Ciliary dysfunction and ultrastructural abnormalities are features of severe asthma

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Background: Epithelial dysfunction has been implicated in asthma pathophysiology, but no studies have directly assessed ciliary function in asthma.
Objective: To study the ciliary function and epithelial ultrastructure of patients with asthma and healthy controls.
Methods: We studied ciliary beat frequency and beat pattern by using digital high-speed video imaging and ultrastructure by transmission electron microscopy of bronchial epithelial strips from 7 subjects with mild, 7 with moderate, and 19 with severe asthma and 9 healthy controls.
Results: The median (interquartile range) ciliary beat frequency was decreased in moderate (6.5 [4.4-8.5] Hz) and severe asthma (6.7 [6.1-7.6] Hz) compared with controls (10.5 [9.7-11.8] Hz; $P < .01$). Dyskinesia and immotility indices were higher in severe asthma (65% [43%-75%]; 6.3% [1%-9.5%], respectively) compared with controls (4% [0%-6.7%; 0%, respectively; $P < .01$). These abnormalities were related to disease severity (ciliary beat frequency, $r_s = -0.68$; dyskinesia index, $r_s = 0.86$; immotility index, $r_s = 0.65$; $P < .0001$). The ultrastructure of the epithelium was abnormal in severe asthma with a reduction in ciliated cells, an increase in dead cells, and ciliary disorientation compared with all other groups ($P < .05$). Compared with patients with mild asthma and healthy controls, patients with severe asthma showed increased ciliary depletion, microtubular defects, mitochondrial damage, and cytoplasmic blebbing ($P < .01$). All of these changes were related to disease severity.
Conclusion: Ciliary dysfunction and ultrastructural abnormalities are closely related to asthma severity. Ciliary dysfunction is a feature of moderate to severe asthma, and profound ultrastructural abnormalities are restricted to severe disease. Whether these changes contribute to the development of severe asthma phenotype remains to be determined. (J Allergy Clin Immunol 2010;126:722-9.)

Key words: *Ciliated epithelium, ciliary beat frequency, ciliary beat pattern, ciliary disorientation, ciliary dyskinesia, refractory asthma, severe asthma*

Asthma affects approximately 300 million people globally.[1] Although the majority of patients have well controlled disease, there remains a subgroup of adults with asthma, accounting for about 10% of patients with asthma, who continue to have debilitating chronic and persistent symptoms despite optimal standard asthma treatment.[2] This group with severe refractory asthma[3] represents those with a high risk of severe exacerbations and asthma-related mortality and accounts for >50% of asthma-related health care costs.[4,5] There has been extensive research to unravel the complex pathophysiology of severe asthma, but it remains uncertain what the immunopathologic hallmarks of severe asthma are.[2,5] In this respect, the role of the dysfunctional respiratory epithelium in severe asthma has been of much recent interest.[6,7]

The ciliated epithelium that covers the surface of the airways forms an immunologically active natural barrier to invasion and injury by inhaled pathogenic organisms and particulate material. The epithelium is lined by an airway surface liquid and the mucus layer. The airway surface liquid provides an ideal environment in which the cilia beat at a frequency of 11 to 14 Hz. The mucus layer that lies above the airway surface liquid is cleared from the airway by the highly coordinated ciliary beating. This process, known as mucociliary clearance,[8] is an essential factor in pulmonary defense.[9] It therefore follows that damage to the respiratory epithelium and ciliary dysfunction causes impaired mucociliary clearance, leading to increased susceptibility to infection and inflammation.

Using an inhaled radio-aerosol technique, mucociliary clearance in asthma in the stable state and during exacerbations was reported to be impaired.[10,11] Optimal mucociliary clearance depends on the structural and functional integrity of the cilia as well as the characteristics of the airway surface liquid and mucus. Submucosal gland hypertrophy and goblet cell hyperplasia are well recognized pathologic features of asthma.[12] The asthmatic airway is also characterized by mucus hypersecretion,[13] abnormal mucus rheology,[12] and tethering of intraluminal mucins to goblet cells in the airway epithelium.[14] Although evidence that supports abnormalities of mucus[12-14] and airway surface liquid[15] provides a plausible mechanistic explanation for the reduced mucociliary clearance observed in asthma, to date no studies have assessed ciliary function directly in asthma or considered its relationship with disease severity. Indeed, impaired ciliary function would have a pronounced effect on mucociliary clearance. In this context, in this observational study, we aimed to characterize the ciliated respiratory epithelium from the lower airways of adults with mild, moderate, and severe asthma compared with healthy controls by assessing the function of cilia and detailed ultrastructure of the ciliated epithelium.

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Abbreviations used
FVC: Forced vital capacity
GINA: Global Initiative for Asthma
IQR: Interquartile range

METHODS

Subjects

Patients with mild (n = 7), moderate (n = 7), and severe (n = 19) asthma were recruited from clinics at Glenfield Hospital, Leicester, United Kingdom, over a 2-year period (2007-2009). Healthy control subjects (n = 9) were recruited from hospital staff and by local advertising. Asthma was diagnosed on the basis of the presence of clinical features consistent with asthma and objective measures of airway hyperresponsiveness and variable airflow obstruction (PC_{20} in FEV_1 of less than 8 mg/mL; increase in FEV_1 by at least 15% after the inhalation of 200 μg salbutamol; or variation in peak flow, expressed as a percentage of the mean, exceeding 20% over a period of 14 days). Asthma severity was classified as mild, moderate, and severe by using the current Global Initiative for Asthma (GINA) guidelines on the basis of the GINA treatment steps[16] (mild asthma, GINA treatment step 1/2; moderate asthma, GINA treatment step 3; severe asthma, GINA treatment step 4/5). Patients with severe asthma also met the American Thoracic Society criteria for refractory asthma.[3] At the time of collection of bronchial epithelial samples, patients with asthma had been free from intercurrent respiratory infections and asthma exacerbations requiring antibiotics and or rescue use of systemic corticosteroids for at least 6 weeks. Healthy subjects had no history of respiratory disease, normal lung function, and normal PC_{20}. All subjects were current nonsmokers, and those who did smoke in the past had a smoking history of less than 10 pack-years. The study protocol was approved by the Leicestershire and Rutland regional ethics committee, and written informed consent was obtained from all subjects.

Measurements

Demographic and medical details including age, sex, and current medications were recorded on all subjects. FEV_1, forced vital capacity (FVC), and FEV_1/FVC ratio were measured on all subjects with a rolling-seal spirometer (Vitalograph Ltd, Buckingham, England). The PC_{20} was computed from the methacholine dose-response curve (the change in FEV_1 in relation to the methacholine concentration) by linear interpolation on a log scale using standard techniques.[17] In patients with asthma, the presence of atopy was determined by allergen skin prick tests for common aeroallergens including *Dermatophagoides pteronyssinus*, dog, cat, and grass pollen. Plasma IgE was measured on patients with moderate and severe asthma. Patients with asthma had single-flow exhaled nitric oxide concentration measured at a rate of 50 mL per second as previously described.[18] Induced sputum was obtained from patients with asthma, and sputum samples were processed as previously described.[19] All subjects underwent flexible bronchoscopy conducted according to the British Thoracic Society guidelines[20] to obtain strips of bronchial epithelium by brushing the bronchus intermedius.

Ciliary beat frequency and beat pattern

Detailed methodology is given in this article's Methods in the Online Repository at www.jacionline.org. Typically, a strip of bronchial epithelium obtained by brushing the bronchus contained a row of adjacent bronchial epithelial cells. Ciliary beat frequency was measured and beat pattern assessed on strips of bronchial epithelium by using a digital high-speed video microscopy system as described previously with nasal epithelial brushings, within 2 hours of sample collection.[21,22] The high-speed video images were analyzed in a blind fashion. The images were re-analyzed by a second observer (A.R.) and blinded on a second occasion by the original observer (B.T.).

The experimental system allowed the ciliary beat pattern to be evaluated in 3 different planes: sideways profile of the ciliated epithelial strip, assessing the ciliated epithelial strip with cilia beating directly toward the observer, and assessing the ciliated epithelial strip from directly above.[21,22] The path taken by a cilium during the beat cycle was analyzed frame by frame. This was characterized and compared with the normal beat pattern[21] seen on digital high-speed video analysis. Dyskinesia was defined as an abnormal beat pattern that included reduced beat amplitude, stiff beat pattern, failure to bend along the length of the ciliary shaft, a flickering or twitching motion, and static cilia. The dyskinesia index was calculated as the percentage of dyskinetic cilia within the sample (number of dyskinetic readings/total number of readings for sample × 100). The immotility index[23] was calculated as the percentage of immotile cilia within the sample (number of immotile readings/total number of readings for sample × 100).

Transmission electron microscopy

The detailed ultrastructure of the bronchial epithelial strips was studied by using transmission electron microscopy as described before with nasal epithelial brushings.[24] Detailed methodology is given in the Methods in the Online Repository. The ciliated epithelium was assessed in a blind fashion for both epithelial and ciliary ultrastructural changes. Epithelial integrity was assessed first by assessing the cell type. The number of ciliated cells, unciliated cells, mucus cells, and dead cells were expressed as a percentage of all cells examined. Disruption and damage to the epithelium was assessed by calculating the percentages of ciliated cells with loss of cilia, cellular projections, cytoplasmic blebbing, and mitochondrial damage among all cells examined. Damage to individual cilium was evaluated by examining ciliary ultrastructure for microtubular and dynein arm defects, and the percentage of cilia with microtubular or dynein arm defects was calculated. Intracellular ciliary orientation, defined as the standard deviation of the angles of lines through the central pair of microtubules of cilia originating from a single ciliated cell, was determined as described previously.[25]

Statistical analysis

Sample size was calculated on the basis of ciliary beat frequency as the primary outcome measure. Seybold et al[26] studied the surface liquid velocity on freshly excised sheep trachea and found that a 16% increase in ciliary beat frequency correlates with a 56% increase in the tracheal surface liquid velocity. Hence, we assumed that an absolute mean difference in ciliary beat frequency of 2 Hz has potential biological significance. It was estimated that, to detect a mean difference in ciliary beat frequency of 2 Hz (with a SD of 1Hz) between 2 groups with a CI of 95% and a power of 80%, a sample size of 6 (n = 6) in each group would be required. Statistical analysis was performed by using GraphPad Prism 5 (GraphPad software Inc, Calif) and SAS/STAT software (SAS Institute Inc, NC). Nonparametric data were described as medians (interquartile ranges [IQRs]). Groups were initially compared by using the Kruskal-Wallis test, and *post hoc* analysis was performed by using the Dunn method. The Spearman correlation was used to assess the univariable relationship between disease severity and abnormalities of ciliary function and epithelial ultrastructure. A P value of <.05 was taken as the threshold for statistical significance in each case. Agreement between the 2 observers was excellent for measurement of ciliary beat frequency (interclass correlation, 0.94) as well as dyskinesia index (interclass correlation, 0.93). Repeatability (agreement within observer) was also excellent (interclass correlation was 0.94 for ciliary beat frequency and 0.99 for dyskinesia index).

RESULTS

The baseline characteristics of the subjects are given in Table I. Ciliary beat frequency was decreased in moderate to severe asthma. The median (IQR) ciliary beat frequency was decreased in moderate (6.5 [4.4-8.5] Hz) and severe asthma (6.7 [6.1-7.6] Hz) compared with controls (10.5 [9.7-11.8] Hz; Kruskal-Wallis $P < .01$; $P < .001$ between groups; Fig 1, *A*). Analysis of ciliary beat pattern showed a higher proportion of dyskinetic and immotile cilia in moderate to severe asthma. The median