcripts that were monitored.

Tschumperlin et al. (109) extended these studies with the use of human airway epithelial cells; in their initial studies, they found that the behavior of human airway epithelial cells in ALI culture is very similar to that of rat tracheal epithelial cells. Furthermore, they used imaging techniques to show that compressive stress reduces the height of airway epithelial cells by ~10%, which forces the cells to expand into the lateral intercellular space (i.e., the space between adjacent epithelial cells). Since the cells were cultured on a porous membrane, liquid in the lateral intercellular space was forced out of the membrane pores; Tschumperlin et al. postulated that the applied mechanical force recapitulated the mechanical impact of buckling in constricted airways (109). In the same report, they suggested that the compressive stress-induced reduction in the volume of the lateral intercellular space, coupled with the continued shedding of ligands into that space, most likely results in an increase in concentration of at least one type of shed ligand [epidermal growth factor (EGF)], which in turn could initiate the observed biological downstream effects. This postulated mechanism of mechanotransduction does not require the presence of a molecular entity that senses the compression; rather, the transduction is triggered by loss of volume in the lateral intercellular space and a continued fixed rate of shedding of ligands into that space.

In a follow-up study, Tschumperlin et al. used finite-element methods to calculate the potential concentrations of ligands in the lateral intercellular space (52). Using reasonable assumptions, they found that alterations in the geometry of the lateral intercellular space could impact the concentrations of constitutively shed ligands inside and below the cell layer. In their model, the maximal change in volume of the lateral intercellular space occurred ~10 min after the application of compressive stress. Finally, they used a three-dimensional imaging technique, with better temporal and spatial resolution than had been available at the time their initial work was done, to observe the evolution of mechanotransduction responses through changes in the concentration of local EGF

ligands such as heparin-binding EGF (HB-EGF) and transforming growth factor  $\alpha$  (TGF- $\alpha$ ) (52). They found that highly localized changes in ligand concentrations can be induced through mechanical loading, depending on both local deformations and the effects of ligand convection. They suggested that these localized ligand concentrations could lead to heterogeneity of cellular responses.

Shiomi et al. (87) continued these studies with the use of airway epithelial cells harvested from mice. Tschumperlin et al. had previously found that bronchoconstriction activates EGF receptor (EGFR) in the airway epithelium in mice; EGFR phosphorylation was induced in isolated murine lungs perfused via the trachea with methacholine but not in lungs perfused with PBS (109). Shiomi et al. found that the application of compressive stress to differentiated mouse tracheal epithelial cells in ALI culture induces phosphorylation of extracellular signal-related kinases 1 and 2 (ERK1 and ERK2) through EGFR activation; this response is similar to the responses detected in rat cells and human cells. The application of compressive stress also induces the expression of genes encoding EGF ligands such as HB-EGF, epiregulin, amphiregulin, and betacellulin. Shiomi et al. used airway epithelial cells derived from mice with a deficiency of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) converting enzyme (TACE) and found that TACE is a critical upstream molecule in the EGFR activation response to compressive stress.

These findings establish that mouse, rat, and human airway epithelial cells in ALI culture all have a predictable biological response to compressive stress. However, the nature of this response and its relationship to airway remodeling require further understanding.

## Extent to Which Compressive Stress Recapitulates Changes Consistent With Airway Remodeling

### EGFR Activation

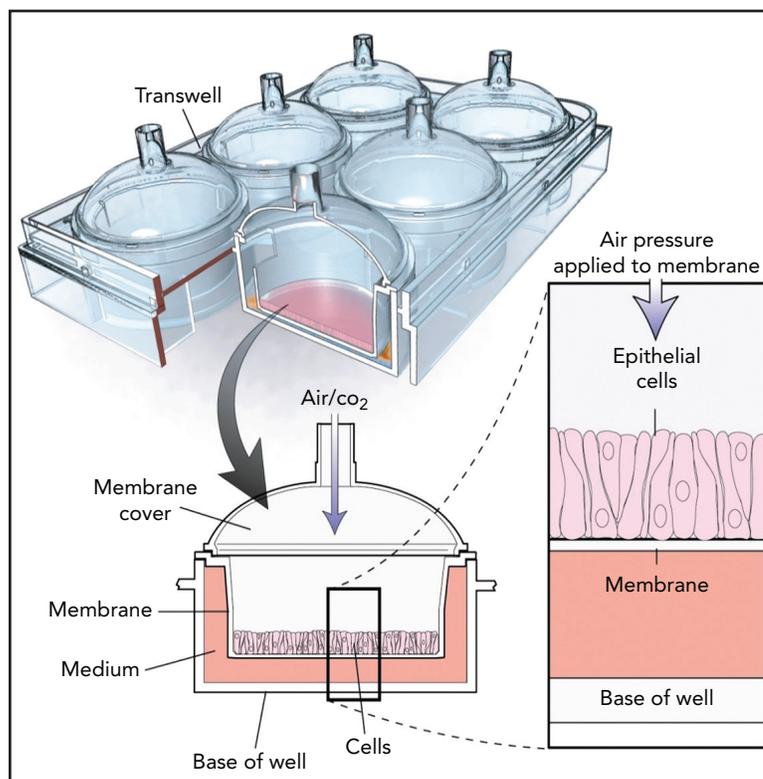
Studies from a number of investigative groups have shown that there are fundamental disorders in the asthmatic airway epithelium; the asthmatic epithelium has an aberrant repair process in which inflammatory signals are sustained by uncontrolled EGFR activation (38, 40, 71, 94). The deformation of airway epithelial cells that is caused by compressive stress not only activates EGFR but also affects EGFR-dependent transcriptomes in bronchial epithelial cells (51), indicating that compressive stress-induced local and transient deformation of epithelial cells recapitulates key characteristics of the asthmatic airway epithelium.

Compressive stress stimulates the phosphorylation of extracellular ERK and the expression of HB-EGF (111). The HB-EGF response to compressive stress is similar to that elicited in the same cells by exposure to TNF- $\alpha$  (1 ng/ml); combined mechanical and inflammatory stimulation is more effective than stimulation with either stimulus alone. Moreover, it has been shown that the induction of HB-EGF is EGFR-dependent; this suggests the presence of a mechanically activated EGFR autocrine loop with positive feedback that involves selected EGFR ligands (11).

### Activation of the Plasminogen System

The plasminogen system consists of serine proteases and their inhibitors; the system is not only involved in the cascade of actions leading to blood clotting but also activated in tissue repair (44). Activation of this system depends on an enzymatic chain reaction that is mainly regulated by two trypsin-like proteases: tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) (53, 82, 116).

In asthma, expression of the u-PA receptor (uPAR) is increased in the airway epithelium (91). Increased uPAR expression leads to the attenuation of wound-repair processes and may in turn contribute to the development and progression of airway remodeling in asthma. Consistent with this



**FIGURE 1. Schematic diagram of the in vitro compressive system** A transepithelial air-pressure gradient at a magnitude of 30 cmH<sub>2</sub>O is applied to primary human bronchial epithelial cells maintained in an air-liquid interface culture. Reprinted from Ref. 110, with permission from *Annu Rev Physiol*.

idea is the observation that the plasminogen level in the airway increases during an asthma exacerbation (97).

Chu et al. (10) found that the application of the compressive stress to human airway epithelial cells in ALI culture induces the expression of genes and proteins related to the plasminogen system, including u-PA, uPAR, plasminogen activator inhibitor 1 (PAI-1), and t-PA, as well as the activity of plasminogen activators. Results from this study further support the idea that compressive stress on airway epithelial cells, in the absence of inflammatory cells, can lead to processes that are characteristic of asthma, such as the induction of changes that are found in the hyperresponsive airway (121) and the initiation of events that lead to subepithelial fibrosis (86). Furthermore, expression of the gene encoding uPAR, *PLAUR*, has been associated with asthma susceptibility, and polymorphisms in that gene have been associated with differences in baseline lung function (92).

### **Collagen Deposition**

In asthma, deposition of extracellular matrix is a component of the thickened subepithelium (18, 41, 56, 74, 75). Roche et al. (75) have shown that the thickened subepithelium is composed of a layer of matrix that is positive for fibronectin and collagen types III and V; the collective amount of these materials is doubled in asthmatic airways (the tissue layer is 10–15  $\mu\text{m}$ ) compared with the amount in normal airways (5–8  $\mu\text{m}$ ).

Swartz et al. (93) expanded the model of compressive stress on airway epithelial cells to include a layer of reporter fibroblasts at the base of the Transwell used for ALI culture. These reporter cells are not exposed to mechanical stress but rather are bathed in a culture medium conditioned by cells in ALI culture that have been exposed to mechanical stress. This model allowed the investigators to examine how compressive stress on cells in ALI culture could facilitate intercellular communication between compressed epithelial cells and reporter fibroblasts (93); application of compressive stress on bronchial epithelial cells in ALI culture was associated with the release of fluid-phase signals and led to the proliferation of reporter fibroblasts and the production of collagen types I and III from these cells (FIGURE 2). As noted above, a cardinal feature of airway remodeling is deposition of collagen types I and III below the basement membrane of the airway (18, 41, 56, 75), and this study establishes that this feature of airway remodeling can be induced in vitro in the absence of inflammatory cells.

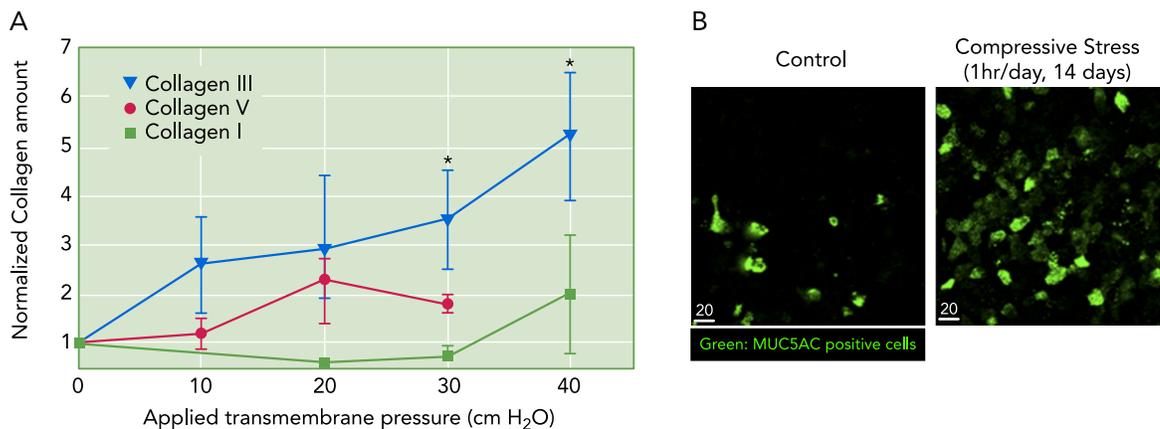
### **Goblet Cell Hyperplasia**

Goblet cells produce mucins, which form hydrated polymer gels (mucus) that line the airways (78, 126). Under normal conditions, mucus provides a pivotal defense against inhaled particles by trapping and facilitating mucociliary clearance, in cooperation with cilia in ciliated cells (50). However, in chronic diseases such as chronic obstructive pulmonary disease and asthma, mucus hypersecretion occurs, in part because of goblet-cell metaplasia and hyperplasia, and contributes to the morbidity and mortality associated with these airway diseases (13, 14, 21, 65, 126). Goblet-cell hyperplasia is a major remodeling event in asthma (16, 17, 37, 41, 56). Culture of primary normal human bronchial epithelial (NHBE) cells recapitulates the differentiated phenotypes of airway epithelial cells that are seen in vivo (79, 124, 128) and is routinely used to study the underlying mechanism of goblet-cell hyperplasia (35, 101, 124, 128). In vitro culture of NHBE cells has shown that cytokines associated with a Th2 response (i.e., IL-4, IL-5, and IL-13) (2, 77, 131), human neutrophil elastase (HNE) (67, 120), and cigarette smoking (76) induce goblet-cell hyperplasia and mucin overproduction.

Park and Tschumperlin (69) reported that mechanical compressive stress can induce goblet-cell hyperplasia in the absence of inflammatory cells and mediators. They applied intermittent compressive stress, mimicking the episodic reversible airway obstruction that occurs during asthma exacerbations, to well differentiated NHBE cells in ALI culture for an hour every day for 14 consecutive days, starting on the 14th day after ALI culture was established. The number of goblet cells was significantly increased in cells exposed to compressive stress, compared with the number in control cells; this was seen as early as 7 days after the initial application of compressive stress. Compressive stress-mediated goblet-cell hyperplasia is dependent on the activation of EGFR and TGF- $\beta$ 2. EGFR is an important signaling molecule in goblet-cell hyperplasia that is induced by other mediators such as IL-13 and HNE (7, 76, 112, 131). Chu et al. previously found that the expression of TGF- $\beta$ 2 is elevated in asthmatic human airways and that TGF- $\beta$ 2 is capable of increasing MUC5AC expression in NHBE cells (12).

### **YKL-40 Expression**

YKL-40, the protein encoded by the Chitinase-3-like protein 1 (*CHI3L1*) gene, has been found in bronchoalveolar (BAL) fluid and serum of patients with asthma. In genetic studies, *CHI3L1* has been associated with asthma in European and American populations (63, 72), with atopy in a Korean



**FIGURE 2. Collagen deposition and goblet cell hyperplasia in response to compressive stress**

A: the application of compressive stress significantly induces collagen type III production from fibroblasts in basolateral conditioned media collected from human bronchial epithelial cells. Reprinted from Ref. 93, with permission from the National Academy of Sciences (Copyright 2001). B: the application of chronic intermittent compressive stress induces goblet-cell hyperplasia in human bronchial epithelial cells. Reprinted from Ref. 69, with permission from the American Thoracic Society (Copyright 2015).

population (89) and with a risk of asthma in a Taiwanese population (107). Ober et al. (63) reported that *CHI3L1* is associated with asthma susceptibility and that an elevated level of circulating YKL-40 is a biomarker for asthma and an accelerated decline in lung function. An increased level of YKL-40 in the serum and BAL fluid is strongly correlated with bronchial hyperresponsiveness and loss of lung function.

Park et al. found that human bronchial epithelial cells in ALI culture are a source of YKL-40 and that the YKL-40 is released in response to compressive stress in a protein kinase C (PKC)-dependent manner (66). They also found that exposure to TNF- $\alpha$  induces the production of YKL-40 in human bronchial epithelial cells.

In asthma, expression of YKL-40 in the airway epithelium is positively correlated with smooth-muscle mass and promotes bronchial smooth-muscle cell proliferation and migration through a protease activated receptor 2 (PAR-2)-dependent mechanism (3). Because YKL-40 has a proangiogenic function, as shown by its promotion of tumorigenesis (85) and endothelial-tube formation (22, 85), the compressive stress-induced expression of YKL-40 might contribute to airway angiogenesis in asthma.

### Exosome Release

Exosomes are small membrane vesicles (40–120 nm in diameter) that are released by all types of cells and are found in biological fluids such as serum, BAL fluid, and extracellular matrix (99). Exosomes contain lipids, proteins, and genetic material such as mRNA and miRNA (61, 114); they constitute an effective vehicle to deliver molecules from one cell to another and thus function as a vehicle for intercellular communication (20, 98, 100, 103).

Park et al. reported that human bronchial epithelial cells in ALI culture release exosomes containing tissue factor in response to compressive stress (68). Exosomes are released basolaterally and contain transmembrane proteins, including EGFR and tissue factor. In a study in which cells were incubated with a PKC inhibitor, bisindolylmaleimide I, the release of exosomes containing tissue factor was dependent on PKC activation.

The importance of exosome release from epithelial cells is an area of active research. Vlahakis and Hubmayr (118) hypothesized that plasma membrane stress failure is a central event in the pathophysiology of ventilation-induced lung injury. They described deformation-induced lipid trafficking (DILT) in alveolar epithelial cells, and hypothesized that DILT is an adaptive mechanism that facilitates membrane growth and ultimately prevents membrane rupture after mechanical stress is applied to the plasma membrane during hyperventilation (119). A disrupted plasma membrane is rapidly resealed, and the resealing process depends on the exocytotic mechanism (102). Togo et al. described the healing process in the disrupted membrane of double-wounded fibroblast: at first wounding, an endocytic process adds the membrane necessary for resealing to the endocytotic compartment, and at second wounding, PKC, which is activated through Ca<sup>2+</sup> entry at first wounding, stimulates vesicle formation from the Golgi apparatus, resulting in rapid resealing of the second membrane disruption (102). Wirtz et al. (127) also found that the transient increase in Ca<sup>2+</sup> is a critical step in exocytosis in mechanically stretched alveolar epithelial cells.

It seems logical to assume that the exocytosis that occurs in alveolar epithelial cells and the release of exosomes from bronchial epithelial cells in ALI culture that occurs after the application of

compressive stress are similar processes in distinct but related tissue types.

### Differential Responses to Compressive Stress in Normal and Asthmatic Cells

Airway epithelial cells from asthmatic airways have clear differences from those found in normal airways, including impaired proliferation of basal and club cells, exaggerated secretion of cytokines and proteins associated with inflammation and remodeling, reduced expression of junction proteins, and aberrant injury-repair responses (39, 56, 58). In one study in which the response to compressive stress in normal cells was compared with the response in cells from asthmatic donors, higher levels of TGF- $\beta$  and granulocyte-macrophage colony-stimulating factor (GM-CSF) were released from cells derived from asthmatic donors (33). Unfortunately, the data on this subject are limited, and more research is needed to clarify the fundamental differences between airway epithelial cells from normal donors and asthmatic donors.

### Role of Bronchoconstriction in Airway Remodeling in Humans

Studies performed by Swartz et al. (93) and by Park and Tschumperlin (69) provide direct evidence that compressive mechanical stress, in the absence of inflammatory cells, can induce key phenotypic changes observed in asthmatic airways. The results of these *in vitro* studies were later validated in humans by Grainge et al. (34). The investigators induced bronchoconstriction in two groups of patients with mild cases of asthma by means of either repeated methacholine challenges or repeated allergen challenges. The challenges were performed four times at 2-day intervals, and transbronchial biopsies were performed 4 days after the last exposure. In addition, half the patients in the methacholine group received the challenges after pretreatment with albuterol to determine whether a bronchodilator is able to modify the histological and biochemical effects caused by the methacholine challenges. Airway remodeling was induced in patients in both the methacholine-alone group and the allergen group; compared with the baseline levels in those patients, there were an increased number of goblet cells (positive Periodic acid-Schiff stain) and a thickened subepithelium (positive stain for antibody against collagen type III) (FIGURE 3). There were no significant differences between the allergen and the methacholine groups with respect to these changes. Infiltration of eosinophils was present in patients in the allergen group but not in those in the methacholine

group, despite the remodeling events. Moreover, the methacholine-induced remodeling events were abrogated by pretreatment with albuterol, which inhibits bronchoconstriction, suggesting that bronchoconstriction alone can induce airway remodeling in humans. This clinical observation validates previous *in vitro* studies and provides strong evidence that compressive stress on airway epithelial cells is an important component of the clinical asthmatic response.

In addition to this study of airway constriction, there is a clinical mirror of mechanically induced airway narrowing in asthma. Numerous studies have shown that the combination of a long-acting beta-agonist (LABA) and an inhaled corticosteroid (ICS) is a far more effective treatment for asthma than high doses of ICS alone (36, 49, 54). These studies suggest that bronchodilation adds therapeutic benefit to inhaled corticosteroids, and it is not unreasonable to assume that this benefit derives from the “virtual anti-inflammatory effect” of bronchodilation. We do not believe that mechanical compression is the sole mechanism by which asthma exacerbates, so it is not surprising that bronchodilators on their own do not have anti-remodeling effects. Rather, we think that bronchodilation adds a dimension to asthma treatment that is not achieved by anti-inflammatory treatment alone. This idea is reinforced by the work of Kips et al. (49), who found that treatment with a combination of a low-dose ICS (budesonide) and a LABA (formoterol) has the same anti-inflammatory effects as treatment with a high-dose ICS (budesonide). In a study performed by Kelly et al. involving mildly asthmatic patients who were challenged with allergens, treatment with a combination of a LABA (formoterol) and an inhaled ICS (budesonide) resulted in fewer myofibroblast numbers and smaller smooth muscle mass than treatment with either component alone (47). If we make the reasonable assumption that the anti-inflammatory effects of ICS are dose-related, then the logical conclusion is that LABA augments the anti-inflammatory effect of ICS, as we contend through an anti-constriction mechanism as reviewed herein.

### Unanswered Questions

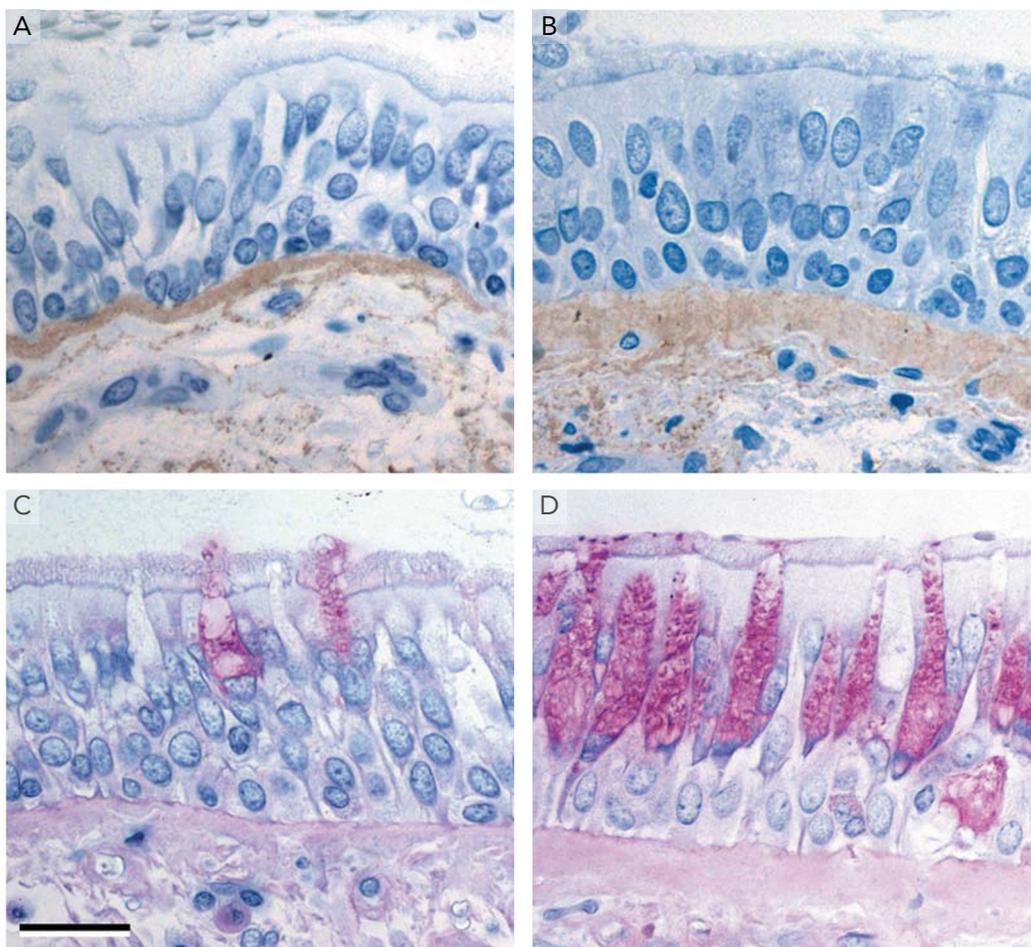
#### *Alteration of Innate Immunity*

Beyond the scope of remodeling, we speculate about unanswered questions and new approaches to explore unknown roles of mechanical stress in lung function and lung disease. For example, does bronchoconstriction impair the innate immune responses of airway epithelial cells? Recent studies have shown that EGFR activation induced by viral infection suppresses the production of interferon- $\lambda$  and CXCL-10, both of which have antiviral

functions in the airway epithelium (45, 113). These studies raise the question of whether bronchoconstriction impairs host defense mechanisms against viral infections through the induction of EGFR in patients with asthma. Grainge et al. have shown that compression induces secretion of IL-8 (33), although the mechanism remains unknown, and Huang et al. have shown that a static compression of A549 cells at a magnitude of 15 cmH<sub>2</sub>O, which is much lower than the magnitude of pressure measured in constricted airways, activates NF-κB (42). Compression-mediated activation of NF-κB is further induced by the pretreatment with jasplakinolide, an actin-polymerizing reagent. These observations suggest that bronchoconstriction itself probably alters the innate immunity of the airway epithelium. Therefore, further studies are needed for a better understanding of the relationship between mechanobiology and innate immunity.

### Collective Migration

Aberrant injury-repair response is a hallmark of asthma, but little is known about if or how mechanical stress contributes to this process. During the injury and repair process, it is well established that airway epithelial cells rapidly migrate to fill denuded areas and then further differentiate to restore normal barrier protective functions (19). It is known that coordinated communication between biochemical and mechanical signals guides the development and the maturation of the epithelium (32). In addition, migration of cells requires the initiation and transmission of physical forces from one cell to its immediate neighbors (1, 48, 96, 104–106, 117, 122). In collective cellular migration, cells act together in a coordinated fashion rather than as individual units (29). This collective behavior is not unique to the airway epithelial layer; it is seen also in tissue-remodeling events that underlie embryonic morphogenesis, wound repair, and cancer invasion (29, 80), in which cells move in



**FIGURE 3. Collagen deposition and goblet cell hyperplasia in response to bronchoconstriction in patients with mild asthma**

A and B: immunohistochemical staining of collagen type III is shown in brown before (A) and after (B) methacholine challenges. C and D: periodic acid-Schiff staining of goblet cells is shown in purple before (C) and after (D) methacholine challenges. Scale bar represents 30 μm. Reprinted from Ref. 34, with permission from the *N Engl J Med*.

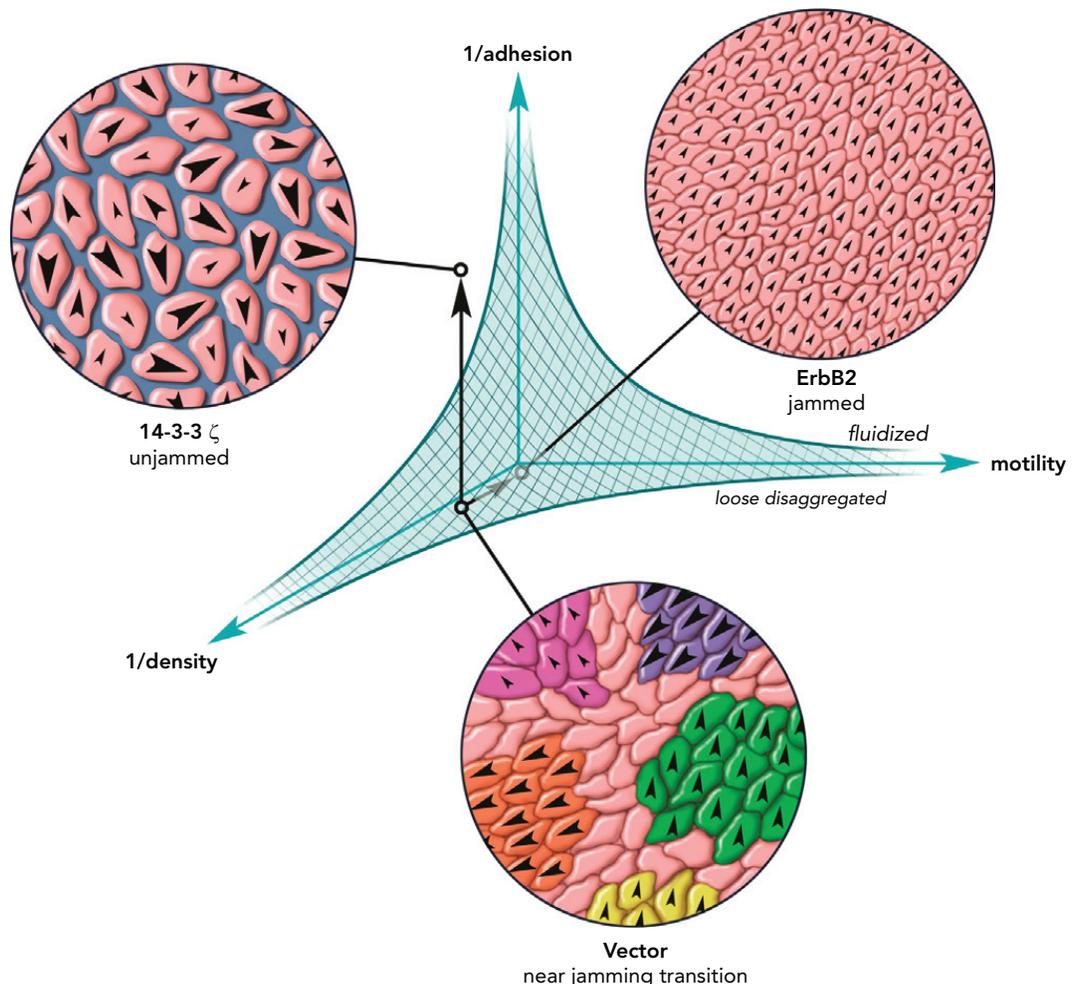
coordinated sheets, ducts, strands, and clusters (28, 29).

The multiple factors contributing to collective migration of cells can include cellular crowding, intercellular force transmission, cadherin-dependent cell-cell adhesion, integrin-dependent cell-substrate adhesion, myosin-dependent motile force and contractility, actin-dependent deformability, proliferation, stretch, and compression (4, 15, 25, 46, 90, 122). New tools are now available for studying the physical forces that each cell exerts on its substrate (9, 95) and the physical forces that each cell exerts on its immediate neighbors (48, 96). These new experimental approaches have led to the discovery that cellular collectives can become jammed, much as coffee beans become jammed in a chute (1, 5, 30, 96, 105). The jammed state is a solid-like state in which intercellular rearrangements are arrested (1, 30, 84, 96). Alternatively, in certain circumstances, the cellular collective can become unjammed and undergo a transition to a fluid-like state in which relatively

rapid intercellular rearrangements are potentiated (84, 96). We have proposed recently that these transitions between solid-like states and fluid-like states of the cellular collective might be governed by a jamming phase diagram (FIGURE 4) (80). However, the existence and nature of cell jamming in human bronchial epithelial cells and its relationship to asthma have yet to be studied.

## Conclusions

Compressive stress in vitro, mimicking the stress generated by bronchoconstriction in vivo, is sufficient to induce cellular changes consistent with airway remodeling, even in the absence of inflammatory cells or mediators. These in vitro studies were subsequently validated in living humans with the use of methacholine challenges. Together, this evidence suggests that bronchoconstriction is not only a consequence of asthma development and airway remodeling but also a rather important contributor. ■



**FIGURE 4.** A jamming phase diagram for the collective migration of the cellular monolayer. In the cellular monolayer, the transition between a jammed (solid-like) state and an unjammed (fluid-like) state might be governed by a jamming phase diagram. Reprinted from Ref. 80, with permission from *Differentiation*.

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