Foreword

The use of biopsy to study airway inflammation

Airway inflammation is a characteristic feature of asthma. The typical components of chronic inflammation are loss of surface epithelium, a thickening of the reticular layer of the sub-epithelial basement membrane (BM) (1), and increased numbers of eosinophils, mast cells and T lymphocytes in the airway mucosa; increased numbers of eosinophils are commonly found even in patients with mild disease, and the number of these cells is correlated with disease severity (2). Cellular infiltration is promoted by increased expression of a number of cytokines, the profile of which is usually consistent with a T-helper 2 (TH2) phenotype, with increased expression of interleukin (IL)-4 and IL-5 (3). Excessive mucus is produced by epithelial goblet cells, and increased vascular permeability also occurs, resulting in mucosal oedema. The airway is narrowed both by the oedema and by hypertrophy of bronchial smooth muscle. Severe steroid-dependent asthmatics may have a different type of inflammation to that in mild to moderate asthma, with greater neutrophilic infiltration; in some of these patients, eosinophilia is absent (4).

Airway remodelling, which is usually consequent to chronic inflammation (1), describes a more persistent process of structural change that is typical of established and possibly under-treated asthma; this may be triggered by damage to the bronchial epithelium (5).

By taking biopsies of bronchial tissue, inflammatory changes can be directly observed using electron microscopy (EM). This allows assessment of the mucosal structure, including the epithelium, BM, lamina propria, and vasculature; this information cannot be gained from bronchoalveolar lavage (BAL) or induced sputum samples. EM also allows study of inflammatory cells such as eosinophils, mast cells and plasma cells. By using high-resolution scanning EM, cell surfaces can be studied in fine detail. Confocal microscopy may also be useful. Biopsy samples can be subjected to freeze-fracture to study the integrity of the tight junction between epithelial cells, through which allergen particles may gain access to antibody presenting cells in the mucosa.

Other techniques are used to process and analyse biopsy samples to generate more information about the underlying cellular and molecular abnormalities present in patients with asthma. One of the most frequently used methods is immunohistochemistry; this entails staining the tissue with conjugated monoclonal or polyclonal antibodies, so that the inflammatory cells can be classified according to the cell surface antigens that they express, e.g. EG1+ and EG2+ for eosinophils (6). This treatment colours the cells so that they can easily be identified and counted by microscopy; this method also allows the activation status of certain cells to be determined. Another widely used tool is in situ hybridization, which is used to detect the presence of intracellular mRNA that codes for synthesis of proteins such as interleukins and interferon (7,8).

The practice of bronchial biopsy is now so widespread that there is a need to standardize biopsy methods, including excision and processing of the sample, fixing and sectioning it, and counting cells (or otherwise analysing the histology). Although the variability introduced by differences in biopsy methods is small when compared with the heterogeneity of asthma patients, standardization would simplify interpretation of study results; until this is achieved, it is important that investigators report in detail the methods used to process and analyse the biopsy. The variability in baseline counts of inflammatory cells between patients, and diurnal variation in levels of inflammatory markers, can be addressed through rigorous study design, as described in the second article of this supplement (9).

Bronchoscopy is acknowledged to carry a medical risk and the invasiveness of the procedure may discourage patients (or volunteers) from participating in biopsy studies; the risk/benefit ratio of the procedure has greatly improved, however, and no longer limits the use of biopsy. An exception to this is transbronchial biopsy which is used for sampling the lower airway and is associated with a higher risk of bleeding and more serious complications. Biopsy may cause epithelial damage, which complicates the interpretation of studies measuring epithelial fragility in asthmatic patients.

BAL is frequently performed at the same time as bronchial biopsy, and is useful for studying the activation status of inflammatory cells. The disadvantage of BAL is that it is a dilute sample, largely derived from the alveolar compartment; significant changes in the number of certain inflammatory cells may be difficult to detect. The proportion of the sample that derives from the airways can be increased by reducing the lavage volume.

Induction of sputum provides a more concentrated source of airway secretions than BAL, deriving mainly from the large airways. This procedure can be performed many times in the same subject and is very reproducible. However, sputum is a complex biological sample; measurement of the component of the fluid phase needs careful validation, and the sample is prone to degradation if not carefully handled and stored. The procedure provides no direct information on the structure of the airway.

Inflammation can also be assessed directly from the macroscopic appearance of the airway surface during bronchoscopy. Other methods are being developed that may help to quantify or assess inflammation in the lung, including exhaled nitric oxide and exhaled breath condensates. However, until these procedures are fully validated for a wide range of clinical applications, bronchial biopsy will remain the best procedure for the sampling of airway tissue and for the direct study of inflammatory changes.

The papers in this issue of Respiratory Medicine form the proceedings of a Biopsy Workshop held in Copenhagen in
November 1999, and cover two main topics. The first is the optimization of biopsy assessments in the context of clinical study design. The two papers review ways in which elements of protocol design can improve the productivity of the biopsy results, and present recommendations on the selection of inflammatory markers that may be monitored in assessments of drug treatment for asthma.

The second topic is the effect of treatment on the inflammatory component of asthma. Three papers review biopsy studies and other investigations that have shed light on the anti-inflammatory profiles of inhaled corticosteroids, long-acting β2-agonists, and the combination of salmeterol with inhaled corticosteroids. These papers provide a timely survey of how the application of bronchial biopsy techniques has allowed an improved evaluation of how modern drug therapies modify the cellular and mechanistic processes of asthma.

References