

Induced sputum and response to glucocorticoids

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Jerry Dolovich has generated substantial new work that has had an international impact on the understanding and treatment of asthma and other airway diseases. For this, he is recognized as a Distinguished Clinician of the American Academy of Asthma, Allergy and Immunology. I have focused on some of his recent work with us using induced sputum to measure indices of airway inflammation noninvasively. The results illustrate that sputum eosinophilia (1) does not necessarily correlate with the severity of symptoms or severity of abnormalities of airway function, (2) does not always occur in exacerbations of asthma, and (3) may be a predictor clinical benefit to steroid treatment, which will be useful in practice. The observations imply that airway inflammation needs to be measured to understand the pathogenesis, pathophysiology, and treatment of asthma and other airway diseases. (*J Allergy Clin Immunol* 1998;102:S102-5.)

I could not have asked for a better honor than to chair this celebration for Jerry Dolovich. Jerry and I have been close friends and collaborators for nearly 30 years. The collaboration has been based on complete trust and similar interests but different skills. We have shared research fellows, and with them and our other staff, have published about 50 original articles, more than 150 abstracts, and a number of book chapters and reviews. Prominent observations that we have made together have included the following. We recognized that IgE-mediated reactions cause late (as well as early) responses.¹ This finding was corroborated by Solley et al² and led to the appreciation that IgE-mediated reactions could cause inflammation. A second of our observations was the recognition that late asthmatic responses are associated with an increase in nonallergic airway responsiveness,³ both features being subsequently confirmed to be associated with airway inflammation.^{4,5} A third observation is the recognition that nonallergic airway hyperresponsiveness is a characteristic of current symptomatic asthma and an important determinant of its severity.⁶ Fourth is the concept of inducers or inciters, inducers being causes of inflammation and disease (eg, allergens and chemical sensitizers) and inciters being triggers of airway constriction (eg, exercise, cold air, and irritant dust and fumes).⁷ A final observation is the recent application of induced sputum examination to study airway inflamma-

Abbreviation used

BAL: Bronchoalveolar lavage

tion relatively noninvasively.⁸

One of Jerry's strengths has been to foster collaborative research between the laboratory and clinical practice. He has stressed that the laboratory work is only relevant if it has relevance in practice. He was instrumental in setting up chest and allergy clinics (which were a forerunner of the Firestone Regional Chest and Allergy Unit), to respiratory medicine and allergy working together in Hamilton, and to the publication of what has been referred to as the first Canadian guidelines for the treatment of asthma.⁹ In this presentation I intend to focus on some of Jerry's recent work with us to measure airway inflammation by induced sputum examination and to illustrate why these measurements are needed in practice and how they can predict whether benefit will result from glucocorticoid treatment.

INDUCED SPUTUM EXAMINATION

Sputum examination for indices of airway inflammation is not new. At around the time that Osler¹⁰ published his first textbook, *The Principles and Practice of Medicine*, and drew attention to the presence of inflammation in asthma, Gollasch¹¹ identified eosinophils in the sputum of patients with asthma. There was a renewed interest in the examination of sputum in the 1950s, but its use in practice and research did not catch on because it was considered to be difficult, and the results were considered to be unreliable. It was not until 9 years ago, when Gibson et al¹² illustrated that the examination of smears of sputum could be reliable and valid and that he, Isabel Pin, and others⁸ showed that sputum induction with an aerosol of hypertonic saline could be applied successfully and safely in asthmatic adults and older children, that there was a renewed interest in this field, which has subsequently spread worldwide.

The method of sputum processing and examination that we now use involves the following steps.¹³ The sputum is selected from the expectorant of sputum plus saliva and is treated with dithiothreitol to break up the mucus and disperse the cells. The cell suspension is then filtered. A total cell count is performed in a hemocytometer, and cell viability is assessed by trypan blue. Cytospins are made and stained with Wright's stain, and a 400 cell differential count of nonsquamous cells is performed. The remaining suspension is centrifuged, and the fluid-phase

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of sputum is stored at -70°C for future molecular measurements. The cytopins can also be stained for metachromatic cells or prepared for immunocytochemistry¹⁴ or in situ hybridization¹⁵ measurements. The cell suspension can also be examined by flow cytometry.¹⁶ In this presentation I will limit further discussion to cell counts.

The cell counts are highly reproducible, valid, and responsive,^{13,17,18} and they are similar in spontaneous and induced sputum.¹⁹ They are not influenced immediately by the induction procedure or treatment with dithiothreitol.²⁰ The filtering of the suspension removes some of the cells but has no effect on the differential count.²¹ Sputum contains more eosinophils and neutrophils than bronchoalveolar lavage (BAL) fluid and less lymphocytes than BAL fluid or bronchial biopsy specimens.^{22,23} It measures a different compartment of airway inflammation than BAL fluid or biopsy specimens. An increase in eosinophils in sputum is a more sensitive and specific marker for asthma than an increase in eosinophils in peripheral blood.²⁴

SPUTUM CELL COUNTS AND CLINICAL FEATURES OF ASTHMA

The clinical features of asthma and other airway diseases are considered to be secondary to airway inflammation and the remodeling that results. It would therefore be reasonable to consider that relationships might be observed between the presence or severity of the inflammation and the clinical features. In an early study using induced sputum, this was observed¹³; the greater the sputum eosinophilia, the more severe the symptoms and the worse the airflow limitation and methacholine airway hyperresponsiveness. A similar observation has been made in cigarette smokers with respect to sputum neutrophils²⁵; the greater the increase in sputum neutrophils, the more severe the chronic airflow limitation. However, there is not necessarily a direct relationship between eosinophils and clinical features of airway disease. This was highlighted by the recognition by Gibson et al²⁶ that some patients who were first seen with a chronic cough had sputum eosinophilia without airflow limitation or airway hyperresponsiveness. Subsequently, in a study to investigate the reliability of flow cytometry to measure lymphocytes, their subsets, and activation markers in sputum, Kidney et al¹⁶ showed no relationship between sputum eosinophils and PC_{20} . These results suggest that whether relationships will be observed will depend on the subject population. Similar varying correlations have been observed between BAL fluid or biopsy measurements of inflammation and clinical characteristics.²⁷ The lack of correlation suggests that objective measurements of airway inflammation are needed to clinically accurately interpret the presence or absence of inflammation.

SPUTUM CELL COUNTS AND EXACERBATIONS IN ASTHMA

Further evidence that direct measurements of inflammation are needed comes from studies of uncontrolled asthma or exacerbations of asthma and comparing the measurements with interpretations based on clinical

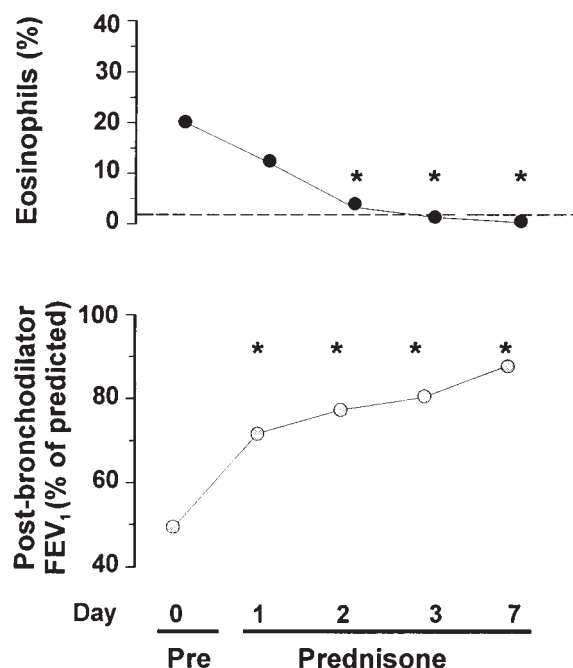


FIG 1. Sputum eosinophils (%) and postbronchodilator FEV₁ (% predicted or previous best) on presentation with a severe exacerbation of asthma and on days 1, 2, 3, and 7 after treatment with prednisone 30 mg daily. Dashed line represents upper limit of normal range. * $P < .05$ from baseline. Sputum eosinophilia is not improved until the second day of treatment, but by day 7 it is within the normal range ($<2.0\%$). FEV₁ improves more quickly.

parameters. Turner et al,²⁸ when recruiting for a treatment study, found that about one quarter of the volunteers with uncontrolled symptoms of asthma had little or no eosinophilia. Fahy et al²⁹ also observed that the majority of patients seen in the emergency department with a severe exacerbation of asthma had no eosinophilia. These observations illustrate that exacerbations in patients with asthma are not always a result of eosinophilic inflammation and raise the possibility that they may also not respond in the same way to treatment. Physicians are going to make incorrect judgements of the presence and type of airway inflammation from clinical features, and this is further supported by the results of 2 recent studies so far published only as abstracts.^{30,31}

SPUTUM CELL COUNTS AND RESPONSE TO STEROID TREATMENT

Although it is recognized that airway eosinophilia is common in asthma and that glucocorticoid treatment reduces eosinophilia in general and generally improves asthma, the association between eosinophilia and the effect of glucocorticoids in airway disease has generally been overlooked. Induced sputum examination has now provided a tool by which the antiinflammatory effects of medications can be studied easily.

In a series of studies with Jerry, Marcia and Emilio Pizzichini, and others, we have examined the effect of

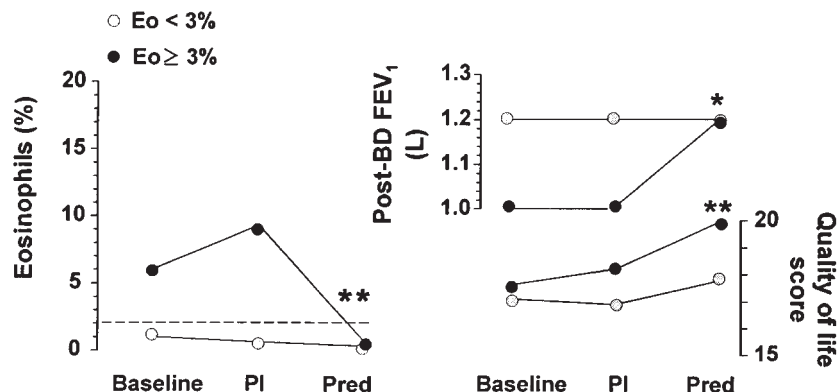


FIG 2. Sputum eosinophils (%), postbronchodilator FEV₁ (L), and quality-of-life score in smokers with severe chronic airflow limitation before treatment, 2 weeks after placebo treatment, and 2 weeks after prednisone 30 mg daily. Those with raised eosinophils at baseline (closed circles) have significant improvement in each outcome after prednisone treatment. **P* = .05. ***P* < .001.

steroid treatment in asthma, smokers with severe chronic airflow limitation, and patients with a chronic cough without asthma or other recognized cause. In one study of 10 patients with a severe exacerbation of asthma, 9 had sputum eosinophilia and 1 did not.¹⁸ Treatment with prednisone improved the eosinophilia and asthma in the 9 but not in the remaining subject without eosinophilia. This latter patient had mucoid sputum with only a mild neutrophilia, and his FEV₁ returned to his usual best slowly over a number of weeks (Fig 1). In another study of 9 patients who were thought to have prednisone-dependent asthma, prednisone dependence was confirmed in 8.³² These 8 had sputum eosinophilia when the prednisone was reduced. The remaining subject had no eosinophilia and the prednisone could be reduced to a level needed to prevent hypoadrenalism. Exacerbations were noneosinophilic and did not require prednisone treatment.³³

In another study 18 smokers or exsmokers with severe chronic airflow limitation (chronic obstructive pulmonary disease) were treated in a single-blind sequential study with placebo for 2 weeks followed by prednisone 15 mg twice daily for 2 weeks.³⁴ Eight had sputum eosinophilia (≥3%), and 10 did not. The presence or absence of eosinophilia was unknown to the investigators. Outcomes included quality of life, FEV₁, and sputum eosinophils. Only in the eosinophilic group were quality of life (mainly in the dyspnea domain) and FEV₁ improved; eosinophilia was reversed (Fig 2). The degree of improvement in FEV₁ was small and may easily have been missed in clinical practice, raising the possibility that it could not improve further because of remodeling.

Gibson et al^{26,35} has shown that patients with a chronic cough with sputum eosinophilia improve on steroid treatment. In a recent study, Pizzichini et al³⁶ have studied a group of patients with chronic cough who had no sputum eosinophilia. The effect of treatment with placebo or budesonide was examined in a randomized, parallel-group, double-blind trial. The steroid treatment produced no improvement in cough scores.

These results therefore suggest that airway eosinophilia, as indicated by induced sputum, is needed for corticosteroid to have a beneficial effect. Measurements in practice may be especially useful to predict a response to steroid in severe exacerbations of asthma, prednisone-dependent asthma, smokers with chronic airflow limitation, and patients with chronic cough.

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