CHEST

NADPH Oxidase-4 Overexpression Is Associated With Epithelial Ciliary Dysfunction in Neutrophilic Asthma

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VIDEO

BACKGROUND: Bronchial epithelial ciliary dysfunction is an important feature of asthma. We sought to determine the role in asthma of neutrophilic inflammation and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases in ciliary dysfunction.

METHODS: Bronchial epithelial ciliary function was assessed by using video microscopy in fresh ex vivo epithelial strips from patients with asthma stratified according to their sputum cell differentials and in culture specimens from healthy control subjects and patients with asthma. Bronchial epithelial oxidative damage was determined by 8-oxo-dG expression. Nicotinamide adenine dinucleotide phosphate oxidase (NOX)/dual oxidase (DUOX) expression was assessed in bronchial epithelial cells by using microarrays, with NOX4 and DUOX1/2 expression assessed in bronchial biopsy specimens. Ciliary dysfunction following NADPH oxidase inhibition, using GKT137831, was evaluated in fresh epithelial strips from patients with asthma and a murine model of ovalbumin sensitization and challenge.

RESULTS: Ciliary beat frequency was impaired in patients with asthma with sputum neutrophilia (n = 11) vs those without (n = 10) (5.8 [0.6] Hz vs 8.8 [0.5] Hz; $P = .003$) and was correlated with sputum neutrophil count ($r = -0.70$; $P < .001$). Primary bronchial epithelial cells expressed DUOX1/2 and NOX4. Levels of 8-oxo-dG and NOX4 were elevated in patients with neutrophilic vs nonneutrophilic asthma, DUOX1 was elevated in both, and DUOX2 was elevated in nonneutrophilic asthma in vivo. In primary epithelial cultures, ciliary dysfunction did not persist, although NOX4 expression and reactive oxygen species generation was increased from patients with neutrophilic asthma. GKT137831 both improved ciliary function in ex vivo epithelial strips (n = 13), relative to the intensity of neutrophilic inflammation, and abolished ciliary dysfunction in the murine asthma model with no reduction in inflammation.

CONCLUSIONS: Ciliary dysfunction is increased in neutrophilic asthma associated with increased NOX4 expression and is attenuated by NADPH oxidase inhibition.

CHEST 2016; 149(6):1445-1459

KEY WORDS: asthma; epithelial cells; NOX4; oxidative stress

ABBREVIATIONS: ALI = air-liquid interface; CBF = ciliary beat frequency; DUOX = dual oxidase part of the NOX/DUOX family; GINA = Global Initiative for Asthma; mRNA = messenger RNA; NADPH = nicotinamide adenine dinucleotide phosphate; NOX = nicotinamide adenine dinucleotide phosphate oxidase part of the NOX/DUOX family; OVA = ovalbumin; ROS = reactive oxygen species

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Normal mucociliary clearance is essential in pulmonary defense.[1] Abnormal mucociliary clearance is a feature of asthma, particularly in severe disease, as a consequence of ciliary dysfunction.[2] This ciliary dysfunction might contribute to the persistent inflammation and susceptibility to infection in the asthmatic airway, as evidenced by higher bacterial DNA level[3,4] and fungal colonization, in particular *Aspergillus fumigatus*.[5]

Importantly, asthma is a heterogeneous disease and in addition to the Th2-mediated eosinophilic paradigm, neutrophilic-predominant inflammation is a feature of one third of patients with asthma.[6-9] Although the cause of neutrophilic asthma is unclear, it is associated with increased presence of proinflammatory and Th1 cytokines in sputum and bacterial colonization.[10] Pathogens can exert direct toxic effects on ciliary function or indirectly via oxidative stress,[11] and stimuli such as infection,[12] pollutants[13] and proinflammatory mediators[14] can induce production of reactive oxygen species (ROS). The nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)/dual oxidase (DUOX) family plays an important role in the generation of ROS and contains seven members: NOX1-5 and DUOX1/2.[15] DUOX expression in the bronchial epithelium has been reported,[16-18] and DUOXs have been shown to be important for neutrophil recruitment to the airways.[19] In addition, we have previously reported that NOX4 expression was increased in airway smooth muscle in asthma, leading to increased ROS production and intrinsic airway smooth muscle hypercontractility.[20] Whether NOX/DUOX expression is altered in the bronchial epithelium and possibly contributes to an increased susceptibility to ciliary dysfunction in asthma is unknown.

We hypothesized that ciliary dysfunction in asthma is due to a combination of an intrinsic abnormality in ciliary function and airway inflammation. To test our hypothesis, we assessed: (1) the ciliary beat frequency (CBF) in fresh ex vivo epithelial cells from patients with asthma with and without sputum neutrophilia, (2) the role of NADPH oxidases in ciliary function and their specificity to the neutrophilic asthma phenotype, and (3) the effects of NADPH oxidase inhibition on ciliary function in a murine in vivo ovalbumin (OVA) sensitization and challenge model.

Methods

A more detailed description of the methods is available in e-Appendix 1.

Subjects

Patients with asthma and healthy control subjects were recruited from a single center (Glenfield Hospital). Asthma severity was defined by using the Global Initiative for Asthma (GINA) treatment steps (mild to moderate asthma, GINA steps 1-3; severe asthma, GINA steps 4-5).[21] The study was approved by the Leicestershire Ethics Committee (UHL 10613), and patients gave their written informed consent.

Epithelial Cells

Primary epithelial cells were isolated from bronchial brushes during bronchoscopy. Experiments were undertaken by using epithelial human bronchial epithelial cells, characterized by cytokeratin 5 and 14 (Abcam) expression.[22] Ciliary function was assessed in ciliated air-liquid interface (ALI) cultures or fresh epithelial strips by using video microscopy as previously described.[23,24]

Immunohistochemistry and Immunofluorescence

Human bronchial biopsy specimens were embedded in glycomethacrylate.[20] Sections were stained by using an 8-oxo-dG monoclonal antibody, anti-NADPH oxidase 4 antibody (Abcam), anti-DUOX1 (Abcam), and anti-DUOX2 (Millipore) or corresponding isotype control (DAKO and ImmunoStep). Staining intensity above isotype control for 8-oxo-dG expression was assessed by using a semiquantitative scoring ranging from none to low, moderate, or high staining (0-3). For NOX4 and DUOX1/2, staining intensity was measured in all areas of epithelium by thresholding using Cell^F software (Olympus). All assessments were made by an observer blinded to the subjects' clinical characteristics. Cytospins of human bronchial epithelial cells were labeled with polyclonal rabbit antibodies to NOX1 and NOX4 (4 μg/mL, Insight Biotechnology; 4 μg/mL, Abcam, respectively) or the corresponding isotype control (BD Bioscience). They were indirectly labeled with an R-Phycoerythrin-conjugated secondary antibody (AbD Serotec). Cells were counterstained with 4′,6′-diamidino-2 phenylindole (1 μg/mL; Sigma-Aldrich).

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Drs Wan and Hollins were joint first authors on this manuscript.

Part of this article has been presented at the British Thoracic Society Winter Meeting, December 5-7, 2012, London, England.

FUNDING/SUPPORT: The following funding bodies were involved in this study: Wellcome Trust Senior Clinical Fellowship (C. E. B.), Airway Disease Predicting Outcomes through Patient Specific Computational Modelling (AirPROM) project (funded through FP7 EU grant), MAARA, NC3R, and the European Regional Development Fund (ERDF 05567) part funded the research laboratories. This article presents independent research funded by the Leicester Respiratory National Institute for Health Research Biomedical Research Unit. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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DOI: http://dx.doi.org/10.1016/j.chest.2016.01.024