

April 1998, Part 1 • Volume 101 • Number 4

Induced sputum examination

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To the Editor:

We are interested in the study by Keatings et al. (J Allergy Clin Immunol 1997;99:693-8) in which induced sputum was used to investigate changes in airway cells and fluid-phase measurements after allergen-induced dual asthmatic responses. The use of sputum for this purpose is relatively new, and as with all new methods there tends to be differences between centers, which may influence the interpretation of results. Therefore we have some questions about the methods, the results obtained, and their interpretation.

First, the sputum induction was performed with a deVilbiss Ultrasonic nebulizer with a very high output (6 ml/min), much higher than that reported by others.^{1 2} We presume that this is the output of the nebulizer minus the tubing that delivers the aerosol to the patient's mouth. The volume of aerosol delivered to the mouth is more relevant. What was this volume per minute?

Second, the duration of saline aerosol has been reported to affect differential cell counts and fluid-phase measurements.³ Early samples of sputum obtained in the first few minutes are said to have a higher proportion of eosinophils and neutrophils, and those produced

later are said to have more macrophages and lymphocytes. In addition, the concentration of fluid-phase eosinophil cationic protein and fibrinogen are significantly lower in the samples taken later. Hence if sputum is obtained more easily after allergen inhalation (which is likely, and therefore the duration of inhalation will be less), increases in markers of inflammation may occur because less saline has been inhaled. In the study by Keatings et al., the duration of saline inhalation was not similar between subjects and between visits (mean [range] at baseline = 15 minutes [6 to 25 minutes] and after challenge = 10 minutes [5 to 18 minutes]).

Third, in processing the sputum the authors have made an attempt to reduce salivary contamination. This contamination will be particularly important in interpreting changes in fluid-phase measurements. Can the authors give some indication of the extent of contamination as indicated, for example, by the percentage of squamous epithelial cells? Was this similar within subjects?

Fourth, the concentration of dithiothreitol seems to be higher than that used by others.²⁴ Is this concentration what was actually used or was the dithiothreitol diluted to 0.1%?

Fifth, the results of the fluid-phase measurements, before and after allergen challenge, are summarized by means and graphically displayed as bars. However, the data is not normally distributed, and means may misrepresent the magnitude of changes. A graph with individual points before and after allergen inhalation would be more informative. This is especially pertinent in relation to IL-5, which was not measurable in all subjects.

Finally, we presume that the units for eosinophil cationic protein in Table II are micrograms per liter rather than milligrams per liter. We also presume that IL-5 levels should be picograms per milliliter rather than nanograms per milliliter. What is the correct value for the correlation between percentage of eosinophils in induced sputum and the maximum fall during the late asthmatic response, $r = 0.79$ as on page 695 or $r = 0.82$ as in Fig. 1, A?

1/8/87934

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