PostScript

LETTERS

Lung cancer in HIV infected patients

Since the beginning of the AIDS epidemic our group has contributed to improving the knowledge concerning the impact of HIV infection in the lung. Several of these contributions have been published in *Thorax* as editorials,¹⁻³ original articles⁴⁻⁵ and a review.⁶ In continuing this effort, we have recently published in *Thorax* a review entitled "Lung cancer in HIV infected patients: facts, questions and challenges".⁷ The main objective of this educational review was to alert pulmonologists to a possible increase in the incidence of lung cancer in the HIV-positive population and to underline the facts that lung cancer occurs particularly in young subjects, may be less directly related to smoking and is probably associated with a worse outcome. This review included 103 references which were almost all original articles, six of which were from our group. However, during the time between submitting it to *Thorax* and publication in the journal, another review on the same topic was also published by our group in *Lung Cancer*.⁸ Even though there are strong similarities between the content of these two articles, the form of them is totally different. Furthermore, the review published in *Thorax* contained 29 additional references which were discussed in the paper. Unfortunately, during the process of reviewing the proof we omitted to include the article in press in *Lung Cancer*. We wish to apologise to the Editor of *Thorax* and the readers of the journal for this omission.

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Safety and tolerability of three consecutive bronchoscopies after allergen challenge in volunteers with mild asthma

Ethical and safety considerations limit the design of studies with more than two consecutive fiberoptic bronchoscopies (FOBs) in patients with asthma. We present data on the safety and

tolerability of three consecutive bronchoscopies at baseline, and 24 h and 7 days after allergen provocation. The study included 15 volunteers with mild asthma (9 men and 6 women); median age 25 (range 19-46) years; percentage predicted forced expiratory volume in 1 s (FEV₁) 97% (range 75.4-125.7%); and a mean provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀) of 2.1 (95% confidence interval (CI) 1.2 to 3.6) mg/ml at baseline FOB, 0.93 (95% CI 0.38 to 2.2) mg/ml at 24 h (p = 0.08) and 0.90 (95% CI 0.45 to 1.8) mg/ml at 7 days (p = 0.03) after the allergen challenge.

FOB was not associated with a significant fall in FEV₁ at baseline (median FEV₁ 93.9% predicted (range 80-120.1%) before FOB and 92.8% predicted (73.6-119.0%) at discharge). However, there was a significant reduction in median (range) percentage predicted FEV₁ after FOB performed both 24 h and 7 days after the inhaled allergen challenge: 94.9% (75.1-111.1%) before FOB and 85.5% (62.4-119%) at discharge, p = 0.04, 24 h post challenge; and 100.1% (70.56-119%) before FOB, falling to 90.2% (66.2-119%) 7 days later, p = 0.009. We found a correlation between the percentage of instilled bronchoalveolar lavage volume recovered and change in FEV₁ at discharge (r = 0.31, p = 0.04) when comparing combined data for all three bronchoscopies.

The median (range) pre-FOB oxygen saturation on room air was 99% (95-100%), with no significant change at discharge (97% (96-100%); p = 0.25). During FOB, the median (range) maximum oxygen saturation recorded was 99% (98-100%), whereas the minimum was 96% (92-100%). End procedure median (range) saturation was 98% (91-100%) on 2-4 l supplemental oxygen. Oxygen saturation on room air 10 min post procedure was maintained at a median (range) of 98% (95-99%).

The median (range) oxygen saturation pre-FOB on room air was 99% (96-100%) at 24 h after the allergen challenge and 97% (94-100%) at discharge (p = 0.02). The peak median (range) oxygen saturation recorded was 99% (98-100%)

and the lowest level recorded was 96% (94-100%). The end median (range) saturation was 97% (93-100%) on 2-4 l of entrained oxygen, whereas the saturation on room air 10 min post procedure was 98% (94-100%).

The median (range) pre-FOB oxygen saturation was 98.5% (96-100%) at 7 days and 97.5% (96-100%) at discharge (p = 0.05). The highest recorded median (range) oxygen saturation was 99.5% (98.5-100%) and the lowest was 96% (92-98%). No clinical consequence as a result of desaturation was seen during the course of bronchoscopy.

We found no significant correlation between the change in oxygen saturation and the percentage volume of bronchoalveolar lavage recovered.

Table 1 summarises the effects of FOB on asthma control the day after bronchoscopy. FOB was associated with increased symptoms on all occasions. A significant fall in FEV₁ was seen only after FOB that was preceded by the allergen challenge (p = 0.002) and was associated with the most significant increases in symptoms (p = 0.001) and corresponding drug usage (p = 0.004). None of these changes required treatment other than inhaled short-acting β_2 -agonists and all had resolved by the second day after FOB. Of the 15 volunteers, 12 returned for follow-up 2-6 weeks after the end of the study. The median (range) percentage predicted FEV₁ was 99.88% (79-109%) at follow-up compared with 95.18% (75.41-114.8%) measured at the study entry screening visit. All 12 of these volunteers were still maintained on short-acting β_2 -agonists only and none reported clinical deterioration of asthma control in the weeks after the study.

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Table 1 Summary of the effect of bronchoscopy on asthma control in terms of percentage predicted forced expiratory volume in 1 s, symptom scores and frequency of reliever drugs recorded the day before and the day after fiberoptic bronchoscopy

FOB	Day before FOB*	Day after FOB*	p Value
FOB1			
FEV ₁ %	84.80 (68.06-120)	87.98 (63.71-113)	NS
Symptom score	0 (0-4)	4.5 (0-10)	0.002
Reliever drug frequency	0 (0-2)	1 (0-8)	0.02
FOB2			
FEV ₁ %	90.17 (67.10-120.4)	82.18 (56.25-111.9)	0.002
Symptom score	0 (0-4)	3 (0-9)	0.001
Reliever drug frequency	0 (0-4)	2 (0-16)	0.004
FOB3			
FEV ₁ %	86.78 (69.06-125.0)	86.43 (68.71-120)	NS
Symptom score	0 (0-10)	2 (0-7)	0.05
Reliever drug frequency	0 (0-10)	1 (0-7)	NS

FEV₁, forced expiratory volume in 1 s; FOB, fiberoptic bronchoscopy; NS, not significant.

The data suggest that, provided an established FOB protocol is followed and the procedure is performed by an experienced group of operators with dedicated aftercare, three consecutive bronchoscopies can be performed in volunteers with asthma, with no occurrence of adverse events. Any deterioration in asthma control seems to be related to increased airway hyper-responsiveness resulting from allergen provocation, combined with bronchoscopy.

*Values are median (range).

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Macrophage enrichment from induced sputum

Since induced sputum has become a widely used non-invasive method of recovering cells from the surfaces of the bronchial airways, isolating specific cell populations will be necessary in order to learn more about their specific role in innate immunity and inflammation in the airways. Several studies have demonstrated the ability to conduct ex vivo analyses on sputum cells such as phagocytosis and surface marker measurements, but these have not been performed on isolated cell types.^{1–3} This study demonstrates the capability to isolate sputum macrophages from human volunteers in order to advance our understanding of macrophage biology in the airways. To this end, techniques that can enrich and isolate cells without significant activation would prove extremely useful. We compared two common methods for isolating and enriching macrophages in sputum: (1) magnetic bead separation; and (2) Percoll gel density gradient centrifugation. Cell purity and markers of cell activation (mRNA tumour necrosis factor α (TNF α) and interleukin-1 β (IL1 β)) were measured at various time points in the isolation process.

Nine healthy subjects underwent induced sputum. Sputum collection and sputum processing has been described in detail previously.⁴ For measuring natural cell activation over time, we incubated the processed sputum cells for 3 h at 37°C and analysed mRNA TNF α and IL1 β at 0 h (baseline) and 3 h. In the positive control experiment we incubated the processed sputum cells with 1 ng/ml LPS (*E coli*, Sigma). For Percoll (Amersham Biosciences) separation, 600 μ l of sputum cell suspension (1×10^6 cells/ml) was layered over Percoll solution (42%) and centrifuged at 560 g for 10 min. Sputum macrophages were removed and incubated at 37°C for 1, 2 and 3 h, respectively, and a pre-incubation sample was also collected. The macrophages were further pelleted and stored at –70°C. For Dynabead separation, CELlection Pan Mouse IgG Kit (Dyna, Norway) was used for immunomagnetic separation of airway macrophages coated with mouse monoclonal IgG2b HLA-DR antibody (Diatec, Norway). Bead coating and cell isolation was performed according to the protocol from the manufacturer. The isolated cells were incubated at 37°C for 1, 2 and 3 h, respectively, and a pre-incubation sample was also collected. The samples were further pelleted and stored at –70°C. Total RNA was extracted (Qiagen) from all the cell samples and reverse transcription was performed (Superscript III, Invitrogen). We used pre-developed PCR primers and probes for TNF α and the house-keeping gene PGK (Applied Biosystems). Specific primers and probes were designed for

Figure 1 Gene expression of tumour necrosis factor α (TNF α) and interleukin 1 β (IL1 β) in airway macrophages in induced sputum. The 0 hour (h) Percoll suspensions are used as baseline for 1 h, 2 h and 3 h Percoll suspension (TNF α or IL1 β Percoll, n = 7). The Dynabeads 0 hour suspension are used as the baseline sample for 1 h, 2 h and 3 h Dynabeads suspensions (TNF α or IL1 β beads, n = 6). In addition, gene expression in the whole cell population is measured at 0 h and at 3 h with (n = 1) or without (n = 5) addition of lipopolysaccharide (LPS). The data are presented as mean (SE).

IL1 β using ProbeLibrary (Exiqon ProbeLibrary). Quantification of mRNA was performed using the ABI Prism 7700 (Applied Biosystems), and the relative standard curve method was used to calculate the relative gene expression.

The results show that the median (range) proportion of macrophages in the pre-isolation sputum sample was 61 (34–70)%. Bead isolation produced 99 (95–99)% macrophage purity compared with 88 (85–94)% with Percoll isolation. mRNA expression of TNF α and IL1 β was measured as markers of cell activation in airway macrophages (fig 1) before and after Percoll isolation, Dynabead isolation, no isolation and lipopolysaccharide (1 ng/ml) stimulation (positive control). Levels of mRNA TNF α and IL1 β were significantly increased as early as 2 h using Percoll isolation compared with 0 h baseline (p = 0.02) and bead isolation (p < 0.01). For bead isolation, mRNA TNF α and IL1 β expression were unchanged throughout the isolation period compared with baseline. At 3 h after bead isolation, macrophage mRNA TNF α expression remained near baseline levels whereas Percoll-separated macrophages showed increased activation near positive control (lipopolysaccharide) levels.

The results from this study show that sputum macrophages can be successfully isolated and enriched with a high degree of purity. Furthermore, the magnetic bead isolation technique results in higher macrophage purity and significantly less cell activation than the Percoll isolation technique. As more researchers begin to use individual sputum cell populations to describe airways cellular phenomena, data presented here will provide important technical information to achieve those research aims.

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