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Mechanical stretch has contrasting effects on mediator release from bronchial epithelial cells, with a rho-kinase-dependent component to the mechanotransduction pathway

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Summary
Introduction: In vivo, the airway epithelium stretches and relaxes with each respiratory cycle, but little is known about the effect this pattern of elongation and relaxation has on bronchial epithelial cells. We have used a model of cell deformation to measure the effect of stretch on inflammatory cytokine release by the BEAS 2B cell line, and to examine the method of mechanotransduction in these cells.
Methods: BEAS 2B cells were cyclically stretched using the Flexercell system. IL-8 and RANTES protein and RNA levels were measured after different elongations, rates and duration of stretch. An inhibitor of Rho (*R*as *H*omologous)-associated kinases was used, to assess the effect of blocking downstream of integrin signalling. Immunofluorescent staining of paxillin was used to study the effect of stretch on the distribution of focal contacts and the organisation of the actin cytoskeleton.
Results: IL-8 release by BEAS 2B cells was increased by cytokine stimulation and stretch, whereas RANTES levels in the cell supernatant decreased after stretch in a dose-, time- and rate-dependent manner. Thirty percent elongation at 20 cycles/min for 24 h increased IL-8 levels by over 100% ($P<0.01$). Blocking rho kinase using Y-27632 inhibited the effect of stretch on IL-8 release by the BEAS 2B cells. Immunofluorescent staining demonstrated that stretch caused dramatic disassembly of focal adhesions and resulted in the redistribution of paxillin to the peri-nuclear region.

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Conclusion: This study demonstrates a marked effect of stretch on bronchial epithelial cell function. We propose that stretch modulates epithelial cell function via the activation of rho kinases. The observation that stretch promotes focal adhesion disassembly suggests a mechanism whereby focal adhesion *turnover* (coordination of assembly and disassembly) is essential for mechanotransduction in bronchial epithelial cells.

Introduction

The bronchial epithelium has an important role in both lung homoeostasis and in modulating airway inflammation. Through the production of pro- and anti-inflammatory cytokines, it is able to affect the migration of leucocytes in the airway submucosa and lumen and modulate the function of mesenchymal cells, including myofibroblasts and airway smooth muscle cells. In various diseases including asthma, cytokine production is increased, stimulated by mediators such as TNFα and IFNγ.[1–3]

The airway epithelium stretches and relaxes during the normal respiratory cycle. Hyperexpansion or hyperventilation of the lung exaggerates this effect, and can result in changes to lung physiology. Deep inspiration causes bronchodilation,[4–8] and inhibition of deep inspiration airway hyper-responsivity.[9,10] Mechanical ventilation causes lung distension and has been linked to changes in the inflammatory profile of the lung. When lung resistance is increased, as in acute respiratory distress syndrome (ARDS), lung compliance falls, magnifying airway pressure and resultant lung deformation. Reducing ventilatory pressures has a significant effect on mortality in patients with ARDS,[11,12] with the Network ARDS study 12 reporting a 22% reduction in mortality of patients with ARDS when a limited ventilatory strategy was employed.

Stretching of the airway therefore has a profound effect on lung function and airway mediator secretion, and has been proposed as a method of initiating a potentially injurious inflammatory response. Studies using isolated animal lungs have shown increased release of inflammatory cytokines compared with controls.[13,14] Work with animal models of ARDS has shown increased levels of both lung and systemic cytokines with higher pressure ventilatory strategies,[15,16] and persistent elevation of plasma cytokines has been shown to be associated with increased mortality in patients with ARDS.[17]

Despite the potential physiological importance of stretch on the bronchial epithelium, most experiments have hitherto been performed using static cultured cells. Recent publications have begun to study the effect of stretch on isolated bronchial epithelial cell lines. For example, Vlahakis et al.[18] used the A549 epithelial cell line to show that cyclic stretch increased production of IL-8 in the absence of structural cell damage. A subsequent paper,[19] also with A549 cell line, reported a stretch-induced increase in IL-8 and transforming growth factor-β1 release after 40% elongation, but no significant increase with 20% or 12%. Oudin and Pugin[20] reported increased IL-8 from BEAS 2B cell cultures exposed to 20% stretch at 20 cycles/min for 8 and 24 h.

We hypothesised that stretching the BEAS 2B cell line, a model of bronchial epithelial cells, would produce varying responses in inflammatory mediator release. We aimed to use this model to assess the role of integrin signalling in transducing responses to mechanical stretch. Integrins are the principal receptors for extracellular matrix (ECM) proteins expressed by the adult human bronchial epithelium. Adult human airway epithelial cells express five integrins constitutively, and are reported to express up to eight with appropriate stimulation.[21,22] As transmembrane receptors, they are key to the formation of links between the ECM, the actin cytoskeleton and various intracellular signalling cascades.[23] When integrins bind to ECM proteins, an increase in lateral clustering and occupancy of integrin–ligand binding sites occurs. It is thought that integrins also undergo a conformational change at this time, enabling the intracellular domain to interact with cytoplasmic focal adhesion molecules, such as talin, paxillin and α-actinin.

In fibroblasts, cyclic stretch stimulates the alignment of the actin cytoskeleton and this has been shown to be dependent on tyrosine phosphorylation of a number of proteins present at the focal adhesion, such as $p130^{cas}$, $pp125^{FAK}$ and paxillin, as well as the activation of RhoA; a GTPase critical for the assembly of focal adhesions.[24] The activity of this catalogue of adaptors, kinases and GTPases is known to impinge upon intracellular signalling pathways (such as the ras–raf–MEK–ERK axis) that control a wide range of cellular functions. Indeed,