

Section II: Drug Modulation of Inflammation

Bronchoscopy as a research tool for the study of asthma pathogenesis and effects of antiasthma drugs

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Fiberoptic bronchoscopic methods have greatly improved our understanding of asthma pathogenesis and of the mode of action of established and experimental antiasthma drugs. It is probably most appropriate to study bronchoalveolar lavage (BAL) and bronchial biopsy simultaneously because there are major differences between the airway lumen and tissue compartments: for example, T cells are the most abundant cells in endobronchial biopsy samples but form only 10% to 20% of total BAL cells, and eosinophils and mast cells are more numerous in the airway tissue than in the lumina. Immunostaining is currently the most reliable method for enumerating cells and assessing their activation state with a panel of cell surface and intracellular activation markers. In situ hybridization can be used to study a cell's capacity for cytokine production. Newer techniques allow immunohistochemistry of adjacent cell sections to co-localize staining with different antibodies, showing for example that mast cells contain preformed cytokines IL-4, IL-5, and IL-6 and tumor necrosis factor- α . A combination of immunohistochemistry and in situ hybridization can colocalize messenger RNA transcription to individual cell types; this approach is useful for T cells, which do not have storage capacity and cannot be shown by immunohistochemistry to contain cytokines. An additional tool to assess cellular activity is reverse transcriptase polymerase chain reaction, which has recently been used to confirm a predominant T_H2 -type cytokine profile in allergic disease. (J Allergy Clin Immunol 1996;98:S41-5.)

Key words: Airway inflammation, bronchial biopsy, bronchoalveolar lavage, drug effects

Until recently, the only means of investigating antiinflammatory actions of drugs in vivo has been bronchial challenge with allergen or individual bronchoactive mediators. The introduction of fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) and bronchial biopsy as a research tool in asthma studies has facilitated the accumulation of vital information on the contribution of various inflammatory cells and their mediators to the disease process.¹⁻¹¹ With >10 years of experience and an excellent safety record,²⁻¹² fiberoptic bronchoscopy is increasingly being used to improve our understanding of the mode of action of established and experimental antiasthma drugs.

The aims of this article are to review the avail-

Abbreviations used

BAL:	Bronchoalveolar lavage
ECP:	Eosinophil cationic protein
LT:	Leukotriene
PG:	Prostaglandin
PCR:	Polymerase chain reaction
VLA:	Very late activation protein

able techniques that have been used in asthma studies and to discuss some of the technical issues that need to be considered when drug studies are planned.

EXPERIENCE WITH BRONCHOSCOPY IN STUDIES OF DRUG EFFECTS

The first use of fiberoptic bronchoscopy to investigate the mode of action of drugs was in a study demonstrating an inhibitory action of cromolyn sodium on airway eosinophils.¹³ Although the po-

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tent clinical effects of inhaled corticosteroids had been appreciated for some time, it is only recently that their antiinflammatory effects in asthmatic airways have been confirmed in bronchoscopic studies.^{10, 14, 15} They have been shown to reduce the numbers of mast cells and eosinophils in the mucosa and the levels of histamine and tryptase in BAL, in addition to attenuating the degree of T-cell activation and expression of adhesion molecules and decreasing the numbers of immunoreactive cells for T_H2-type IL-4 and IL-5^{10, 14, 15} (Howarth PH, personal communication). Comparable effects have also been demonstrated with oral corticosteroids.¹⁶

In the search for drugs that could take the place of corticosteroids as prophylactic agents or permit reduction of their doses, evidence has accrued from bronchoscopic studies to suggest that, in addition to acting as a bronchodilator and respiratory stimulant, theophylline, a nonspecific phosphodiesterase inhibitor, may have antiinflammatory actions that are apparent at doses previously considered to be subtherapeutic.¹⁷ After the withdrawal of theophylline, which resulted in clinical deterioration, there was an increase in the number of activated T cells in the airway mucosa that was associated with a reduction in the same cells in blood.¹⁸ With the finding that premedication with theophylline reduced mucosal eosinophilia resulting from allergen challenge,¹⁹ this result suggests that theophylline may inhibit the blood-to-airway trafficking of inflammatory cells that results from allergenic stimulation.

It is important to note that fiberoptic bronchoscopy has been shown to be a safe and reliable investigative tool even in subjects with highly reactive airways.^{2, 12} However, the procedure must be conducted by experienced staff, preferably in facilities that are dedicated to research. When performed with local anesthesia, it is well tolerated, and volunteers usually agree to undergo a repeated procedure after allergen challenge or as part of a drug trial. Careful monitoring of vital signs and oximetry are strongly recommended to avoid the risks of excessive bronchoconstriction and arterial blood oxygen desaturation.²⁰ Although current guidelines recommend the use of bronchodilator premedication,²⁰ it has been shown that even when this step is omitted, bronchoscopy can be completed safely and without serious side effects.¹² Nevertheless, it seems prudent to use bronchodilators, except in cases in which the effects of allergens are being explored because those effects may be altered by premedication.

RELATIVE VALUES OF BAL AND BRONCHIAL BIOPSY

When deciding on the method for investigating the effects of established and novel antiasthma compounds, careful consideration needs to be given to the site of action and likely cellular targets of the drug. Some drugs, such as corticosteroids, exert potent effects on cell recruitment and survival by inhibiting the production and action of cytokines; others may act by inhibiting mediator generation or their effects on target cells. Although there definitely is a case for studying drug effects in peripheral blood, especially in severe disease, it is evident that there are differences between the lungs and peripheral blood with respect to the activity of inflammatory cells, and it cannot be assumed that the observations in blood mirror the events in the lungs. Although studies of blood have shown evidence of cell activation in more severe forms of asthma,²¹ in mild disease it is likely that because of a dilution factor the levels of cell activation and soluble mediators in the blood poorly reflect those in the lungs. Thus in mild asthma, clear differences exist between the circulation and lung compartments in the level of T-cell activation, suggesting that this process occurs in the airway mucosa.⁸

In addition, there appear to be significant differences between the airway lumen and tissue compartments. Although BAL samples a larger surface area and possibly provides a more uniform sample, it does so at the expense of contamination with cells derived from the alveoli.²² There are also major differences in the relative numbers and activities of various cells that constitute the inflammatory cell infiltrate in the two compartments. In BAL, macrophages are the most numerous cells. Although these cells can be triggered by IgE to produce an array of mediators pertinent to asthma (such as leukotrienes [LT] LTB₄ and LTC₄; prostaglandins [PG] PGD₂ and PGF_{2α}; IL-1β; tumor necrosis factor-α; and oxygen free radical),²³ they differ from their counterparts in other organs in being relatively poor as antigen-presenting cells. It is unclear whether this characteristic reflects maturation and phenotypic differentiation as a safeguard against excessive stimulation by environmental antigens. Moreover, it is uncertain whether these cells have functional properties similar to those of macrophages resident in the mucosa. What is certain, however, is that the major antigen-presenting cells in the lungs are the dendritic cells, which form an intricate network in the mucosa, expressing abundant class II major histocompatibility complex

antigens but having little phagocytic capacity.²⁴ As a further indication of the differences between these two compartments, dendritic cells constitute only 0.1% of the total cell population in BAL.²⁴

Whereas in endobronchial biopsy specimens T lymphocytes are the most numerous inflammatory cells,¹⁰ in BAL they constitute only 10% to 20% of the total cells.^{8, 25} In comparison with blood, BAL-derived T cells are less responsive to mitogens, a feature that may indicate the *in vivo* effects of the local environment involving resident macrophages.²⁴ Nevertheless, when compared with T cells from nonatopic control subjects, airway T cells, both in BAL and in biopsy samples, can be shown to be more activated.⁸ This index of cellular activity has been successfully used to study the antiinflammatory effects of inhaled corticosteroids.¹⁴ After treatment with beclomethasone dipropionate, the extent of T-cell activation is reduced, and, of interest, some of this effect can be seen to spill over into the circulation.¹⁴ There is increasing evidence from BAL and biopsy that a greater proportion of T cells in asthmatic persons transcribe for cytokines of the T_{H2} type (IL-4 and IL-5),²⁶ a finding that has important implications for the maintenance of the allergic response in asthma through the action of T cells on eosinophils and mast cells. It has been suggested that the T_{H2} profile of cytokines is a selective target for corticosteroids¹⁶ and may be resistant to inactivation in some people with severe asthma whose lymphocytes display corticosteroid resistance.²⁷

Thus future studies probably will concentrate on the ability of novel drugs to inhibit selectively T_{H2}-type cytokines without affecting those belonging to the T_{H1} profile. However, increased levels of a plethora of cytokines, including IL-2, a T_{H1} cytokine, can also be demonstrated in unchallenged and allergen-challenged airways,²⁸ pointing to the complexity of the cytokine network. Furthermore, it is likely that additional cytokines and chemokines will be discovered and their presence associated with clinical disease or the physiologic abnormalities of asthma.

Both eosinophils and mast cells are more numerous in the airway tissue than in the lumina,^{3, 8} but it is unclear whether their functional properties are different in the two compartments. Until this issue has been resolved, it seems appropriate to study both BAL and biopsy samples simultaneously. The number of activated eosinophils, identified in the mucosa by their immunostaining for the cleaved form of eosinophil cationic protein (ECP), correlates with the level of airway hyperre-

sponsiveness.²⁹ The enhanced activation of eosinophils in asthma can also be demonstrated in BAL samples, in which higher concentrations of ECP and major basic protein are found.^{30, 31}

CURRENTLY AVAILABLE METHODS

Initial studies have used classical stains, such as Giemsa, or electron microscopy^{32, 33} to enumerate and observe the ultrastructure of inflammatory cells in the airways. In general these techniques have now been supplanted by immunostaining methods, which are more reliable for enumerating cells and assessing their state of activation through use of a panel of cell-surface and intracellular activation markers. In addition, the use of *in situ* hybridization has made it possible to study the capacity of cells to produce cytokines.²⁶

Immunohistochemistry techniques can be used in tissue that has been frozen or embedded into paraffin or resins, such as araldite and glycol methacrylate; the latter resin preserves a greater number of surface epitopes. Resin-embedded tissue can be cut into 2- μ m thin sections, which, compared with frozen sections, offer better resolution and preservation of cell morphologic features. In addition, several sections can be cut through the same cell, enabling immunohistochemical analysis of adjacent sections to colocalize staining with different antibodies.³⁴ This approach has shown that mast cells contain preformed cytokines IL-4, IL-5, IL-6 and tumor necrosis factor- α ,³⁴ a finding that may have important implications for drugs that have an effect on these cells. Staining of adjacent sections with monoclonal antibodies against a panendothelial vessel marker and adhesion molecules has shown enhanced adhesion-molecule expression after allergen challenge.³⁵ This technique can be applied to other inflammatory cells, including eosinophils, and can be extended further by combining immunohistochemistry and *in situ* hybridization to localize messenger RNA transcription to individual cell types. This approach is particularly useful for T cells, which do not have the capacity for storage of cytokines, which thus cannot be demonstrated by immunohistochemistry.³⁴

A variety of methods have been used to quantify the cellular infiltrate. Early descriptive studies using semiquantitative methods have been superseded by methods that express results in terms of cell numbers per unit area³ or length of basement membrane.²⁹ One approach has been to focus on the superficial layer of the mucosa, which may be more relevant because of its proximity to external

stimuli.²⁹ The other approach, taking into consideration the response of the entire mucosa, has been to calculate the area of the whole tissue section, excluding the submucosal glands, smooth muscle, and blood vessels.^{3, 10} It is unclear whether either method has any advantages, and studies applying the two methods show similar findings.

Whereas some drugs are effective at reducing cell numbers in the airways, others may act predominantly on their activity and have less effect on cell accumulation. This factor must be taken into consideration when defining outcome measures in studies of the antiinflammatory effects of drugs. Although inhaled corticosteroids neither reduce the numbers of T cells in the airways nor change the balance between CD4⁺ and CD8⁺ subsets, they reduce the numbers of eosinophils and mast cells, with part of the effect attributable to their action on T-cell activation.¹⁴ Cell activity can be assessed by studying indicators such as cell surface markers (by immunohistochemistry on biopsy samples and flow cytometry on BAL cells) and soluble mediators in BAL. The majority of T cells recovered by BAL or seen in bronchial biopsy samples express CD45RO, a marker previously considered to indicate memory cells, with no differences between persons with and without asthma.²⁵ A smaller proportion of airway T cells are positive for the IL-2 receptor, an indicator of recent cell activation, and for the class II human leukocyte antigen HLA-DR.⁸ Both of these activation markers are expressed on a greater proportion of airway T cells in asthma.^{8, 29} In addition, other late activation markers, such as very late activation proteins (VLA) VLA-1, VLA-4, and VLA-6, can be demonstrated. To study the level of activity of other inflammatory cells, a range of mediators currently can be measured in BAL samples. These mediators include histamine, tryptase, prostanoids (e.g., PGD₂ and its metabolite 9 α ,11 β -PGF₂; 6-keto-PGF_{1 α} , the stable metabolite of prostacyclin [PGI₂]; and thromboxane B₂, the stable metabolite of thromboxane A), LTC₄, LTD₄, and LTE₄, ECP, eosinophil-derived neurotoxin, and major basic protein. Some of these mediators are specific markers for one cell type (e.g., tryptase is produced only by mast cells); some are produced by related but distinct cell types (e.g., histamine is produced both by mast cells and by basophils); and other mediators are derived from a variety of cell types (e.g., LTC₄ is produced by mast cells, basophils, macrophages, and eosinophils).

More recently, reverse transcriptase polymerase chain reaction (PCR) has been used to detect

transcripts for a range of cytokines. This development adds another tool to the assessment of cellular activity. Although it has not yet been used in the context of clinical trials, preliminary studies have confirmed the observations of *in situ* hybridization of a predominant T_{H2}-type cytokine profile in allergic disease (Howarth PH, personal communication).

CONCLUSIONS

A wide range of methods are currently available to study the antiinflammatory actions of drugs in conjunction with fiberoptic bronchoscopy. The method to be used needs to be selected carefully, according to the likely effects of the drug being studied. Most of these techniques have already been put to use in bronchoscopic studies involving drugs. Attempts are being made to improve the sensitivity of these methods and to quantify more precisely inflammatory responses such as cytokine production by using semiquantitative PCR. Very sensitive methods, such as *in situ* PCR, are being developed to enable detection of minimal amounts of messenger RNA, although the relevance of these approaches to the study of asthma pathogenesis and drug effects remains to be determined.

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