Monitoring central and peripheral airway inflammation in asthma

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Introduction

Airway inflammation plays an important role in asthma and its measurement has greatly improved our understanding of the mechanisms that underlie this disease. It can be assessed directly by means of bronchoscopy through analysis of bronchoalveolar lavage fluid (BALF) and biopsies, and also through analysis of induced sputum. The latter method is the least invasive and can be performed repeatedly, allowing inflammatory processes to be monitored over time. Further non-invasive methods used to detect airway inflammation include the analysis of various components of exhaled air, in particular nitric oxide (NO).

It has been demonstrated that inflammatory processes in asthma occur not only in the central airways, but also in the peripheral airways and lung parenchyma (1). Treatment of inflammation, either via the systemic or the inhaled route, may not reach all regions of the lung to a sufficient extent; therefore monitoring inflammation in different areas of the airways may help to optimize treatment.

The aim of this review is to compare the different methods used to assess inflammation throughout the airways, in particular the ability to attribute the inflammatory processes to either central or peripheral airways.

Central versus peripheral airways

Central airways are all those down to the subsegmental bronchi, i.e. the fourth or fifth generation, with a diameter of 4–5 mm and length of 8–10 mm. Beyond this, down to the ninth generation, the airways have a diameter of 2–4 mm and a length of 3–6 mm and are referred to as small bronchi. Airways with a diameter less than 2 mm are often called distal or small airways, comprising terminal and respiratory bronchioles, down to a diameter of 0.5 mm and representing the 14th to 16th generation (2).

Bronchoalveolar lavage

The method of bronchoalveolar lavage (BAL) was introduced in 1970. Interestingly, and in a similar way to the situation experienced today with the method of induced sputum, it was first thought of solely as a research tool, owing to the difficulties in performing and standardizing the procedure (3,4). Nowadays, the method is well accepted in clinical practice as a valuable tool for diagnosis and monitoring of a wide range of airway and lung diseases (5).

Bronchoalveolar lavage is used primarily to sample the alveolar region. Commonly, it is performed by sequentially adding four to five portions of 20–50 ml of isotonic saline to the middle lobe. To reduce the contribution from components of the peripheral airways, it is sometimes recommended to introduce the saline in larger volumes (3). However, it seems reasonable to deliberately investigate samples from the peripheral airways by recovering a first, small (20 ml) portion of saline immediately after introduction. The comparison of the first portion, termed bronchial wash (BW), and subsequently obtained portions of BALF has revealed a gradient in cellular composition along the bronchial tree, with more neutrophils and fewer macrophages in the peripheral airways compared with the alveoli (6,7). Using special balloon catheters it is also possible to temporarily isolate compartments within the central airways and to selectively sample fluid from these regions (8). Such studies have demonstrated that the central airways show a higher proportion of neutrophils than the peripheral airways and the alveolar region.

A recent randomized open-label study has used BAL to examine the anti-inflammatory effect of two different preparations of the inhaled corticosteroid, beclomethasone dipropionate (BDP 400 µg bd), administered via a press-and-breathe metered dose inhaler on cells of the peripheral airway (9). Alveolar macrophages were harvested using BAL from 28 healthy volunteers before and after a 14-day course of inhaled BDP or placebo and then placed in primary culture. The two formulations of BDP that were compared included a chlorofluorocarbon (CFC)-free preparation, which utilized hydrofluoroalkane-134a as propellant (QVAR™, HFA-BDP, 3M Pharmaceuticals, St Paul, MN, U.S.A.) and a CFC-containing BDP (CFC-BDP). The cultured alveolar macrophages were then stimulated to release a key inflammatory mediator, tumour necrosis factor-α, through incubation with a bacterial glycolipid preparation. It was found that glycolipid-induced tumour necrosis factor-α production from the harvested alveolar macrophage cells was significantly reduced compared with baseline following HFA-BDP treatment, while treatment with CFC-BDP and HFA-placebo had no effect. These
findings suggest that alveolar deposition of inhaled HFA-DBP is capable of modulating the inflammatory potential of alveolar macrophages. This study also clearly demonstrates how BAL techniques can be used to investigate the effects of asthma treatments on inflammatory processes in the small airways.

In summary, the advent of flexible bronchoscopes has enabled several techniques to collect samples from different regions of the airways. Down to the subsegmental bronchi the location can be precisely determined and visualized. Beyond this, the region sampled must be assigned to the peripheral airways or the alveolar space. The introduction of ultrathin bronchoscopes, which can advance further down the airways, may improve this situation, although currently they seem to be limited to visual inspection (10). Bronchoscopy has been, and will remain, a valuable tool to assess airway inflammation and has potential for direct assessment of topical anti-inflammatory effects, however it is rather invasive and therefore of limited applicability when airway inflammation is to be monitored repeatedly.

**Bronchial biopsies**

Biopsies can be taken at different depths of the airways, from bronchial mucosal biopsies of the large airway to transbronchial biopsies of the peripheral airways and lung parenchyma. Unfortunately there is little data concerning a comparison of cellular composition of biopsies taken in different depths of the central airways. It is known that the variability between biopsies from the same individual is smaller than the variation between individuals (11), and thus seems to be a prerequisite of any use of biopsies to describe individual patterns of inflammation.

What is available, are comparisons between endobronchial and transbronchial biopsies. These studies provided evidence on differences in inflammation between central airways versus peripheral airways and the alveolar region. For example, patients with nocturnal and non-nocturnal asthma showed higher numbers of eosinophils per volume of tissue in the small airway/alveolar region than in bronchial mucosal biopsies (12). However, of fundamental importance is careful discrimination between which part of the sample is analysed. Haley et al. demonstrated a similar distribution of eosinophils along the airways of patients with asthma, but only when the outer region of the airway, defined as the area between smooth muscle and alveolar attachment, was compared (13). The inner airway regions, defined as the area between basement membrane and smooth muscle, showed the inverse course, with higher numbers of eosinophils in the large as compared to the small airways.

Compared with forceps biopsies, brush biopsies sample a larger area of the airway, but sub-basal cell structures are less well preserved. Brush biopsies mainly comprise epithelial and goblet cells, and for these cell types no differences were observed between samples taken from the carina and samples taken from subsegmental bronchi (14).

**Induced sputum**

Unlike bronchoscopy, the analysis of sputum, either induced or spontaneously produced, is a less invasive way to obtain material from the airways. This method therefore has regained major interest as a tool to measure airway inflammation in large numbers of subjects or repeatedly. Sputum is characterized by a much higher percentage of neutrophils than found in BW and BALF. Therefore it is likely that the sputum obtained during an induction is derived mainly from the central airways (15). Indeed, the percentage of neutrophils in sputum corresponds very closely to those found in lavage fluid of the central airways (8,6). When sputum composition was compared with that of airway secretions sampled by bronchoscopy in the proximal airways, again very similar percentages of neutrophils were found with both techniques (16). In addition, the inhalation of a bolus of radiolabelled particles into the central airways resulted in much higher radioactive counts in sputum as compared to the counts when particles were deposited peripherally (17).

Data from our own laboratory indicate that sequential sputum inductions sample different depths of the airways. The cellular composition changes when sputum samples produced during three consecutive inhalation periods are compared (18). The highest percentage of neutrophils occurred in the first sputum sample, while the lowest proportion occurred in the sputum that was produced during the third induction period (Fig. 1). At the same time the percentage of macrophages increased, indicating that the sputum originated from more peripheral airways. This phenomenon has also been observed when using other induction protocols (19,20) Addition evidence for the hypothesis on sequential sampling of progressively more peripheral airways has been supplied by Gershman et al. who demonstrated that the concentration of mucin decreased and that of surfactant increased in consecutive sputum samples (19). This change in sputum composition may be considered in two ways. On one hand it points out the need for standardization of the time of sputum induction. On the other hand it offers a chance to deliberately sample different regions of the central airways by an easy to perform and acceptable method. It might be of interest to compare the gradient in cellular composition between different diseases. Using a slightly different protocol we demonstrated that proportions of neutrophils did not change in patients with COPD, and that the change was much smaller in patients with asthma as compared to healthy subjects (Fig. 2) (21).

In summary, sputum induction is a non-invasive way to assess inflammation within the central airways. It can be performed repeatedly and therefore has the advantage of enabling airway inflammation to be monitored, e.g. in response to treatment. However, as compared to the more invasive bronchoscopy, the analysis of sputum does not accurately locate the origin of the sample within the central airways. It may only provide a relative location by comparing samples produced during consecutive induction periods.
FIG. 1. Mean percent cell counts in sputum for three consecutive sputum sampling periods within one induction procedure. The decline in the percentage of neutrophils was statistically significant in the healthy (□) and asthmatic subjects (■) (P < 0.001), the pattern of change being different between groups (interaction term, P < 0.05). The increase in the percentage of macrophages was also statistically significant (P < 0.001). Data according to Marshall et al. (18).

FIG. 2. Changes in sputum composition during sputum induction in different groups of patients (21). Mean and SEM of 11 healthy subjects, 10 patients with COPD, 11 patients with mild asthma (group 1: β2-agonists only), 12 patients with mild to moderate asthma (group 2: β2-agonists and inhaled steroids) and seven patients with moderate to severe asthma (group 3: inhaled and oral steroids) are given. The change in the percentage of neutrophils in sputum obtained in the two induction periods was significant in healthy subjects and patients with moderate asthma (**P<0.01, *P<0.05). □: Inhalation period 1; ■: inhalation period 2.
Comparison of BALF, BW, biopsies and induced sputum

A number of studies compared the data from bronchoscopy (BALF, BW, biopsies) and induced sputum within the same subjects (22-25). In most cases the result was that sputum composition correlated better with the composition of BW than with the composition of BALF, while the correlation between the composition of sputum and that of biopsies was rather weak. Since evidently these methods sample different regions of the airways and lung, these results seem plausible.

Exhaled air

The detection of markers of disease in exhaled air, as guided by the physician's nose, has a long tradition in medicine. Nowadays, extremely low levels of gaseous components such as NO can be measured by fast analysers, and minute amounts of substances collected in the breath condensate can be quantified via enzymatic reactions.

For most compounds detectable in the breath condensate, either volatile or non-volatile, the discussion about their actual concentration, their reproducibility, their origin within the lung or airways and their interpretation has only just begun. For hydrogen peroxide (H₂O₂), at least, the flow dependence of the detected concentration in breath condensate seemed to indicate its origin from the central airways (26). However, variability appeared to be high despite all attempts to standardize the breathing manoeuvres that were required.

So far, only the measurement of NO has been standardized in official guidelines (27). It is well known that the concentration of exhaled NO is higher in asthmatic patients as compared to healthy subjects (28) and that levels of exhaled NO are increased during late phase responses to allergen (29). The treatment of asthma with corticosteroids results in a reduction of the exhaled NO, which is very likely due to a down-regulation of the inducible nitric oxide synthase (iNOS), the enzyme thought to be responsible for the largest part of NO produced within the airways (30). Recently, however, it has also been demonstrated that steroids also normalize the pH value that is measured in the breath condensate of asthmatic patients. This is possibly an indication for an additional mechanism affecting the concentration of NO in exhaled air (31).

As NO is produced in the nasal cavities in very high concentrations, exceeding the concentrations detectable in the lower airways by a factor of 100 to 1000, it requires special means to measure only the NO produced in the airways and lung. Commonly, patients exhale against a resistance to assure the closure of the velum, to exclude the nasal NO. This results in an accurate measurement of the NO originating from the lung, and the NO level measured at a well-defined expiratory flow rate can be used phenomenologically to support the diagnosis of airway diseases or the assessment of the effects of anti-inflammatory treatment.

However, localization of the source of the exhaled NO within the lung requires a special set-up and the use of mathematical models for the analysis of NO signals (32-34). Nitric oxide could be derived from the airways or from the alveolar region. The latter is rather unlikely, since the diffusing capacity for NO in the alveoli is very high. The conclusion that NO is derived from the airways is supported by direct measurements of NO via bronchoscope, which showed very similar concentration as those detected in expired air (35). Furthermore, the NO concentration reaches a true plateau level during exhalation which is flow-dependent (Fig. 3). To explain this flow dependence, a two compartment lung model has been introduced. In this model the alveolar space is connected to a tube, which represents the airways, through which the air is exhaled. During a slow passage through the tube the air has much time to pick up NO, as produced in the airway wall, resulting in high exhaled concentrations. Conversely, after a fast passage there will be a lower concentration in the exhaled air. In addition, the diffusion of NO into the air depends on the concentration gradients between the airway wall and lumen. These relations can be expressed in a mathematical model that describes the values observed at different flow rates fairly well (33).

As a result, the level of exhaled NO is related to the level of production within the airways and to certain parameters such as the airway diffusing capacity for NO.

When the breathing manoeuvre includes a sudden change of expiratory flow rate, the model can be used to estimate the volume of the airways within which NO is produced (36). As NO is considered to be a marker for airway disease, this production volume might correspond to the region of ongoing inflammation. The measurement involves an

Fig. 3. The upper panel shows six different NO-concentration profiles obtained during exhalation of the same volume of air at different flow rates. In the lower panel the average NO concentrations of the plateau levels are plotted against expiratory flow rate.
experimental setup that allows instantaneous changes to air flow during exhalation. This 'step-response' will ideally result in a NO signal with two plateaux. After a sudden reduction in air flow there is a time-lapse before the exhaled NO concentration begins to rise from its initial steady state value. This allows the deadspace of NO production, from the lips to the site where NO production starts within the airways, to be estimated. The time needed to reach the second steady-state (plateau) value gives the volume in which NO is produced within the airways. The volumes are obtained by multiplying the respective times with the respective flow rates.

We used this method in a pilot study where we compared 2 weeks of treatment with BDP (200 μg day⁻¹) delivered as HFA-BDP or CFC-BDP from metered-dose inhalers in 15 steroid naive patients with mild asthma (37). After HFA-BDP treatment there was a trend towards a decreased bronchial NO production volume, suggesting that future non-invasive technique might be capable of a volumetric assessment of the site of airway inflammation. This reduction in NO production volume following HFA-BDP treatment was correlated with a significant reduction in NO levels and a significant improvement in lung function.

Summary
Invasive methods involving bronchoscopy allow identification of the site of airway inflammation, although within the peripheral airways further distinctions are difficult. The method of induced sputum refers only to the central airways, and the analysis of exhaled NO to the airways as a whole. Therefore there is still a need for more refined tools to assess airway inflammation.

References


