

The effect of methacholine challenge on the cellular composition of induced sputum

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Analysis of induced sputum is currently being used in clinical studies to characterize the airway secretions of

asthmatic subjects. These studies also often involve the concurrent assessment of bronchial responsiveness to inhaled methacholine. In some cases methacholine challenge is performed immediately before sputum induction. Although 2 reports of the effect of inhaled methacholine on bronchoalveolar lavage cell counts did not reveal any significant changes,^{1,2} there is no available data on the effects of a standard methacholine challenge on the constituents of induced sputum. Therefore to determine the effect of inhaled methacholine on the cellular components of induced sputum from asthmatic sub-

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TABLE 1. Characteristics of induced sputum collected with and without a preceding methacholine challenge

	No preceding methacholine challenge	Preceding methacholine challenge
Volume (mL)	8.5 ± 1.5	8.1 ± 0.9
Total cell count (× 10 ⁵ /mL)	15.8 ± 3.0	11.7 ± 1.5
Squamous cells (%)	34.9 ± 4.1	38.4 ± 3.8
Squamous cells (× 10 ⁵ /mL)	4.6 ± 1.1	3.8 ± 0.4
Eosinophils (%)*	7.0 ± 2.3	5.7 ± 1.8
Eosinophils (× 10 ⁵ /mL)	1.4 ± 0.63	0.52 ± 0.16
Macrophages (%)*	44.8 ± 4.2	37.8 ± 4.4†
Macrophages (× 10 ⁵ /mL)	6.4 ± 1.0	4.8 ± 0.9
Neutrophils (%)*	41.6 ± 4.4	49.7 ± 5.1†
Neutrophils (× 10 ⁵ /mL)	7.2 ± 1.9	5.4 ± 0.8
Lymphocytes (%)*	1.7 ± 0.3	1.3 ± 0.3
Lymphocytes (× 10 ⁵ /mL)	0.25 ± 0.06	0.15 ± 0.04
Epithelial cells (%)*	4.9 ± 1.0	5.6 ± 1.5
Epithelial cells (× 10 ⁵ /mL)	0.61 ± 0.11	0.83 ± 0.30

*Percentage of nonsquamous cells.

†Significantly different from samples collected with no preceding methacholine challenge.

jects, we analyzed induced sputum collected with and without a preceding methacholine challenge.

METHODS

Subjects

Twenty-two nonsmoking, asthmatic adults (14 men and 8 women) with a mean age of 29.8 ± 1.3 (SEM) years, an FEV₁ of 93.7% ± 2.8% of predicted value, and a mean methacholine reactivity (PC₂₀FEV₁[M]) of 0.52 mg/mL (range, 0.1 to 9.4 mg/mL) were enrolled in the study. The subjects had symptoms of mild asthma, 1 or more positive skin prick test responses to aeroallergens, and required only as-needed inhaled bronchodilator therapy. Each subject signed a consent form approved by the Committee on Human Research at the University of California, San Francisco.

Protocol

The study involved 3 visits, the first of which was for asthma characterization studies (allergen skin testing, spirometry, and methacholine challenge). In 12 subjects the second visit (at least 48 hours after visit 1) was for methacholine challenge and was followed 15 to 20 minutes later by sputum induction; these subjects then underwent sputum induction alone at the third visit (4 to 10 days after visit 2). In 10 subjects the second visit was for sputum induction alone; these subjects then underwent a methacholine challenge followed by sputum induction at the third visit (4 to 10 days after visit 2).

The maximum dose of inhaled methacholine administered was the dose that caused at least a 20% fall in FEV₁. Spirometry, sputum induction, methacholine challenge, and allergen skin testing were performed according to previously published methods.³ Briefly, for sputum induction, subjects were pretreated with albuterol 360 µg, and they then inhaled 3% saline for 20 minutes, coming off the mouthpiece at least every 5 minutes to spit saliva into a saliva container (later discarded) and sputum into a sputum container. The sputum cell differentials are presented as the percentage of nonsquamous cells in the induced sputum.

Statistical analysis

Data is presented as means ± SEM unless otherwise specified. Comparisons of sputum data on visits with or without a prior methacholine challenge were made with either the paired *t* test or the Wilcoxon signed-rank test as appropriate. The study had a power of approximately 80% to detect an absolute difference of 2% in percentage of eosinophils. A *P* value of less than .05 was considered significant.

RESULTS AND DISCUSSION

The median dose of methacholine administered during the methacholine challenges that preceded sputum inductions was 0.63 mg/mL (range, 0.16 to 10.0 mg/mL). The volume of induced sputum, the total cell count, and the eosinophil percentage were not significantly different among induced sputum samples collected with and without a preceding methacholine challenge (Table I). In contrast, the neutrophil percentage in induced sputum collected immediately after a preceding methacholine challenge was significantly higher than the neutrophil percentage in induced sputum collected without a preceding methacholine challenge (Table I).

The eosinophil percentage is a commonly reported outcome of induced sputum analysis in asthma. The data reported here suggest that there is no significant effect of methacholine challenge on sputum eosinophils when methacholine is administered in doses required to determine the PC₂₀. This data is in accordance with studies that have examined the effects of methacholine challenge on the cellular constituents of bronchoalveolar lavage.^{1,4} Methacholine may have the potential to induce airway eosinophilia in some instances, however, especially when delivered in very high doses.²

The neutrophil percentage, but not the neutrophil number, in induced sputum was significantly increased after a preceding methacholine challenge. This finding may represent a nonspecific effect of the methacholine challenge on large airways because other studies have shown that airway neutrophilia occurs after control challenges of the airway with isotonic solutions.⁵ Whatever the mechanism of this effect, it will be important to control for it in studies in which both the methacholine reactivity and sputum neutrophil percentage are outcomes.

In summary, we found that a preceding methacholine challenge has no significant effect on the eosinophil percentage in induced sputum but is associated with a significant increase in the sputum neutrophil percentage.

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REFERENCES

- Kraft M, Bettinger CM, Wenzel SE, Irvin CG, Ackerman SJ, Martin RJ. Methacholine challenge does not affect bronchoalveolar fluid cell number and many indices of cell function in asthma. *Eur Respir J* 1995;8:1966-71.
- Diamant Z, Hiltermann JT, van Rensen EL, Callenbach PM, Veselic-Charvat M, van der Veen H, et al. The effect of inhaled Leukotriene D₄

- and methacholine on sputum cell differentials in asthma. *Am J Respir Crit Care Med* 1997;155:1247-53.
3. Gershman NH, Wong HH, Liu JT, Mahlmeister MJ, Fahy JV. Comparison of two methods of collecting induced sputum in asthmatic subjects. *Eur Respir J* 1996;9:2448-53.
 4. Soderberg M, Lundgren R, Bjermer L, Angstrom T. Inhaled methacholine does not influence the cellular composition of bronchoalveolar lavage fluid. *Allergy* 1993;48:173-6.
 5. Liu MC, Hubbard WC, Proud D, Stealey BA, Galli SJ, Kagey-Sobotka A, et al. Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics. *Am Rev Respir Dis* 1991;144:51-8.