Profiles of proinflammatory cytokines in sputum from different groups of severe asthmatic patients

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Background: Severe asthma represents a heterogeneous group of patients whose characteristics of airway inflammation are poorly known.

Objective: To evaluate the sputum cytokine profiles of different phenotypes of severe asthma.

Methods: Severe asthmatic patients (n = 45) were divided into 3 groups: frequent exacerbations, persistent bronchoconstriction, and both features. Two other groups (9 patients with untreated mild asthma and 10 control subjects) were also studied. Selected sputum portions were assayed for differential cell count, supernatant interleukin 5 (IL-5), granulocyte-macrophage colony-stimulating factor, IL-8, and eosinophil cationic protein.

Results: There were no statistically significant differences among the 3 severe asthma groups in terms of sputum inflammatory cell percentages, IL-8 levels, and eosinophil cationic protein levels, although IL-8 levels tended to be higher in patients with persistent bronchoconstriction. Sputum concentrations of granulocyte-macrophage colony-stimulating factor and IL-5 were significantly higher in patients with frequent exacerbations compared with the other 2 groups. Levels of IL-5 and IL-8 were higher in severe asthmatic patients compared with mild asthmatic patients and controls, whereas sputum eosinophil percentages were intermediate between those of mild asthmatic patients and controls.

Conclusions: Proeosinophilic cytokine levels are increased in severe asthmatic patients with frequent exacerbations but not in severe asthmatic patients with persistent bronchoconstriction, suggesting that different cytokine profiles could be associated with different phenotypes of severe asthma.

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INTRODUCTION

Severe asthma represents a low percentage of the population of asthmatic patients, but it represents a high economic cost in the care and treatment of asthma.[1,2] Severe asthmatic patients are defined, according to international guidelines, as a subgroup of asthmatic patients with low pulmonary function or a high frequency of severe asthma symptoms in the absence of regular asthma treatment or asthmatic patients who require regular bronchodilators and high-dose inhaled or systemic corticosteroids for controlling asthma, sometimes with poor response to standard care.[3] They represent a heterogeneous group of patients, defined also as "refractory asthmatic patients" or "difficult-to-treat asthmatic patients."[4]

Several theories have been proposed to explain this clinically severe state. An increased "drive" of eosinophil and lymphocyte inflammation, with inflammation-induced changes in the binding affinity of the glucocorticoid receptor, has been proposed.[5,6] In contrast to this theory, severe asthma may represent a pathologic entity distinct from the milder levels of asthma, with a high level of neutrophils.[7] Neutrophils are relatively resistant to corticosteroids, and, consequently, the increased neutrophilic inflammation might explain the poor response to corticosteroids.

Studies of cells and soluble markers of inflammation were performed in biopsy samples from the airway wall and in bronchoalveolar lavage (BAL) fluid during bronchoscopy in severe asthmatic patients.[8] In BAL studies, the importance of eosinophilic inflammatory markers has been demonstrated for eosinophil and eosinophil cationic protein (ECP) levels.[9,10] On the other hand, neutrophilia was found in BAL fluid and biopsy specimens.[11] However, considerable heterogeneity was noted in the ranges for the eosinophil and neutrophil populations.[11]

Bronchoscopy is an invasive procedure and is not easily applicable in patients with severe asthma.[11] In contrast, induced sputum is a noninvasive method, and it can be used in patients with severe asthma.[12–14] Using this technique, severe asthmatic patients differed from other less severe asthmatic patients in sputum eosinophil, neutrophil, and ECP levels.[15]

Some studies report that different phenotypes can be observed in this group of severe refractory asthmatic patients. Although they all meet the international definition of severe asthma, a group of severe asthmatic patients can show wide variability in airway obstruction across time, whereas another group can be more chronically severely obstructed.[16,17] To our knowledge, no data have been reported on the difference in

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biological markers among these different phenotypes of severe asthma. Different phenotypes in patients with early- or late-onset asthma or patients with prominent eosinophilic inflammation and those without eosinophilic inflammation can be distinguished, suggesting different pathologic processes.[18]

In this study, we measured the percentages of inflammatory cells, particularly eosinophils and neutrophils, and the concentrations of ECP and some proinflammatory cytokines in the sputum of patients with severe uncontrolled asthma. We chose interleukin 5 (IL-5), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-8 to investigate the pattern of cytokines as markers of eosinophilic and neutrophilic inflammation.[19] The aim of this study is to evaluate whether different phenotypes of severe asthma are associated with different profiles of proinflammatory cytokines.

METHODS

Participants

Forty-five patients (19 men and 26 women; age range, 23–79 years) with severe asthma were recruited from the Pneumology Section of the Cardio-Thoracic Department, University of Pisa, during a 3-year period. The diagnosis of asthma had been established in all the patients at the time of the first observation by symptoms of wheezing, chest tightness, dyspnea, and cough in the presence of reversible airflow obstruction (>12% improvement in forced expiratory volume in 1 second [FEV_1] after salbutamol) or bronchial hyperresponsiveness to methacholine (provocation dose of methacholine that caused a decrease in FEV_1 of 20%, <1.0 mg). To be included in the study, patients were required to attend follow-up visits after diagnosis for at least 3 years to evaluate longitudinally the severity and the control of the disease. During this follow-up, patients were seen regularly every 4 months and every time an exacerbation occurred to evaluate for additional treatment. At each visit patients were evaluated regarding the level of asthma treatment required to maintain good control of asthma, the frequency of symptoms and exacerbations, and the level of pulmonary function, measuring FEV_1 before and after taking an inhaled short-acting β_2-agonist.

At enrollment into the present study, all the patients had a diagnosis of severe asthma. All of them reported current asthma symptoms and showed reversible airway obstruction (increase in FEV_1 after 200–400 μg of salbutamol of >12% from the baseline value). Severity of asthma was evaluated according to the recommendations of the international guidelines[3]; in particular, asthma was defined as severe in the presence of 1 or more of the following findings: (1) the persistence of daily asthma symptoms despite regular treatment with high-dose inhaled corticosteroids and long-acting bronchodilators, (2) the presence of at least one exacerbation requiring oral corticosteroids per year for 2 or 3 years, and (3) FEV_1 less than 20% of predicted during a stable phase of the disease during regular treatment, including short-acting β_2-agonists (postbronchodilator FEV_1).

All the patients were examined at least 1 month after asthma exacerbations. At the time of the study, all 45 patients were being treated with long-acting inhaled β_2-agonists and inhaled corticosteroids (≥1,000 μg of beclomethasone dipropionate daily or the equivalent); in addition, oral theophylline (n = 28), anticholinergics (n = 5), antileukotrienes (n = 8), and oral corticosteroids at low doses (≤5 mg/d of prednisone or the equivalent; n = 4) were also used. Thirty-two of 45 patients were atopic, 23 have had a smoking history (with <10 packs per year), and 8 were current smokers at the time of clinical evaluation. Atopic status was defined as having positive skin prick test reactions to common aeroallergens. Only 3 of 45 patients (all with frequent exacerbations) were sensitive to aspirin.

A group of untreated individuals with early-onset mild persistent asthma at the time of their first examination (n = 9) and a group of controls (n = 10) underwent a similar evaluation, which included sputum induction for inflammatory markers, and they were used as control groups. Mild asthmatic patients had normal baseline FEV_1 values and a positive response to methacholine challenge testing (provocation dose of methacholine that caused a decrease in FEV_1 of 20%, <1.0 mg). The study was approved by the medical ethics committee of the University Hospital of Pisa, and all the patients gave informed consent.

Study Design

All the patients who fulfilled the diagnosis of severe asthma after follow-up of at least 3 years were recruited. Other criteria for eligibility were compliance with functional measures and acceptable sputum recovery. Each patient was examined during a stable period, on 2 different days. On the first day, patients provided a detailed clinical history to evaluate the frequency of symptoms and exacerbations, the number of short-course treatments with oral corticosteroids in the past year, and pulmonary function measurement during regular treatment. On the second day, after 2 weeks of peak expiratory flow monitoring of symptom scores and the use of rescue medication, spontaneous or induced sputum collection was performed after bronchodilator testing; on this occasion, patients had discontinued regular treatment for 24 hours.

For clinical and functional evaluation, patients were assigned to 3 different groups: group 1 included patients with frequent exacerbations of asthma (≥3 per year); group 2, patients with persistent bronchoconstriction (postbronchodilator FEV_1 ≤70% of predicted) without frequent exacerbations; and group 3, patients with features of both groups. Persistent bronchoconstriction was defined as an FEV_1 consistently less than 70% of predicted during all measurements performed in the 3 years of follow-up, including measurements obtained after a short course of high-dose oral corticosteroids. Patients with mild asthma and controls were examined using the same protocol schedule.