

Assessment of airways inflammation in chronic bronchitis

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Chronic bronchitis is a clinical syndrome, defined on the basis of sputum production [1, 2]. Investigations have demonstrated that airway inflammation is central to the clinical changes associated with chronic bronchitis. Histological investigations of surgical and autopsy specimens have provided material to determine the site and nature of the airway inflammation. Sampling of the airways via flexible fiberoptic bronchoscopy has also advanced understanding of the nosology and cell biology of airway inflammation, and promises to provide a means for rational assessment of therapeutic interventions.

Histological investigations of resected lungs, and lungs obtained at autopsy, have correlated the presence of airways inflammation with airways obstruction in chronic bronchitis. In studies reported by Costo et al. [3], airway inflammation was found to be a distinguishing characteristic in the airways of smokers with obstruction, compared to smokers without obstruction. More recently, these findings have been confirmed by Mullen et al. [4], who demonstrated that airway inflammation correlated with hypertrophy of mucus glands and best distinguished smokers with chronic bronchitis from smokers without chronic bronchitis. Thus, pathological investigations confirm the central role of airways inflammation in the clinical manifestations of chronic obstructive pulmonary disease (COPD). However, pathological investigations are obviously limited in their applicability for testing of therapeutic modalities directed towards the treatment of airways inflammation.

Sputum has been used for assessment of airways inflammation in chronic bronchitis [5]. In particular, sputum cytology has been used to determine the presence or absence of intraluminal neutrophilia, and can, to some degree, assess the intensity of the inflammation. Moreover, mediators of airways inflammation can be assessed in sputum [6, 7]. However, measurements in sputum are limited by several factors. Sputum is not available from normal individuals. The collection of expectorated sputum is inevitably associated with contamination by oral contents. Sputum is very heterogeneous. Finally, quantification of sputum contents is difficult; sputum is difficult to physically manipulate, and measurements expressed as concentrations may not in fact accurately reflect the degree of hydration of the sputum, the extent of contamination with saliva, or the rate of elimination of sputum from the lower respiratory tract. In spite of these limitations, sputum has been successfully employed to assess airways inflammation, and to measure the effect of therapeutic interventions upon lower respiratory tract inflammation [8, 9].

Alternatively, bronchoscopic techniques can be used to sample the lower respiratory tract. Flexible fiberoptic bronchoscopy allows direct visualization of the mucosa of the first four to six generations of the airways. Consistent with inflammation elsewhere, inflammation of mucosal surfaces is associated with erythema and oedema. In addition, when inflamed, mucosal surfaces increase secretion and become more susceptible to physical trauma [10]. Taking advantage of these characteristics, a semiquantitative scale has been developed for the visual assessment of airways inflammation [11]. Using the "bronchitis index", cigarette smokers who deny symptoms of chronic bronchitis have been found to have abnormal appearance of the airways (mean=8.5, range=4–18) compared to normals (mean=2.3, range=0–12), but have significantly less severely abnormal appearance of the airways compared to smokers with chronic bronchitis (mean=12.2, range=3–31) [12]. In addition, the bronchitis index has been noted to improve with therapeutic interventions [13, 14].

Bronchoalveolar lavage (BAL) has been used to quantify the cellular and biochemical markers of airways inflammation in a number of airway diseases, including chronic bronchitis, asthma, cystic fibrosis and bronchiectasis (in normal hosts). Bronchoalveolar lavage can be performed in a manner which allows for enrichment with airways contents. It has been demonstrated that the initial fluid infused, following wedging of the bronchoscope into an airway, tends to preferentially sample the airways [15, 16]. This preferential sampling is dependent upon infusion of small volumes. The adequacy of the preferential sampling of airways is reflected by BAL fluid content with greater numbers of airway epithelial cells [17], and higher concentrations of proteins such as lactoferrin and lysozyme, which are secreted by airway serous cells, than

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in the alveolar sample [18]. Using such criteria, volumes of 20 ml or less provide samples enriched, perhaps tenfold, with airway contents.

Cigarette smoking and chronic bronchitis have both been shown to influence BAL findings [19]. Bronchoalveolar lavage in young smokers has demonstrated elevated recovery of lavage fluid cells, in particular macrophages and neutrophils [20]. Martin et al. [21] demonstrated that chronic bronchitis was associated with neutrophilic inflammation, and that the first instilled 50 ml of lavage fluid contained higher amounts of neutrophils than later instilled aliquots. Employing 20 ml aliquots to sample airways contents has demonstrated that chronic bronchitis is associated with airways neutrophilia [12]. The intensity of the airway neutrophilia was positively correlated with airways obstruction, sputum production, and smoking history. In a separate study, an elevated proportion of goblet cells, presumably reflecting goblet cell metaplasia, was recovered by lavage of chronic bronchitics [22]. The number of goblet cells was strongly correlated with the forced expiratory volume in one second (FEV1), corroborating previous observations [23]. Furthermore, BAL has disclosed elevated alveolar albumin [12], and proteins felt to be derived from airways epithelial serous cells, lactoferrin and lysozyme [18], in association with chronic bronchitis. Therapeutic interventions, including smoking reduction [13], theophylline [24], and inhaled beclomethasone [14], have been shown to ameliorate these indices of airway inflammation in chronic bronchitics.

Bronchoalveolar lavage is not without its limitations. It is clearly more invasive than collection of expectorated sputum. No standard technique is universally accepted for the performance of BAL [25, 26]. A number of different techniques have been employed by investigators, which may account for slight differences in results. Indeed, differences resulting from technical considerations have been advantageously exploited by investigators. A more important limitation is that there is no gold standard for the reporting of BAL data. The variable dilution of the epithelial lining fluid of the lower respiratory tract during BAL remains problematic. Various endogenous markers for dilution have been used, particularly albumin and urea [27]. However, both the concentration of albumin [14, 28], and of urea in BAL fluid are subject to systematic errors [29, 30]. One approach to this dilemma is to express data as a concentration of lavage fluid, normalized to albumin, and as an estimated concentration in epithelial lining fluid, using the urea method [31]. Expressed in this manner, and taking into account the limitations of albumin and urea as endogenous markers for dilution, comparisons can be made between subject groups.

Sampling of the endobronchial mucosa can be performed via the flexible fiberoptic bronchoscope, by direct brushing or biopsy. Brushing the bronchial wall with a cytological brush, and agitating the cells in saline or medium, yields dispersed cells and clumps of bronchial mucosal cells. The cells can be further dispersed with proteases, or manipulated as clumps. The epithelial cells can be quantitated and freshly preserved, or maintained in cultures. Advances in molecular biology and immunohistochemistry have produced very sensitive techniques, which can be fruitfully applied to the epithelial cells harvested by endobronchial brushing.

Endobronchial brushing is slightly more invasive than BAL, and is limited by the relatively small area of endobronchial anatomy which can be sampled. However, brushing provides a representative cross-section of epithelial cells, directly sampled in situ. Thus, investigations of cells sampled by brushing do not suffer the experimental artifacts theoretically present for BAL, which harvests a population of epithelial cells that are presumably loosely adherent or non-viable. Endobronchial brushing of subjects with chronic bronchitis has disclosed an increased percentage of epithelial goblet cells, compared to normal nonsmokers [32]. The sensitive methodologies of molecular biology are ideally suited for the investigation of cells obtained by brushing. Ribonucleic acid (RNA) has been extracted from the cells and probed with reagents to detect messenger ribonucleic acid (mRNA) for inflammatory mediators [33], and brushed epithelial cells have been used as targets for transfection experiments [34]. Immunohistochemistry can be applied to delineate both membrane-bound and cytoplasmic antigens.

Sampling of the airway epithelium by endobronchial biopsy adds a further degree of invasiveness, and is even more limited in the surface area of bronchial mucosa which is sampled. However, endobronchial biopsy has the unique property of preserving mucosal anatomy. This property of biopsies permits assessment of pathological changes, which are not appreciated by brushings. Biopsies can also be used to quantitate inflammatory cells within the mucosa and adjacent submucosa. As for the epithelial cells obtained by brushing, endobronchial biopsies provide effective samples for immunohistochemistry and molecular biology.

Bronchial biopsy has been used to good advantage, especially for the investigation of airway inflammation associated with asthma. Biopsies have confirmed the involvement of eosinophils, even in mild asthmatics [35]. In mild asthma endobronchial biopsies have indicated ultrastructural changes suggestive of epithelial fragility with hyperreactivity [36]. Endobronchial biopsy has also proved to be of value in the assessment of therapeutic interventions. Biopsies performed before and after 10 yrs of daily therapy with inhaled steroids demonstrate resolution of inflammation and reduced epithelial damage [37]. The successful application of endobronchial biopsies in the study of asthma holds promise for the wider application of invasive sampling of bronchial epithelium for therapeutic investigations in other airway inflammatory diseases, such as chronic bronchitis and cystic fibrosis.

As newer therapeutic strategies for the treatment of lower respiratory tract inflammation are developed, the importance of the assessment of airways inflammation and its response to therapy will continue to grow. In subjects with chronic bronchitis and fixed obstruction, demonstrations of therapeutic efficacy by means of traditional pulmonary function tests would require long-term investigations with large numbers of subjects. In
contrast, the demonstration of amelioration of airways inflammation has been shown to be a practical alternative. Improvement in morbidity and mortality are, of course, the ultimate yardsticks for therapeutic efficacy in COPD. Demonstration of efficacy in the therapy of airways inflammation may, however, provide the rationale and justification for extended longitudinal studies for specific therapeutic interventions.

References

