Editorials

Sputum induction to assess airway inflammation: is it an inflammatory stimulus?

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The last five years have seen growing interest in the non-invasive assessment of airway inflammation in asthma and other conditions associated with airway inflammation. A number of techniques have been advocated including measurement of nitric oxide in exhaled air,^{1,2} inflammatory mediators in the exhaled condensate,³ and markers of inflammation in induced sputum.⁴⁻⁶ Induced sputum is particularly promising since it provides specific information on both cellular and molecular aspects of airway inflammation and does not require expensive equipment. Furthermore, the methodology of sputum induction and processing have been unusually well validated and a number of markers of airway inflammation reliably discriminate between normal and asthmatic subjects, are repeatable over a one to two week period, and are responsive to change.⁴⁻⁷

One aspect of the measurement of induced sputum markers of airway inflammation that has not been extensively investigated is the short term repeatability of cell counts and mediator concentrations. This is an important question since induced sputum seems particularly well suited to repeated measurement over short periods of time. Two studies reported in this issue of *Thorax* address this subject. Holz *et al*⁸ evaluated the effects of repeat sputum induction within 24 hours on cell counts and sputum fluid phase eosinophilic cationic protein (ECP) concentrations in 10 normal subjects and 19 subjects with asthma and found that the sputum differential neutrophil count and ECP concentration increased significantly from the first to second induction in both patient groups. Nightingale *et al*⁹ also report an increase in the sputum neutrophil count 24 hours after a sputum induction in a more limited study of eight normal subjects. Both studies showed an associated decrease in the sputum differential macrophage count but no significant change in the eosinophil, lymphocyte, and epithelial cell counts. In the study by Holz *et al* the absolute sputum neutrophil count increased in the second sample whereas the absolute counts of other cell types were unchanged in keeping with a selective influx of neutrophils into the airways.⁸

The studies provide some insight into the time course of the effects of sputum induction on sputum cell counts. Nightingale *et al* showed that the sputum neutrophil count is increased as early as eight hours after the first sputum induction.⁹ A very rapid effect seems unlikely since sputum collected at intervals during a 30 minute sputum induction does not show a progressive increase in the neutrophil count⁸ and the sputum neutrophil count of spontaneous and induced sputum is similar.¹⁰ A prolonged effect of sputum induction is also unlikely since the sputum neutrophil count is repeatable over a one to two week period.^{6,11}

Bronchoalveolar lavage (BAL) using sterile isotonic saline causes an influx of neutrophils into the lavaged and non-lavaged airways of animals¹² and man¹³ and there may be parallels between this and the effect of induced sputum. In man the effect of BAL is seen as early as two hours after the first lavage, is maximal at seven hours, and has resolved by 72 hours.¹³

Neither study provides insight into the mechanism of the effect of sputum induction on sputum cell counts. Possibilities include release of pro-inflammatory mediators in response to changes in the airway lining fluid volume or osmolarity or, less likely, airway mucosal trauma secondary to prolonged coughing. Mediator release is unlikely to involve mast cells since the effect of sputum induction is seen equally in normal subjects and subjects with asthma and is not inhibited by salbutamol. The consequences of the apparent inflammatory response to sputum induction have not been assessed in the current studies although others have shown no change in airway responsiveness 24 hours after sputum induction,¹¹ suggesting that it is not functionally important.

It may be that the magnitude of the effect of sputum induction on neutrophils in the sputum depends on the total amount of hypertonic saline delivered to the airway or on its rate of delivery. The fact that the increase in the sputum neutrophil count in the study reported by Nightingale *et al* (where the total dose of hypertonic saline administered was 67.5 ml at a rate of 4.5 ml/min) was greater than that reported by Holz *et al* (total dose 51 ml; rate 1.7 ml/min) would support this view. Less intensive sputum induction protocols such as that described by Pin *et al*⁶ and modified by Pizzichini *et al*^{6,7} and our group¹⁴ deliver between a quarter and a third of the total amount of hypertonic saline at 0.9 ml/min yet achieve very similar success rates and are more acceptable to patients.^{14,15} It is possible that these protocols do not cause an increase in sputum levels of neutrophils. A recent study by Pizzichini *et al*¹⁶ provides some support for this view by showing that the sputum neutrophil count remained stable in subjects with acute severe asthma who had frequent sputum inductions over one week. Until more is known on the relationship between the dose and rate of delivery of hypertonic saline and the change in sputum neutrophil count it would be sensible to induce sputum using a low output ultrasonic nebuliser delivering as little hypertonic saline as possible.

The effect of sputum induction on sputum neutrophils clearly limits the value of sputum induction in the short term serial assessment of neutrophilic airway inflammation. The findings are particularly relevant to studies where sputum induction is performed before and after allergen

challenge and it is possible that the increase in the sputum neutrophil count seen by some after allergen challenge¹⁷ is an effect of sputum induction rather than allergen. Investigators planning similar studies need to be aware of this potential confounding factor and should take care to study appropriate control groups.

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