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EDITORIAL

Investigative bronchoscopy in asthma and other airways diseases

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In this issue of the European Respiratory Journal the guidelines for the "Investigative use of bronchoscopy, lavage and bronchial biopsies in asthma and other airways diseases" are published. They will also be published in three other prominent journals (American Review of Respiratory Diseases, Chest, Journal of Allergy and Clinical Immunology) [1]. The document was produced by a group of experts under the sponsorship of the American National Institute of Health. After appropriately stating that invasive bronchoscopy has very little use in clinical asthma and that the guidelines are limited to research application, this document reviews the techniques and the past experience obtained with them. Subsequently, it provides indications and contraindications, and prudently and appropriately suggests that invasive bronchoscopy provides material very difficult to examine productively and, thus, that only investigators who can guarantee an adequate and valuable use of this precious biological material should perform invasive bronchoscopy in their patients. Anticipating that the major risk factor for the patient is the experience of the bronchoscopist and, thus, that only expert bronchoscopists should perform bronchoscopy in asthmatics, the guidelines provide detailed indications on safety, potential hazards, patient evaluation before and after bronchoscopy, safety parameters to be monitored, and procedures. Finally, the guidelines provide two short, but comprehensive, paragraphs on limitation and future needs.

Overall, the guidelines provide a very useful reference for investigators of the area. They encourage extension of the use of bronchoscopic techniques to patients with airways diseases other than asthma, where the experience is very limited and also, with due care, to patients with more severe airflow obstruction. In this editorial we briefly review the results obtained with bronchoscopic techniques in the last 10 yrs, and try to predict the areas that will be investigated in the future, taking full advantage of this timely, well-balanced, clear and valuable document.

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Background and perspectives

Investigative bronchoscopy has been increasingly and safely used in subjects with asthma in the last 10 yrs [2]. Studies in other airways diseases have been very limited [3]. The findings of bronchoscopy and associated techniques are not diagnostic in asthma and the therapeutic use of bronchoalveolar lavage or bronchial aspiration is still controversial [2, 4]. Thus, investigative bronchoscopy, apart from individual therapeutic applications, is of very little clinical value in asthma [1, 2], but it can be productively used as a tool for clinical studies. Indeed, investigative bronchoscopy has been used almost exclusively during research protocols, and important discoveries have been made by analysing bronchoalveolar lavage (BAL) fluid and cells, tracheobronchial biopsies and, more recently, by placing devices to measure airway temperature, tracheobronchial blood flow, and airway physiology in central as well as peripheral airways [1].

The increasing interest in investigative bronchoscopy has been stimulated by the development of the concept that asthma is not, or at least is not only, a disease characterized by reversible bronchospasm due to contraction of airway smooth muscle, but also by a peculiar pattern of airway inflammation [5]. Thus, interest has progressively moved from the traditional measurements of airway physiology (*e.g.* lung function tests, bronchoprovocation) to the pathological correlates, first looking at peripheral blood, sputum and bronchial aspirates, and then to lavage and tracheobronchial biopsies.

The first studies including investigative bronchoscopy in asthmatics were performed on patients with mild to moderate asthma using BAL, both in baseline condition [2, 6, 7] and after bronchoprovocation [8, 9]. These studies showed that in BAL obtained from asthmatic subjects in baseline conditions, there is a small but significant increase in the number of inflammatory cells, in particular of metachromatic cells and eosinophils, and a slight increase in some inflammatory mediators compared to normals (reviewed in [2]). Studies performed after bronchoprovocation showed that allergen or chemical sensitizer induced asthmatic reactions, and late asthmatic reactions in particular, are associated with: a) a marked increase in inflammatory cells, particularly eosinophils and/or neutrophils; b) a change of lymphocyte subpopulations; c) an

increase of macrophages, and of their state of activation [8–10]; and d) a marked increase of inflammatory mediators and proteins in BAL fluid, suggesting the development of an acute inflammatory reaction of the airways associated with microvascular leakage of plasma proteins and oedema formation [2]. Interestingly, even if in baseline condition the number of inflammatory cells was only slightly changed, when examined *in vitro,* some of these cells were shown to have markers of activation in asthmatics compared to normals [7, 11–13].

The bronchoprovocation and BAL techniques used in these early studies might have investigated changes not only of the airways but also of the alveolar spaces. Apart from the lack of specificity of sampling between airways and alveolar spaces, one additional limitation of BAL in the investigation of airways diseases is its intrinsic low sensitivity, due to the fact that large volumes of fluid, *e.g.* three aliquots of 50 ml each, are instilled both in the airways and in the alveoli. To increase the recovery from the bronchi, smaller volumes of fluid have been used and new techniques have recently been developed. These techniques consist in lavaging isolated airway segments created either with a double balloon bronchoscope or with a double balloon-tipped catheter inserted through a double lumen bronchoscope [14]. They are extremely promising because lower volumes improve the sensitivity by reducing the dilution of airway lining fluid and cells, the fluid recovered coming only from the airways; and because they allow the consistent recovery of cells and mediators in the same airway segment before and after bronchoprovocation.

Following the pioneering work of LAITINEN *et al.* [15], who examined tracheobronchial biopsies obtained through the rigid bronchoscope, and who demonstrated severe damage to the airway mucosa in asthmatics, there has been an increasing number of studies on tracheobronchial biopsies obtained through the flexible fibreoptic bronchoscope, almost all confirming their findings and extending their observations [16, 17]. In addition to epithelial damage, the examination of bronchial biopsies enabled investigators to discover that even mild asthmatics have an increased number of eosinophils and metachromatic cells in the airway mucosa, and an increased number of activated lymphocytes. Also, that both resident mucosal cells and/or inflammatory cells expressed the capacity to produce, or indeed produced, inflammatory mediators [18, 19]. In this respect, the application of the modern techniques of cell and molecular biology, combined with the traditional pathological studies, look very promising, not only for the characterization of asthma but also for the understanding of its pathogenesis.

Finally, BAL and/or airway mucosal biopsies have recently been examined before and after treatment with anti-inflammatory agents or bronchodilators. These studies have shown that the improvement of symptoms and lung function, which is invariably associated with anti-inflammatory treatment, is also associated with a decrease in the degree of inflammation of the airway mucosa, neither effect being obtained with bronchodilators [20–23]. These results further support the modern approach to asthma therapy, which suggests aerosolized anti-inflammatory agents as the first-line therapy of asthma [24, 25].

Future applications

Although, at present, it is not possible to predict a clinical application for investigative bronchoscopy in airways diseases, it may be important to examine the correlations between non-invasive techniques such as sputum examination, and BAL and/or tracheobronchial biopsies in the search for biochemical, immunological and cellular markers to be used in daily clinical practice. Indeed sputum, and particularly sputum induced by inhalation of aerosolized hypertonic solutions [26], has been shown to be an excellent, non-invasive examination, both in the diagnosis of neoplastic and infectious diseases and, more recently, of airways diseases including asthma [26–29]. If some of the information obtained with lavage and biopsies is proven to be obtainable with the examination of sputum, investigative bronchoscopy will have provided the basis for a major diagnostic, and possibly prognostic, tool in daily clinical practice. This approach might be particularly valuable for the management of airways diseases induced by environmental factors (*e.g.* occupational asthma), both to predict the effect of exposure or of cessation of exposure, and/or to screen susceptible individuals [30–32].

Another area which might be productively investigated is the role of viral and/or bacterial infections in asthma. The role of viral infections in the pathogenesis and/or exacerbation of asthma is still controversial [33]. Modern techniques of molecular biology, *e.g. in situ* hybridization, might be used to identify viral genome in cells and tissues obtained with bronchoscopic techniques, as well as with collection of sputum [34].

The immunological basis of asthma remains uncertain [35]. Although antibody, particularly immunoglobulin E (IgE) mediated immunity seems to play a significant role, there is increasing evidence that cell, particularly T-cell, mediated immunity may have an important role in the regulation of the airway inflammation associated with asthma [36]. In fact, even if it was generally considered that the main role of T-lymphocytes in IgE-dependent hypersensitivity was the induction and regulation of IgE production by B-lymphocytes, it is now recognized that antigen, after appropriate uptake, processing and presentation, mainly by dendritic cells [37–39], but also by macrophages [37] and possibly other cells [37], including epithelial cells [40], may directly stimulate very specific subsets of T-lymphocytes, resembling the Th2 type described by Mossman (reviewed in [41]), to release cytokines capable of recruiting and stimulating other inflammatory and effector cells [12]. Thus airway mucosal T-cells, in addition to a fine

regulation of IgE synthesis, mediated by the production of interleukin-4 (IL-4) and interferon-γ (IFN-γ) [41], might also directly contribute to the allergic inflammation of the airways present in asthma. Advances in cell culture techniques, monoclonal antibodies and recombinant deoxyribonucleic acid (DNA) technology and protein chemistry applied to material obtained with lavage, biopsies or sputum might greatly help to clarify the immunology of asthma.

Neurogenic inflammation and neuropeptides have been shown to play an important role in experimental airway hyperreactivity and inflammation [42] and, more recently, in human asthma [43, 44]. Neuropeptides and their regulatory enzymes may be measured in tissues [42–45], particularly in biopsies [44], providing another important tool for the investigative approach to the pathogenesis of asthma.

Adhesion molecules have been identified, and their role in the inflammatory process partially characterized [46]. Adhesion molecules are cell surface glycoproteins specifically involved with the attachment, signal transfer, activation and migration/degranulation of cells into or around sites of inflammation. Modulation of one or more of these "adhesive" interactions might provide a novel approach to the treatment of inflammatory diseases [46]. The prevention of adhesion of inflammatory cells to endothelial cells, or of their migration through the epithelium may inhibit airway inflammation, whereas the maintenance of integrity of intercellular adhesion molecules, which keep columnar cells attached to basal cells, may prevent epithelial desquamation, both characteristic features of asthma. Interestingly, in an animal model of asthma, the inhalation of monoclonal antibodies against neutrophil and eosinophil ahesion molecules prevents, respectively, neutrophil infiltration of the airway mucosa and the late asthmatic reactions induced by allergen inhalation, or eosinophilia and airway hyper responsiveness associated with repeated inhalation of allergens [47, 48]. These molecules may be examined directly in the airway tissue either using monoclonal antibodies or messenger ribonucleic acid (mRNA) probes [49, 50], and thus may be helpful in investigating changes present in asthmatic subjects.

Finally, for the clinical perspective, novel information obtained in pivotal studies with investigative bronchoscopy or sputum examination, information which has already significantly contributed to the characterization of asthma [16, 28, 43], prediction of its natural history [32], and evaluation of its treatment [20–23], should be more carefully used in clinical and epidemiological studies in order to evaluate potential applications in clinical practice.

In conclusion, the guidelines published in this issue [1] provide useful directions for the proper use of investigative bronchoscopy and we hope that they will stimulate researchers of the area to take full advantage of these modern bronchoscopic techniques, and by applying modern cell and molecular biological techniques to perform studies which may increase our understanding of asthma. We also hope that the advancement of knowledge will be associated with a significant improvement of diagnosis, classification, prevention and treatment of asthma.

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