Nature of airway inflammation and remodeling in chronic cough

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Background: Chronic cough may be a result of asthma and nonasthma causes, but it is unclear whether there are specific inflammatory or remodeling changes.

Objective: We determined airway mucosal changes in patients presenting with asthmatic cough and cough associated with nonasthmatic causes.

Methods: Patients with chronic cough of nonasthmatic (n = 33; postnasal drip/rhinitis in 6, gastroesophageal reflux in 5, bronchiectasis in 3, and idiopathic in 19) and asthmatic (n = 14) causes and 15 healthy controls underwent fiberoptic bronchoscopy. Morphometry of bronchial biopsies and capsaicin cough sensitivity were assessed.

Results: Compared with controls, submucosal eosinophils and neutrophils were increased in patients with asthmatic cough ($P < .005$) and submucosal mast cells in patients with nonasthmatic cough ($P = .01$). Subbasement membrane thickness, goblet cell area, vascularity, and vessel size were also increased in both groups. Smooth muscle area was higher only in patients with nonasthmatic cough ($P = .0007$ vs control and $P = .019$ vs asthmatic cough). None of the pathologic changes were related to the duration of coughing. Cough sensitivity was heightened in patients with nonasthmatic cough compared with controls and patients with asthmatic cough. The degree of goblet cell hyperplasia and epithelial shedding positively correlated with cough sensitivity in patients with nonasthmatic cough ($r = 0.43$; $P = .01$; and $r = 0.40$; $P = .02$, respectively).

Conclusion: Features of airway wall remodeling are prominent in the airways with nonasthmatic as well as asthmatic cough. These are linked to chronic cough rather than to asthma. Mast cell hyperplasia rather than eosinophilia is distinctive for nonasthmatic cough. (J Allergy Clin Immunol 2005;116: 565-70.)

Key words: *Chronic cough, airway inflammation, airway remodeling, cough sensitivity*

Chronic cough is a common clinical problem.[1,2] Asthma, postnasal drip or rhinosinusitis, and gastroesophageal reflux have been recognized as the most common causes of chronic cough.[2,3] In some patients, no cause can be identified despite thorough investigations and empiric treatment,[4-6] a group denoted as having idiopathic chronic cough. Overall, patients with a chronic cough may be divided into patients who have asthma or asthma-related diagnoses such as cough-variant asthma,[7] and nonasthma-related diagnoses. The simplicity of this approach is related to the fact that the former group of patients responds well to inhaled or oral corticosteroid therapy, whereas the latter group does not.[8]

Abbreviations used
ASM: Airway smooth muscle
C5: Concentration that caused 5 or more coughs
TRPV-1: Transient receptor vanniloid potential-1
UK: United Kingdom

Airway inflammation and remodeling are established features of asthma.[9] Postmortem investigations in patients with asthma have shown infiltration of inflammatory cells including eosinophils, edema in the submucosa, subbasement membrane thickening, goblet cell hyperplasia, airway smooth muscle (ASM) hypertrophy and hyperplasia, submucosal gland hyperplasia, vascular proliferation, and airway wall thickening.[10,11] Many of these features have also been reported in endobronchial biopsies of patients with asthma.[12-15] Infiltration of eosinophils and thickening of subbasement membrane in the bronchial mucosa are also reported in patients with cough-variant asthma, who lack typical features of classic asthma such as wheezing or airflow obstruction, indicating that this condition is a variant of asthma.[16,17] Similar pathologic features are observed in eosinophilic bronchitis, which unlike cough variant asthma does not demonstrate airway hyperresponsiveness or bronchodilator responses to β-adrenergic agonists.[18]

There is limited information regarding the mucosal changes of chronic nonasthmatic cough. An increase in mononuclear cell infiltrate without increase in submucosal fibrosis has been reported,[19] whereas in bronchoalveolar lavage fluid, an increase in histamine levels, mast cells, and eosinophils was shown.[20] In induced sputum, increased levels of IL-8 and neutrophils and of prostaglandins D_2 and E_2 have been reported.[21] In addition to these mediators, cys-leukotrienes, leukotriene B4, myeloperoxidase, and TNF-α have been detected in patients with persistent cough.[22]

Patients with nonasthmatic cough are characterized by increased cough sensitivity to the irritant capsaicin

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irrespective of the associated cause or being in the idiopathic group.[6] The mechanisms by which the cough sensitivity is increased is unknown. We hypothesize that there may be airway wall remodeling features together with inflammatory changes in the airways of patients with nonasthmatic chronic cough, as observed in asthmatic cough patients; these features may also be different. We therefore investigated the histological changes in the airways of patients with chronic cough of nonasthmatic origin by using endobronchial biopsy, and compared these with those from patients with asthmatic chronic cough and healthy controls.

METHODS

Subjects

We studied 47 patients with chronic cough (14 asthmatic and 33 nonasthmatic) of at least 8 weeks' duration referred to our cough clinic. Diagnostic investigations included chest radiograph, pulmonary function test, methacholine challenge, 24-hour esophageal pH monitoring, and chest and sinus computed tomography.[1] Patients with airway hyperresponsiveness (PC_{20} $FEV_1 \leq 4$ mg/mL), diurnal variation of peak expiratory flow ($\geq$20%), or $\geq$15% increase of FEV_1 after β-agonist, and also response of coughing to inhaled bronchodilator and corticosteroid therapy, were diagnosed as having asthma responsible for chronic cough. They had cough as the sole or predominant symptom (cough-variant or cough-predominant asthma).

Chronic cough caused by gastroesophageal reflux was diagnosed by 24-hour esophageal pH monitoring and efficacy of 12-week course of proton-pump inhibitor and dietary changes. Chronic cough was attributed to postnasal drip/rhinosinusitis when symptoms and objective diagnosis of postnasal drip and/or rhinosinusitis were present and nasal corticosteroids and/or nasal anticholinergics were effective against cough. Bronchiectasis was considered when patients had productive cough and typical findings of bronchiectasis on high-resolution computed tomography. Coughing in such patients responded to some extent to antibiotics and/or chest physiotherapy. Some patients had no identifiable cause of cough despite additional investigations including bronchoscopy and intensive therapeutic trials for asthma, gastroesophageal reflux, and postnasal drip/rhinosinusitis, and were labeled idiopathic. All patients showed a PC_{20} $FEV_1 > 16$ mg/mL. We grouped these patients together as having nonasthmatic cough for analysis. Patients who complained of sputum rarely or only occasionally were considered to have a dry cough, whereas those who produced sputum usually were classified as productive coughers.

We also studied 15 normal noncoughing volunteers. All participants were current nonsmokers, and the number of exsmokers, defined as those who stopped smoking for 3 or more years at the time of study, is shown in Table I. Exsmokers had less than 5 pack-years of smoking. The study was approved by the ethics committee of our institution. All subjects gave informed consent to participate in the study.

Bronchoscopy and bronchial biopsy

Bronchoscopy was performed as previously described.[23] Briefly, subjects were pretreated with intravenous midazolam (5-10 mg). Oxygen was administered via nasal prongs throughout the procedure. Using local anesthesia with 2% lidocaine to the upper airways and larynx, a fiberoptic bronchoscope (Olympus BF 10; Key-Med, Herts, United Kingdom [UK]) was passed through the nasal passages into the trachea. Three to 5 mucosal biopsies were taken from the segmental and subsegmental bronchi of the right lower lobe.

Specimens were fixed in 10% formalin, embedded in paraffin wax, and cut into 4-μm sections for staining with hematoxylin and eosin (for measurement of subbasement membrane thickness, epithelial shedding, smooth muscle and submucosal gland area, and total submucosal area), Alcian blue–periodic acid-Schiff (goblet cell area), and toluidine blue (mast cells in lamina propria and smooth muscle). They were also stained with mAb to factor VIII (Dako Cytomation, Cambridge, UK) after pretreatment with proteinase K (Dako Cytomation) for evaluation of blood vessels. Specimens for immunohistochemical analyses were immediately placed in optimal cutting temperature embedding media, snap-frozen in isopentane precooled with liquid nitrogen, and stored at −70°C. Sections (6 μm) were cut on a cryostat and placed on poly-L-lysine–coated microscope slides. For staining of eosinophils and neutrophils in lamina propria, mouse mAbs to major basic protein (Bradsure Biological, Loughborough, UK) and neutrophil elastase (Dako Ltd, High Wycombe, UK) were used. Then, a biotinylated mouse immunoglobulin followed by avidin-biotin complex was added. Diaminobenzidine was used for 5 minutes, and the slides were counterstained with hematoxylin and mounted.

Tissue quantification

Tissue cell counts and morphometry were performed in a blinded fashion on randomly assigned slides by 1 investigator.

Areas of total epithelium, goblet cells, subbasement membrane, total submucosa, blood vessels, submucosal gland, and smooth muscle were identified by morphologic examination, and the area was calculated by using a computer analysis system (KS-300; Zeiss, Oberkochen, Germany). The region of interest was traced manually by using a mouse, and the area was derived.

Eosinophils, neutrophils, and mast cells were counted in the bronchial submucosa (lamina propria) in a zone excluding submucosal gland and smooth muscle. Mast cells in airway smooth muscle were also enumerated. Cells were counted within the bundles of smooth muscle but not in the adjacent areas.[18] All available fields were examined, and the results were expressed as the total number of cells per square millimeter of submucosa or smooth muscle. Eosinophils or neutrophils in airway smooth muscle were not counted because they were too scant to be analyzed.

Subbasement membrane thickness (μm) was quantified by measuring total length of basement membrane present in the biopsy that was overlaid by at least a basal epithelial cell layer. The thickness was computed from the area and the length.[13] Goblet cell area (%) was determined as the proportion of Alcian blue–periodic acid-Schiff—positive area within the total epithelial layer. Epithelial shedding was assessed as the total length of basement membrane covered with no epithelial cells divided by the total length of the membrane.[14] Blood vessels were counted and the individual areas measured; vascularity (%) defined as area occupied by blood vessels per square millimeter of submucosal area, vessel number per square millimeter, and the mean blood vessel size (μm^2) were measured for each patient. The areas of the bronchial submucosa occupied by submucosal gland and by airway smooth muscle were determined as the percentage of gland or smooth muscle area over the total submucosal area.[15] All parameters were examined in sections from 2 to 4 different biopsies and the mean of these measurements recorded.

Capsaicin challenge

As previously described,[24] coughs were counted for 1 minute after single-breath inhalations of 0.9% sodium chloride and capsaicin solutions of increasing concentrations (0.98 to 500 μmol/L). They