



Pathology

What is the nature of asthma and where are the therapeutic targets?

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The characterisation of chronic asthma as an inflammatory condition of the human airway, associated with heightened airway responsiveness to a variety of bronchial stimuli, has led to the clarification of therapeutic strategies. These strategies have focused on bronchodilation and attenuation of airway inflammation. Inhaled corticosteroids effectively reduce chronic inflammation and produce substantial symptomatic relief in most patients. This article examines the pathophysiology of asthma and discusses the interpretation of current methods of assessment, and the targets and actions of inhaled anti-asthmatic drugs in relationship to central and peripheral airway events.

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Introduction

Asthma is a chronic airway disease characterised by episodic wheezing, coughing and chest tightness associated with airflow obstruction. For many years, asthma was regarded as a disease involving abnormal contractility of airway smooth muscle and thus solely treated with bronchodilator medication. However, improved understanding of the disease process has altered this perception, such that asthma is now regarded primarily as an inflammatory airway disorder. This realisation has led to consequent changes in the approach to therapy and therapeutic targets, with the recommended prophylactic use of anti-inflammatory therapy (1). Mast cells, eosinophils, epithelial cells and lymphocytes are implicated as central to the disease process, while at the molecular level, numerous inflammatory mediators, cytokines, chemokines, adhesion molecules and transcription factors have received widespread attention. Despite a greatly improved understanding of the pathogenesis of asthma, there are still many aspects which remain to be fully elucidated, not least of these being the relative contribution of inflammation in the large

and small airways to clinical disease expression. This review examines the interpretation of current methods of assessing asthma, details the inflammatory process, and discusses the targets and actions of inhaled anti-asthmatic drugs in relationship to large and small airway events.

Pathophysiology of Asthma and Chronic Obstructive Pulmonary Disease

Assessment of airway obstruction

Objective measurements of airflow obstruction can be obtained by lung function tests. Many measurement techniques are available, including, for example, body plethysmography and flow volume loops, as well as spirometry and peak expiratory flow. For clinical purposes, simple peak flow monitoring with a hand-held peak flow meter is most commonly used to assess airflow obstruction, either as a reduced peak expiratory flow rate (PEFR) or as an exaggerated diurnal variation in peak flow. More formal lung function testing requires spirometry, to measure the percentage reduction in the volume of air exhaled during the first second of forced expiration (FEV_1), or a reduction in the ratio of FEV_1 to forced vital capacity (FVC). An indication of what is represented by these parameters can be obtained from examining the changes in cross-sectional areas in different parts of the airway, which vary from 10^6 cm² at the alveolar

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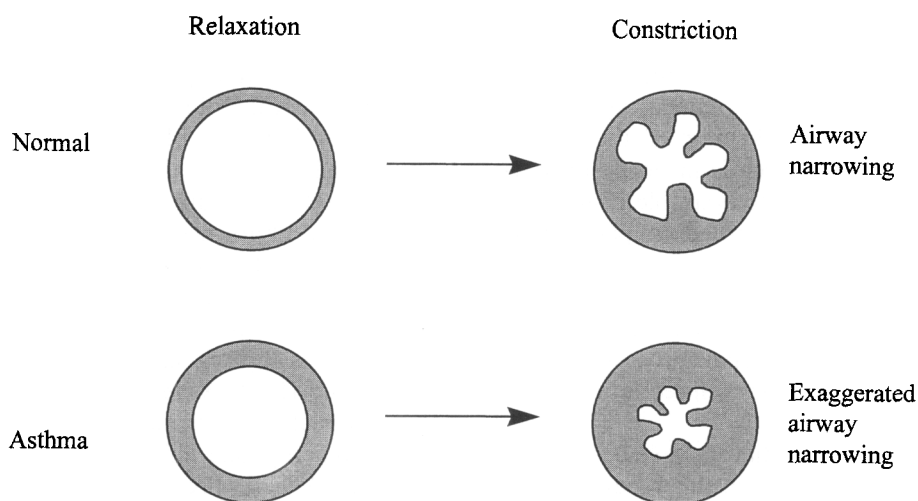


FIG. 1. Illustration of how bronchial hyperresponsiveness may be linked to airway wall thickening. Adapted from Ref. 2.

level down to 3 cm^2 in the trachea. The trachea is thus the major site of airflow limitation, and changes here will be reflected by the peak flow rate. Although FEV_1 reflects events in both large and small airways, it is widely recognised that much loss of small airway function is silent and does not influence lung function measurements. It can be demonstrated mathematically that large percentage changes in peripheral resistance have relatively little impact on airways resistance and thus, by implication, on peak flow and FEV_1 measurements. Both parameters are therefore unreliable reflections of events in the small airways. The criteria for small airway obstruction are abnormal ventilation-distribution, combined with normal elastic and flow-resistive properties, and density-independence of maximal expiratory flow and flow resistance. A simple parameter such as FVC may provide information on gas trapping which frequently accompanies small airway disease, but will also be modified by changes in lung volume and may thus be misleading (2).

Density dependence has been used to assess sites of airflow obstruction. It is a difficult and often unreliable technique, based on airflow in the large airways being turbulent, and therefore density dependent, whereas the slower flow in small airways is laminar and density independent. A change in density of the inspired air, such as breathing an 80% helium mixture, will alter the measurements of resistance and can be used to partition resistance between the large or small airways. Measurements of this type made on smokers and non-smokers in the late 1960s (2) indicated that in smokers, obstruction in the small airways predominated, whereas in non-smokers, uncomplicated obstructive airway disease was mostly

associated with the large airway events. In patients who suffered recurrent episodes of infection, both sites were found to be affected.

Ventilation scans provide an alternative way of assessing small airway obstruction. Such scans indicate that in asthma, much greater aerosol deposition occurs in central airways relative to peripheral airways, suggesting a defect in small airways ventilation. Similar results have been obtained from penetration indices (ratio of central airway deposition to peripheral airway deposition) determined from planar imaging. Studies of this type suggest that small airways disease is a feature of asthma, and that the simple measurements of lung function used clinically, focus more on large airway events and improvements in these parameters may not be reflected by small airway pathology.

Structural changes to the airway: distribution and relationship to bronchial hyperresponsiveness

Structural changes that could contribute to possible airflow defects are known to occur throughout the airways of asthmatics, being evident in large and small ($<2 \text{ mm}$ internal perimeter) airways (3,4). These include sub-epithelial fibrosis with increased deposition of type III and IV collagen, smooth muscle hyperplasia and hypertrophy, and neovascularisation, which together contribute to thickening of the airway wall. The importance of this thickening has been highlighted by mathematical modelling (5), which suggests that it may contribute to the increased bronchoconstrictor response to inhaled stimuli (bronchial hyperresponsiveness) by

disproportionately reducing the size of the airway lumen during smooth muscle contraction (Fig. 1). In large airways, the radius of the lumen will not be dramatically affected by thickening of the airway wall, nor will the airflow (which is inversely proportional to $1/\text{radius}^4$), whereas comparable changes in small airways will have a much greater impact. These structural changes to the airway wall, which are clearly visible in electron micrograph studies of biopsy specimens from asthma patients, are thought to be the result of exaggerated repair processes occurring in response to inflammatory cell recruitment and tissue damage.

A number of fibrogenic mediators have been identified which could be implicated in this repair process (6). These include growth factors such as transforming growth factor beta (TGF- β), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (BFGF), cytokines such as interleukins 1 and 4 (IL-1, IL-4), tumour necrosis factor alpha (TNF- α), and mediators such as endothelin, histamine and tryptase. Many of these factors have been shown to be increased in bronchoalveolar lavage samples recovered from asthmatic airways and will reflect inflammatory events including mast cell degranulation, T-lymphocyte activation, epithelial cell activation, eosinophil influx and indirect interaction between eosinophils and the airway matrix. Proteoglycan molecules are a component of this matrix and consist of a central protein core to which sulphated glycosaminoglycan (GAG) side-chains are attached. These side-chains bind to, and inactivate, cytokines, so that the proteoglycan molecules function as cytokine storage sites. TGF- β also has a close relationship with the matrix protein, decorin. TGF- β induces synthesis of this protein, which in turn binds and inactivates the $\beta 1$, $\beta 2$ and $\beta 3$ isoforms of TGF- β , providing a depot of this potent growth factor within the matrix layer. GAG side-chains can be cleaved from the protein core by enzymes, particularly metalloproteases and heparinases. The metalloprotease MMP-9, which co-localises to eosinophils, has been implicated in this process from *in vitro* studies. Migration of eosinophils through the airway epithelium and underlying proteoglycan scaffold of the submucosa, during leucocyte recruitment, may thus bring about local release of growth factors such as TGF- β . BFGF is also proteoglycan-bound and may be released by a similar process, thus implicating eosinophils indirectly in fibroblast proliferation.

Leucocyte recruitment and airway inflammation

The recruitment of leucocytes from the circulation to the tissues is a central feature of the inflammatory

response, in which the vascular endothelium is recognised as having an important role (7). The initial stage of recruitment is 'rolling margination', during which, leucocytes from the bloodstream are slowed down under the influence of the adhesion molecules (adhesins) P- and E-selectin, expressed on the endothelial cell surface. Preformed P-selectin is stored intracellularly in Weibel-Palade bodies, from where it is rapidly mobilised by inflammatory mediators such as histamine or platelet-activating factor (PAF), whereas E-selectin is up-regulated by cytokines including TNF- β and IL-1 β . As leucocytes become marginated, they interact with chemokines such as interleukin-8 (IL-8), macrophage inflammatory protein 1 α (MIP-1 α) and RANTES presented on the glycocalyx, which mediate cell activation and increase expression of binding ligands such as leucocyte function-associated molecule-1 (LFA-1), and other proteins, including metalloproteases. The newly expressed ligands increase adhesion by binding firmly to adhesion molecules from the immunoglobulin supergene family, e.g. intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), present on the endothelial surface. In this context, the interaction that occurs in allergic inflammation between the ligand, very late antigen-4 (VLA-4) and VCAM-1 is of particular interest, as VLA-4 is expressed on eosinophils, lymphocytes and basophils, but not on neutrophils, and thus confers some specificity for cell recruitment. In contrast, LFA-1-ICAM interactions are non-selective, being common to all leucocytes, including neutrophils. Regulation of expression of VCAM-1 is also interesting as it is not only influenced by the cytokines, TNF- α and IL-1 β , which affect many aspects of adhesion expression, but is upregulated by IL-4 and IL-5. These cytokines are characteristic of T_H2 lymphocytes, which are also associated with allergic inflammation, and implicates them in a co-ordinating role in the allergic inflammatory response. Hamid *et al* (8) have described up-regulation of mRNA for IL-3, IL-4, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) in bronchoalveolar lavage from asthma patients, and found IL-5 message co-localised to CD2+ cells, providing for evidence for upregulation of T_H2-associated cytokines being consistent with expansion of a specific T-lymphocyte subpopulation.

Once adhesion has occurred, cells diapedese through the inter-endothelial cell tight junctions and migrate through the tissue under the chemotactic influence of cytokines and chemokines, possibly facilitated by metalloproteases. IL-3, IL-5 and GM-CSF are thought to be particularly important in eosinophil recruitment and activation, as they stimulate proliferation and maturation of progenitor

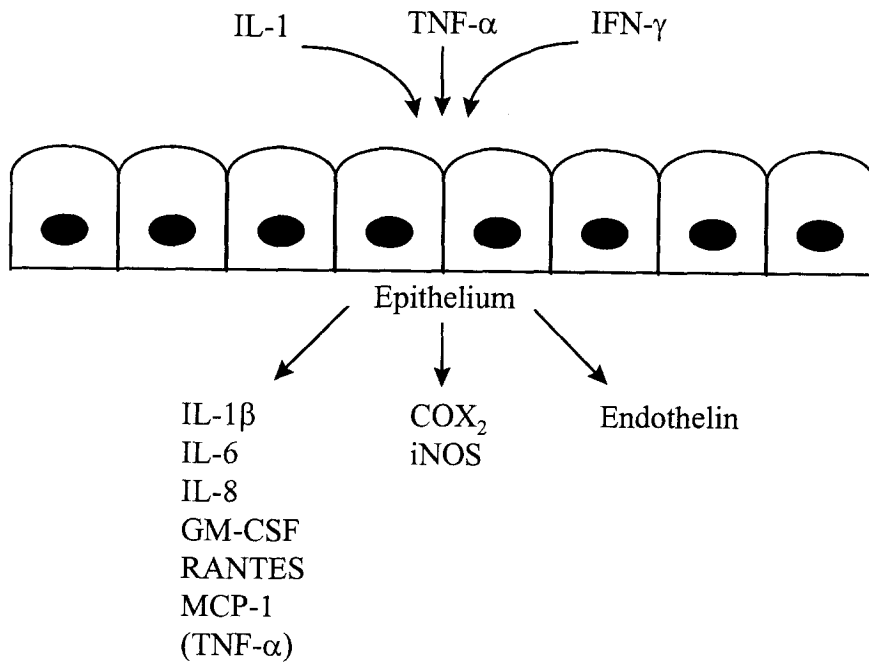


FIG. 2. Schematic diagram illustrating the chemokines, chemoattractants and enzymes released by the epithelium following activation.

cells in the bone marrow, enhance the response of eosinophils to other chemotactic stimuli, despite possessing only weak intrinsic chemotactic activity, and prolong the survival of eosinophils in the tissues by inhibiting apoptosis. Post-mortem studies reveal that tissue eosinophilia is equally present in small and large airways in asthma and that large airway sampling will be reflective of small airway events (9).

The role of epithelial cells

Activated epithelial cells are recognised as an important site for production of cytokines and chemokines, which affect recruitment of a variety of cells including macrophages and eosinophils. Chemokines are thought to regulate the movement of eosinophils after their initial attraction by IL-5. For example, the known eosinophil chemoattractant and activator, RANTES is expressed by epithelial cells, but attention has also focused on IL-8, a classical neutrophil chemoattractant. A recent study using endobronchial allergen challenge (10) found that immunoreactive RANTES levels in lavage fluid increased at allergen challenge sites compared to control sites, and this correlated with eosinophil influx. In the case of IL-8, local release of IL-4 and tryptase can lead to formation of an IL-8–secretory IgA (sIgA) complex, which is a potent eosinophil chemoattractant and activator found at increased levels in asthma. This complex formation illustrates the duplicity of action among cytokines and the role of such interactions in

modulating activity at the local level. Eotaxin is another candidate chemokine for study, and survival of eosinophils is also enhanced in the epithelium by production of GM-CSF. Enzymes such as inducible cyclo-oxygenase (COX₂) and inducible nitric oxide synthase (iNOS), as well as the peptide, endothelin, are also produced by epithelial cells (Fig. 2). Endothelin is a potent bronchoconstrictor and a growth factor for fibroblasts, while iNOS mediates the production of nitric oxide (NO), a regulatory molecule that is thought to be generated in the lower airways and has been widely reported to occur in elevated levels in exhaled air from asthma patients.

The influence of sampling techniques on disease assessment

Both direct and surrogate markers for airway inflammation can be measured by current clinical procedures. For example, exhaled NO is a surrogate marker which may reflect epithelial activation. Direct measurements such as bronchial lavage, bronchoalveolar lavage and induced sputum, provide indices of luminal events, whereas biopsy reflects tissue events within the airways. These two compartments may be separate and give different information about the disease state. This is illustrated by recent correlation analyses, where comparisons between luminal mast cell numbers and lavage tryptase showed a correlation, whereas submucosal cells and lavage

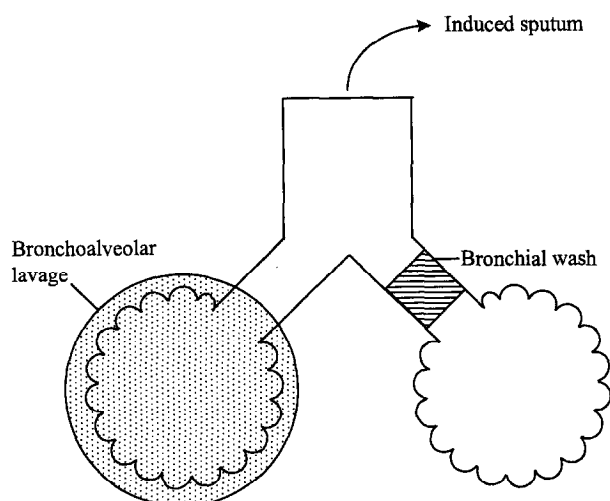


FIG. 3. The influence of technique on the site of airway being sampled.

did not. Similar observations have been made with eosinophil numbers and lavage ECP levels (11).

For luminal events, sampling techniques involve either a small wash of a proximal airway site via a bronchoscope (bronchial wash), or flooding the airway with a larger volume to recover both proximal and distal airway samples (bronchoalveolar lavage). Bronchoscopic lavage of this type bypasses the larger airways above the bronchoscope, which are probably implicated in airway obstruction and induced sputum will provide a better measure of events in these areas (Fig. 3). The various sites of sampling give different information, as evidenced from a study by Fahy *et al* (12) comparing cell counts in samples taken from asthma patients (Table 1). In these studies, the highest counts of epithelial cells, and lymphocytes occurred in bronchial wash and bronchoalveolar lavage, respectively, whereas eosinophil numbers were highest in induced sputum. Induced sputum also

TABLE 1. Percent composition of cells in airways samples taken using different sampling techniques from asthma patients. Data from Ref. 12

| Cell type | Sampling technique | | |
|------------|--------------------|----------------|------------------------|
| | Induced sputum | Bronchial wash | Bronchoalveolar lavage |
| Epithelial | 1.1 | 4.2 | 0.3 |
| Eosinophil | 1.9 | 0.9 | 0.2 |
| Macrophage | 30.0 | 90.0 | 93.0 |
| Neutrophil | 36.0 | 1.9 | 0.8 |
| Lymphocyte | 0.4 | 1.8 | 2.9 |

contained the highest number of neutrophils, but fewer macrophages than from either of the other two sites.

The induced sputum technique has been used to assess the different cellular responses and regulatory effects occurring in chronic bronchitis and asthma. A comparative study (13) found increased numbers of macrophages/monocytes, mast cells and eosinophils in asthma patients and increased numbers of neutrophils in chronic bronchitis patients. There were correspondingly higher levels of GM-CSF and IL-5 in the fluid phase from asthma patients, and increased IL-8 levels in those with chronic bronchitis.

Cellular Targets for Drug Intervention Strategies

The action of bronchodilators and location of receptors

A number of effective inhaled therapies for asthma act on different components of the disease process. Bronchodilators such as β_2 -agonists and anticholinergics interact with β_2 -adrenoceptors and muscarinic receptors, respectively, to bring about rapid symptomatic relief. However, the distribution of these receptors varies. Muscarinic receptors occur almost exclusively in proximal airways, whereas β_2 -adrenoceptors are located on a variety of cells including smooth muscle, epithelial cells, Clara cells, mucous cells, endothelial cells, mast cells, T-cells, pneumocytes and autonomic ganglia, although they also are distributed to a higher degree in central airways (14). β_2 -agonists consequently have pleiotropic therapeutic effects in addition to smooth muscle relaxation, such as increasing mucociliary transport, inhibiting mediator release (particularly from mast cells), suppressing endothelial permeability and oedema formation and modifying ganglionic neurotransmission. However, β_2 -agonists do not inhibit chronic inflammation, suggesting that the receptors may be uncoupled in inflamed tissues, or that the cells implicated in this component of the disease state do not contain functional β_2 -adrenoceptors.

Location of corticosteroid receptors and drug action

Although the anti-inflammatory action of corticosteroids is well recognised clinically, the key cellular targets in asthma and the mechanisms at the molecular level have yet to be established (for review see

Ref. 15). Mast cells, for example, lack corticosteroid receptors and degranulation is not inhibited by these agents *in vitro*, but both mast cell numbers and concentrations of inflammatory mediators, such as histamine and tryptase, decline during corticosteroid therapy *in vivo*. Other effects of steroids include decreased eosinophil infiltration, probably as a result of modification of the cytokine profile and increased apoptosis. Oral prednisolone is known to decrease the T_{H2} cell population, while inhaled fluticasone propionate decreases immunoreactivity for IL-4 and IL-5, the major eosinophil regulators, in biopsy specimens. Lymphocytes are particularly sensitive to steroid regulation and exhibit decreased expression of the activation markers, CD25 and HLA-DR, in addition to reduced T_{H2} cytokine production. The effect of steroids on cell recruitment is also evident at the endothelial cell level, which shows decreased expression of the adhesion molecules involved in rolling margination (E and P-selectin) and in expression of ligands for VLA-4 and LFA-1.

The molecular action of steroids occur at intracellular glucocorticoid receptors (GR) which are widely distributed in cells throughout the airways. Once bound to the GR, the glucocorticoid-receptor complex is translocated to the nucleus, where it interacts with the glucocorticoid-responsive element of target genes to modify the expression of pro-inflammatory cytokine production and increase the synthesis of other proteins such as I κ B, a regulator of the transcription factor, nuclear factor κ B (NF- κ B). Steroids may also affect the action of other transcription factors known to be important in asthmatic inflammation, such as AP-1. As steroids are administered topically by inhalation, the epithelium is likely to be a primary target in the response. For example, there is evidence that cytokine-related epithelial events such as endothelin expression (determined by immunohistopathology), and production of COX₂ and iNOS, are increased in asthma and down-regulated in asthma patients on inhaled steroids (16).

Summary

Progression of the asthmatic disease process can be summarised in four stages: immune activation, cell activation and recruitment, tissue damage, and tissue remodelling.

Immune activation following allergen exposure results in antigen presentation by mucosal dendritic cells (Langerhans cells) to T-lymphocytes and their activation, with release of cytokines. In the primary response, there are T-B cell interactions with the

production of antigen-specific IgE and subsequent IgE-mediated hypersensitivity reactions. The secondary immune response thus involves mast cells, due to their cell surface expression of IgE, as well as T-lymphocyte responses, with both mediator and cytokine release.

Structural cells of the epithelium and endothelium are recognised as important in the cell activation and recruitment processes which follow immune activation. Leucocytes, predominantly lymphocytes and eosinophils, are recruited from the blood into the tissues, where, together with cytokine and chemokine production from epithelial cells and mast cells, they function as effectors in bronchoconstriction and clinical disease expression. This inflammatory process is thought to lead to tissue damage, particularly epithelial disruption, which triggers tissue remodelling, giving rise to fibroblast proliferation, collagen deposition, and structural changes to the smooth muscle and vasculature of the airways.

Inhaled corticosteroids are particularly effective against chronic inflammation as they modify T-lymphocyte activation, decrease mast cell numbers, decrease epithelial and endothelial cell activation and reduce eosinophil recruitment. Evidence for their ability to reverse chronic structural changes is more speculative. Most studies, for example, have failed to find any effect of steroid therapy on the increased collagen deposition. However, studies on reversal of structural changes by anti-inflammatory therapy may need to be conducted over a prolonged period of time.

In conclusion, in asthma there are abnormalities of both the large and the small airways. The standard tests of lung function are probably a poor guide to small airways disease, which can therefore be easily overlooked, and the contribution of small airways disease to clinical disease expression is poorly understood because of lack of availability of good markers.

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