

Use of induced sputum to examine airway inflammation in childhood asthma

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Airway inflammation is important in the pathogenesis of asthma, during which it may lead to symptomatic exacerbations and increases in asthma severity, as well as contribute to future decline in asthma status. The use of induced sputum has emerged as an important and useful technique to study airway inflammation. It has particular advantages in the study of childhood asthma because it is noninvasive and allows samples to be collected on repeated occasions in children over 7 years of age. The results of cell counts are reliable when the sputum is processed in a standardized manner involving selection from saliva, cell dispersion, and quantitative cytology. Children with asthma have increased eosinophils and mast cells, which may persist even with high doses of inhaled corticosteroid therapy. During a severe exacerbation of asthma, there is an intense and heterogeneous inflammatory response involving eosinophil and neutrophil accumulation and activation. Characterization of the relevance of airway inflammation in children with asthma is important. (*J Allergy Clin Immunol* 1998;102:S100-1.)

There is now general agreement that chronic airway inflammation and airway hyperresponsiveness are important features of asthma in adults. These 2 factors interact to lead to the variable airflow obstruction and episodic respiratory symptoms that characterize asthma. The importance of airway inflammation in adult asthma is acknowledged by its inclusion in the definition of asthma in recent asthma management guidelines.¹ The role of inflammation in pediatric asthma is less well established. As a reflection of this, pediatric asthma management guidelines define asthma in terms of episodic respiratory symptoms.²

There are important reasons to study airway inflammation in childhood asthma. There is an increasing tendency to use inhaled corticosteroids as preventive therapy in childhood asthma, as is the case with adult disease. It is therefore necessary to establish whether airway inflammation is the same in childhood asthma as it is in adult asthma to prevent children unnecessarily being exposed to the effects of corticosteroid use. Furthermore, key insights into the pathogenesis of asthma may be missed by restricting studies of airway inflammation in

asthma. Asthma frequently begins in childhood and becomes chronic by adulthood. It is necessary to evaluate the role of airway inflammation in children in order to study the role of airway inflammation in the genesis of asthma. In adults it may be difficult to dissect the mechanisms and features of early inflammation from more chronic components involving tissue repair.

ASSESSMENT OF INFLAMMATION

Typically, airway inflammation has been assessed by bronchoscopic studies by means of biopsies, bronchial brushings, and bronchoalveolar lavage. A limited number of these studies have been conducted in children. They indicate that the presence of airway eosinophilia and mast cell activation correlates with airway hyperresponsiveness.^{3,4} Bronchoscopy is comparatively invasive and of limited use to study large numbers of children with asthma on repeated occasions. Recently, the technique of sputum induction has been applied to the study of airway inflammation in asthma,⁵ and this is eminently suitable for assessment of airway inflammation in childhood asthma.

The use of an ultrasonic nebulizer to generate a dense aerosol of either hypertonic or normal saline can be used to induce sputum from children over the age of 7 years. This technique has been successfully applied to induce sputum from normal children, children with stable asthma,^{5,6} and children first seen in the emergency department with acute asthma.⁷ Spirometry is measured periodically during the challenge to monitor the safety of the procedure, and β_2 -agonist is given as pretreatment to prevent bronchoconstriction during hypertonic saline inhalation. With this technique, sputum can be obtained from over 80% of children over the age of 7 years. Although not systematically evaluated, success below the age of 7 years may be limited by spirometric technique and a low tidal volume, which limits the dose of saline that can be delivered.⁸

Once the sputum is obtained, it needs to be processed in a standardized manner. This involves selection of lower respiratory portions of sputum, which are dispersed with dithiothreitol, to create a single cell suspension from which a total cell count is performed and cyto-centrifuge preparations are made. These slides are then suitable for quantitative cytology, cytochemistry, and immunocytochemistry. When processed in a standardized manner, sputum cell counts are reproducible^{5,9,10,11} and compare favorably with bronchial washings.¹²

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INDUCED SPUTUM IN CHILDHOOD ASTHMA

The results of induced sputum from normal children show that the predominant cell is the macrophage; eosinophils and mast cells are seldom seen. Eosinophils comprise less than 2.5% of cells in normal children.

In contrast, in children with stable asthma there are increased numbers of eosinophils and mast cells. There is a range of sputum eosinophil counts in children with stable asthma. The significance of this range has yet to be defined. It will be important to establish whether there is an association between the degree of asthma control, asthma severity, and sputum eosinophil counts in childhood asthma. Biopsy studies in adult asthma suggest that the degree of airway eosinophils is related to asthma severity.¹³ Furthermore, the effects of steroid therapy on sputum eosinophils in childhood asthma needs to be defined. In adults with chronic cough, sputum eosinophilia is reduced by corticosteroid therapy.¹⁴

Asthma is frequently associated with periodic exacerbations. These vary in severity, but can be severe enough to require emergency treatment or hospitalization and can even cause death. It is important to evaluate the role of airway inflammation during exacerbations of asthma. To do this, induced sputum methodology was adapted for study in the emergency department.⁷ Children seen in the emergency department were first treated with β_2 -agonist. Those with an FEV₁ greater than 40% of predicted value then underwent sputum induction with ultrasonic nebulization of normal saline. Normal saline was used to minimize any bronchoconstriction. This technique was found to be safe in this setting, where the average fall in FEV₁ was 5%. A satisfactory sputum sample was obtained in each of the children tested acutely. The examination of induced sputum cell counts in acute asthma revealed several important features.⁷ First, the total cell count was significantly increased compared with subjects with stable asthma and normal subjects. There was an approximate 10-fold increase in the average total cell count in acute severe asthma, reflecting the presence of an intense airway inflammatory response in acute asthma. This inflammatory response was heterogenous and included eosinophils, mast cells, and neutrophils. Mast cells and eosinophils were present as reported in stable asthma.⁵ The eosinophils stained positive for eosinophilic cationic protein, suggesting eosinophil activation, and there was a fall in cell count as asthma resolved. The intensity of the eosinophil infiltrate was correlated with the severity of the airflow obstruction. In addition, however, large numbers of neutrophils were present. This implies that different cytokines may mediate the inflammatory response in acute severe asthma. In this regard, the recent observations that viruses cause infective exacerbations is important because of the potential for this to lead to IL-8 secretion and neutrophil influx. These findings indicate that acute severe asthma in childhood is also associated

with airway inflammation, but that it has different features than stable asthma. There is an intense inflammatory cell infiltrate that is heterogenous, including neutrophils, eosinophils, and mast cells.

CONCLUSION

There is a need to conduct studies in children to establish the role of airway inflammation in childhood asthma. From these studies we may gain new insights into the pathogenesis of asthma that may not be possible from studies in adults. Research to date indicates that induced sputum is a suitable way to obtain airway secretions for the study of airway inflammation in childhood asthma. Eosinophils and mast cells form the characteristic inflammatory cell profile in stable childhood asthma, and there is evidence of intense airway inflammation, which includes neutrophils, in acute severe asthma.

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