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Asthmatic and Normal Respiratory Epithelial Cells Respond Differently to Mechanical Apical Stress

To the Editor:

Mechanotransduction is increasingly appreciated as being relevant to cell activation. This is pertinent to asthma, in which airway smooth muscle contraction, leading to bronchoconstriction, compresses airway epithelial cells. Several studies have shown that compressive force comparable with that arising *in vivo*, with bronchoconstriction, promotes bronchial epithelial cell activation *in vitro*, with generation and release of growth factors and cytokines (1, 2). We have demonstrated the relevance of mechanotransduction to asthma *in vivo*, with repeated bronchoconstriction inducing epithelial activation and promoting airway remodeling changes (3). These findings are supported by *ex vivo* work demonstrating up-regulation of contractile proteins and the activation of transforming growth factor β (TGF- β) after airway narrowing (4). It is, however, unclear whether epithelial responses to bronchoconstriction reflect a normal airway response to mechanotransductive stimuli or whether there is an altered response in asthmatic bronchial epithelial cells.

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Author Contributions: C.G. designed the study, performed the experiments, analyzed the data, and wrote the manuscript. P.D. and L.L. performed the experiments and analyzed the data. D.D. and P.H. designed the study and wrote the manuscript.

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The epithelium in asthma is structurally and functionally abnormal, with increased production of mucus, higher permeability, a greater sensitivity to oxidants, and a deficient innate immune response to virus (5, 6). Interaction of an abnormal epithelium in asthma with underlying mesenchyme may result in deviation of immune, inflammatory, and structural responses down abnormal pathways in response to external stimuli (7). Although response to compression has not been assessed in asthmatic epithelial cells, an abnormal response to physical stress may provide an additional pathway for asthma pathogenesis.

We hypothesized that mature primary bronchial epithelial cells (BECs) cultured *in vitro* from volunteers with and without asthma would respond similarly to a compressive stimulus mimicking bronchoconstriction and that the epithelial response to bronchoconstriction *in vivo* was a normal response to this stimulus.

To test this hypothesis, BECs were obtained by brushings at bronchoscopy from adult volunteers, both with and without a diagnosis of asthma, to establish primary epithelial cell cultures. Patients with asthma ($n = 13$) had a physician diagnosis of asthma and had either a provocative concentration of methacholine, causing a 20% fall (PC_{20}) in the forced expiratory volume in 1 second of less than 8 mg/ml, or were receiving high-dose maintenance inhaled corticosteroids. Healthy volunteers ($n = 11$) had no respiratory disease and a methacholine PC_{20} higher than 16 mg/ml (see online supplement). All participants gave written informed consent, and the study was approved by the local research ethics committee. BECs were cultured and taken to an air-liquid interface (ALI) at passage 2, as previously described (5, 6). Cultures were used at ALI day 21 when confluent and when the transepithelial resistance was greater than $3,000 \Omega \cdot \text{cm}^2$. Sixteen hours before compression, ALI medium was replaced with minimal medium, comprising a 1:1 mixture of Dulbecco's modified eagle medium and bronchial epithelial basal medium plus insulin, transferrin, penicillin, and streptomycin. Cell compression was performed by increasing air pressure above the ALIs to 30 cm H_2O pressure, using humidified 5% CO_2 in air for 1 hour (1, 2). Cells were also subject to sham compression. Basal medium was analyzed 24 hours after compression. Two transwells from each volunteer were used in each of the sham and compression

groups of the study. Total TGF- β ELISA (R&D Systems, Abingdon, UK) and bead-based multiplex analysis (Invitrogen, Paisley, UK) were performed on basal medium to manufacturer's instructions.

Results

There were no differences in mediator measures between the groups with and without asthma after sham compression. Interleukin 8 (IL-8) and vascular endothelial growth factor (VEGF) were significantly increased after compression in both normal and asthma-derived cells, whereas TGF- β and granulocyte monocyte colony stimulating factor (GM-CSF) were only increased after compression of cells derived from donors with asthma, with this difference being significant within and between groups (Table 1, Figure 1).

Discussion

We identified no difference between asthmatic and healthy epithelial cells in the basal output of IL-8, VEGF, GM-CSF, or TGF- β , consistent with other publications, but did reveal the differing potential of asthmatic and healthy BECs to respond to compressive force. We demonstrate that bronchial epithelial cell responses to mechanical compressive stress are a normal physiological mechanism, with similar release of IL-8 and VEGF from healthy and asthma-derived cells, whereas TGF- β and GM-CSF concentrations in basal medium only increased after mechanical compression of cells from individuals with asthma. This is the first study demonstrating a differential response of asthmatic and normal BECs to mechanical compression.

The reason for the additional response in asthmatic cells is currently unclear, but in comparison with healthy airway epithelium, the asthmatic epithelium is morphologically distinct, with reduced expression of adhesion molecules including E-cadherin and ZO-1 and increased expression of the epidermal growth factor receptor (8, 9). During apical compression of epithelial cells, there is a reduction in the volume of the lateral intercellular space, leading to increased activation of the epidermal growth factor receptor by heparin-binding epidermal growth factor (2, 10, 11). Potentially, this pathway underlies the

Table 1. Results of Compression of Air-Liquid Interface Cultures from Normal Subjects and Patients with Asthma

	Normal				Asthma				<i>P</i> Value for Between-Group Change
	Sham Compression	Active Compression	Change	<i>P</i> Value for Within-Group Change	Sham Compression	Active Compression	Change	<i>P</i> Value for Within-Group Change	
VEGF, pg/ml	38.2 (26.1 to 61.5)	63.5 (45.7 to 92.2)	17.2 (4.1 to 30.7)	0.014	54.3 (40.0 to 75.6)	75.1 (62.1 to 100.2)	22.1 (1.2 to 39.6)	0.039	0.64
IL-8, ng/ml	15.94 (11.89 to 20.28)	23.37 (16.96 to 26.15)	5.53 (1.94 to 9.06)	0.014	19.71 (9.13 to 26.06)	23.23 (14.24 to 27.44)	3.52 (0.48 to 6.04)	0.048	0.27
TGF- β , ng/ml	191.9 (120.0 to 231.6)	211.2 (119.1 to 266.7)	-4.3 (-35.4 to 29.8)	1.00	163.1 (89.7 to 266.9)	218.8 (111.5 to 330.1)	35.0 (10.4 to 103.1)	0.011	0.03
GM-CSF, pg/ml	60.1 (49.6 to 63.3)	53.2 (49.4 to 60.1)	0.0 (-7.3 to 7.1)	0.81	49.6 (47.6 to 56.7)	69.9 (55.0 to 80.5)	13.1 (5.4 to 20.0)	0.002	0.002

Concentrations of cytokines in basal media from primary bronchial epithelial cells after compression for 1 hour at 30 cm H_2O pressure. All measurements are expressed as median (interquartile range). *P* values within group were calculated by Wilcoxon signed-rank test, the difference in the change between asthma and normal cells calculated using Mann-Whitney *U* testing.

Definition of abbreviations: GM-CSF = granulocyte monocyte colony stimulating factor; IL8 = interleukin 8; TGF- β = transforming growth factor beta; VEGF = vascular endothelial growth factor.

Figure 1. Cytokine release into the basolateral medium of air-liquid interface cultures from donors with and without asthma 24 hours after apical compression with 5% CO₂ in air at 30 cm H₂O pressure for 1 hour. GM-CSF = granulocyte monocyte colony stimulating factor; IL-8 = interleukin 8; ns = not significant; TGF-β = transforming growth factor β; VEGF = vascular endothelial growth factor. Bars indicate median values and interquartile ranges. **P* < 0.05, calculated using Wilcoxon signed-rank test within groups and the difference in the change between asthma and normal cells using Mann-Whitney *U* testing.

additional TGF-β responses to compression we observed. In our study, compression did not lead to increased TGF-β release from normal cells, although this has previously been described (2). The ALI culture duration (day 10–14, rather than at day 21 in our study) and the small numbers of donors (1, 2) used previously may explain the discrepancy in the observed results. We were unable to identify any significant correlation between clinical measures of asthma (PC₂₀ to methacholine, percentage predicted forced expiratory volume in 1 second, or reversibility) and change in TGF-β or GM-CSF concentrations with compression.

TGF-β and GM-CSF release after airway compression may contribute to epithelial mast cell and eosinophil recruitment, both of which are features of the asthmatic disease process (12, 13). In addition, TGF-β, through mesenchymal signaling, is likely to promote myofibroblast transformation and enhance collagen synthesis and subbasement membrane collagen deposition, as well as induce increased smooth muscle contractile protein expression (4, 14), all features of airway wall remodeling. Epithelial VEGF and IL-8 release has relevance in asthma with regard to angiogenesis and neutrophil recruitment. Such release may also explain the reported airway neutrophilic inflammation and vascular remodeling described in nonasthmatic individuals with chronic cough (15).

This study provides, for the first time, a direct comparison of the response of primary epithelial cells from volunteers with and without asthma to compressive force and further adds to the weight of evidence for the relevance of airway mechanical compressive forces induced by bronchoconstriction to the asthmatic disease process. ■

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Inhaled Antibiotics or Inhaled Hazard?

To the Editor:

Palmer and Smaldone are to be applauded for their recent study of the effects of inhaled antibiotics on the eradication of multidrug-resistant organisms in intubated patients with suspected pneumonia in the intensive care unit (1). Their study is a two-arm study in a single center. However, are these authors able to conclude whether the inhaled antibiotics reduced the acquisition of multidrug-resistant organisms in the treated group? Is it possible that the inhaled placebo increased the acquisition of multidrug-resistant organisms in the control group (2) as a paradoxical effect (3)? Is it possible to distinguish between the contextual effects versus the counterfactual effects of topical (or inhaled) antibiotic without an untreated reference group as a third arm (4)? ■

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Reply

From the Authors:

We appreciate Dr. Hurley's thoughtful query on our study of the effects of inhaled antibiotics on the eradication of multidrug-resistant organisms (MDROs) in the respiratory cultures of intubated patients (1). In our study, we had four main observations: existing MDROs in the treatment group were eradicated, new resistance to the inhaled antibiotic in the treatment group was not seen, existing MDROs in the placebo group were not eradicated, and new MDROs emerged in the placebo group.

Dr. Hurley asks whether there might be a paradoxical effect of the inhaled treatment that increased MDRO infection in our control group. He argues that without a third study arm (e.g., no placebo), we cannot prove that at least some of our observations were not the result of an effect of our treatment on our placebo arm.

Dr. Hurley's concern arises from his exhaustive analysis of trials of selective digestive decontamination (SDD; his analysis included 37 observational studies and 58 investigations with control and intervention arms) (2). *Staphylococcus aureus* emergence in patients with ventilator-associated pneumonia during these studies was seen in both the intervention and control arms of the groups receiving topical paste placebo or active drug paste. The mean isolate proportion (percentage of ventilator-associated pneumonia microbiology per 100 isolates) was 22 in the control arms and 44 in the intervention arm. Regarding SDD, we share Dr. Hurley's concern.

He postulates that the increased *S. aureus* observed in the intervention arms could cause more cross-contamination and