# Cell Jamming in the Airway Epithelium

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## Abstract

Hallmarks of asthma include chronic airway inflammation, progressive airway remodeling, and airway hyperresponsiveness. The initiation and perpetuation of these processes are attributable at least in part to critical events within the airway epithelium, but the underlying mechanisms remain poorly understood. New evidence now suggests that epithelial cells derived from donors without asthma versus donors with asthma, even in the absence of inflammatory cells or mediators, express modes of collective migration that innately differ not only in the amount of migration but also in the kind of migration. The maturing cell layer tends to undergo a transition from a hypermobile, fluid-like, unjammed phase in which cells readily rearrange, exchange places, and flow, to a quiescent, solid-like, jammed phase in which cells become virtually frozen in place. Moreover, the unjammed phase defines a phenotype that can be perpetuated by the compressive stresses caused by bronchospasm. Importantly, in cells derived from donors with asthma versus donors without asthma, this jamming transition becomes substantially delayed, thus suggesting an immature or dysmature epithelial phenotype in asthma.

**Keywords:** unjamming; cell mechanics; phase transition; bronchoconstriction

(Received in original form July 28, 2015; accepted in final form September 13, 2015)

*Author Contributions*: J.-A.P. and J.J.F. wrote and revised the manuscript.

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Ann Am Thorac Soc Vol 13, Supplement 1, pp S64–S67, Mar 2016

DOI: 10.1513/AnnalsATS.201507-476MG
Internet address: www.atsjournals.org

The lung routinely faces a variety of environmental stresses. With every breath, for example, irritants such as allergens, bacteria, viruses, or environmental pollutants can enter the lung (1, 2). These irritants trigger innate defense mechanisms, but these mechanisms can sometimes become dysregulated. Moreover, with every breath, the lung changes its size appreciably (3–5), and thereby stretches and distorts each of the lung's multiple cellular constituents (6–8). Under normal physiological conditions, these mechanical stresses, in turn, are known to activate cellular signaling cascades (6, 9, 10) that guide branching morphogenesis and alveolar proliferation *in utero* and in early life (11, 12). For example, mechanical stress is upstream of the Hippo pathway that includes the activation of kinases, MST1/2 and LATS1/2, and the inactivation of cofactors YAP/TAZ (13). More interestingly, Varner and colleagues showed that a purely physical mechanism can control the pattern of the airway branching during embryonic lung development (14). But under certain pathologic conditions, such as during ventilator-induced lung injury or during bronchospasm, excessive mechanical stresses can impair normal lung function and cause aberrant airway repair after injury (4). Here we focus on mechanical stresses that arise spontaneously within the airway epithelium as a result of bronchospasm and the role these stresses play in airway remodeling in asthma.

How does mechanical stress impact airway epithelial cells? We now know that mechanical stress initiates the activation of signaling cascades and the release of fibrotic and inflammatory mediators (15–17). But as described below, compressive stress also causes mature airway epithelial cells to move in collective fluid-like swirls, whereas otherwise they are solid-like and virtually immobile. In this article, we discuss these phenomena in relation to the process of airway remodeling in asthma.

## Activation of Epithelial Cells, Compressive Stress, and Bronchoconstriction

The transmural stress that distends the airway wall is supported by the outward tethering recoil of the lung parenchyma and is well approximated by the transpulmonary pressure (6, 18). During bronchoconstriction, however, the airway becomes compressed by the contraction of airway smooth muscle, and the airway epithelium then buckles into a rosette pattern (19). Finite-element modeling (20) shows that during maximal

bronchoconstriction, airway smooth muscle can exert a compressive stress on the airway epithelial layer of approximately 30 cm $H_2O$, which is much greater than the compressive stresses imposed on the epithelial layer during normal breathing or exercise.

Using rat tracheal epithelial cells maintained in air–liquid interface (ALI) culture, Ressler and colleagues showed that compressive stress causes rapid and robust epithelial cellular responses, including messenger RNA expression of early growth responsive protein 1, endothelin 1, and transforming growth factor β1 (21). Tschumperlin and colleagues then showed that primary human airway epithelial cells behave in a similar fashion, and suggested that associated squeezing of the lateral intercellular space (LIS) between epithelial cells, combined with shedding of epidermal growth factor into that space, leads to a novel mechanism of mechanotransduction (22). Using computational modeling, they concluded that the volume of the LIS changed maximally at 10 minutes after compression, at which time the concentration of heparin-binding epidermal growth factor (HB-EGF) in the LIS became readily increased (23). Subsequently, using a murine model, Shiomi and colleagues showed that activation of tumor necrosis factor α (TNF-α) converting enzyme is critical in the epidermal growth factor receptor (EGFR) activation response to compressive stress (24).

In bronchial epithelial cells, activation of EGFR and changes in EGFR-dependent transcriptomes are impacted by deformation of airway epithelial cells (25). The activation of EGFR results in the induction of the HB-EGF expression and phosphorylation of extracellular signal–regulated kinase (26). In the same cell, compared with responses elicited by exposure to TNF-α (1 ng/ml), the responsiveness of HB-EGF expression to compressive stress is similar. Mechanically activated EGFR initiates a positive feedback autocrine loop that involves the induction of EGFR ligands (27).

Together, these results establish that compressive mechanical stress mimicking bronchoconstriction initiates signal transduction pathways and provokes the production of asthma-associated mediators known to be implicated in airway remodeling.

## Compressive Stress and Airway Remodeling

In asthmatic airways, airway epithelial cells display impaired proliferation of basal and club cells, together with exaggerated secretion of proinflammatory and profibrotic mediators and proteins, a reduced level of junctional proteins, and aberrant injury-repair responses (28–30). In cells from donors with asthma, there is evidence indicating higher levels of transforming growth factor β and granulocyte/macrophage colony-stimulating factor (31) in response to compressive stress, suggesting an exaggerated response of asthmatic cells to mechanical stress. In asthma, thickening of the subepithelium, which is composed of fibronectin and collagen types III and V, is a major remodeling event (32). Swartz and colleagues showed that the application of compressive stress significantly induces collagen type III production from fibroblasts that were conditioned with basolateral media collected from compressed normal human bronchial epithelial cells (15). In asthma, goblet-cell hyperplasia or metaplasia is also a major remodeling event (28, 33–36). Using primary normal human bronchial epithelial cells in ALI culture, Park and Tschumperlin showed that goblet-cell hyperplasia is induced by intermittent compressive stress (16). Under normal conditions, mucus produced from goblet cells protects the lung against inhaled irritants by trapping those irritants and then clearing them toward the airway opening through the action of the mucociliary escalator (37). With goblet cell hyperplasia or metaplasia, however, mucus hypersecretion can contribute to airway obstruction, which in turn is responsible for the morbidity and mortality that are associated with chronic airway diseases such as chronic obstructive pulmonary disease and asthma (38–42).

These *in vitro* studies suggested that airway remodeling can be induced independent of inflammatory cells, and these *in vitro* studies were later validated in humans *in vivo* (17); in patients with mild asthma, Grainge and colleagues (17) showed that repeated bronchoconstriction causes an increased number of goblet cells and a thickened subepithelium. As such, it is now clear that mechanical stress acting on the airway epithelium is sufficient by itself to induce a host of changes associated with the remodeled asthmatic airway.

## Unanticipated Implications for Asthma Therapy

Even in the complete absence of augmented inflammatory cells or mediators, key phenotypic changes as observed in asthmatic airways can be induced by compressive mechanical stress alone (15–17). Could it be, therefore, that amelioration of bronchospasm might also attenuate remodeling events triggered by mechanotransduction pathways, and thus add to asthma treatment strategies in a dimension that was unanticipated previously? In that connection, numerous studies have shown that the combination of a long-acting β2-agonist (LABA) and an inhaled corticosteroid (ICS) is a far more effective treatment for asthma than high doses of ICS alone (43–45). For example, the combination of a low-dose ICS (budesonide) with a LABA (formoterol) has no more antiinflammatory effect than does a high-dose ICS (budesonide) alone (45). Similarly, post-treatment of allergen challenged individuals with asthma with LABA (formoterol) together with inhaled ICS (budesonide) reduces the number of myofibroblasts and the mass of airway smooth muscle compared with either treatment alone (46). The combination of LABA (salmeterol) with ICS (fluticasone propionate) also showed the greatest improvement in asthma endpoints, including the magnitude of change in morning peak expiratory flow, asthma symptoms, and rescue β2-agonist use, compared with ICS (fluticasone propionate) alone (47). Combination therapy can achieve better asthma control and a reduction of exacerbation risk and it is more effective in adults than in children (48).

Together, these findings suggest that LABA acting through its amelioration of bronchospasm may augment the antiinflammatory effect of ICS.

## Jamming and Unjamming of Bronchial Epithelial Cells

Using primary human bronchial epithelial cells derived from donors without and with

asthma and studies in ALI culture, Park and colleagues have recently established that a pseudostratified layer derived from donors without asthma is quiescent (49). Cellular motions are relatively small, cellular rearrangements among neighboring cells are rare, and each cell remains virtually caged by those immediate neighbors. Statistical analyses of these motions confirm that such a layer is solid-like and jammed. However, application of an apical-to-basal mechanical stress mimicking the compressive effect of bronchospasm (16, 17, 22) is sufficient to trigger large cellular motions and cooperative cellular rearrangements. Cells move chaotically, but the motions exhibit cooperative packs and swirls; such a layer is fluid-like and unjammed. When they examined the more complex process of progressive layer maturation, they found an innate tendency of the maturing layer to transition from an immature, fluid-like, unjammed phase in which cells readily rearrange and flow, into a mature, solid-like, jammed phase in which cells become virtually frozen in place (50–52). As compared with donors without asthma, however, in the maturing layer derived from donors with asthma this jamming transition is delayed substantially or disrupted altogether. Whether this delay arises from layer injury, immaturity, or dysmaturity remains unclear.

## Where Do We Go from Here?

Open questions remain concerning unknown roles for mechanical stress in lung function and lung disease. The potential bronchoconstriction in the innate immune responses of the airway epithelial is particularly interesting because EGFR activation caused by viral infection suppresses the production of IFN-λ and CXCL-10, both of which are antiviral in the airway epithelium (53, 54). Although the mechanism remains unknown, compression of bronchial epithelial cells induces secretion of IL-8 (31), and compression of A549 cells activates nuclear factor-κB (55). These observations suggest that the innate immunity of the airway epithelial cells may be altered by bronchoconstriction itself. And as described above, the compressive stresses generated by bronchoconstriction are sufficient to induce cellular changes consistent with airway remodeling, even in the absence of inflammatory cells or mediators (15–17). Together, this evidence suggests that bronchoconstriction is not only a consequence of asthma progression and associated airway remodeling, but also a rather important contributor to these processes. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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