Allergology International (2001) **50**: 281–294

Review Article

Airway inflammation and repair in asthma

Ratko Djukanovic

Division of Inflammation and Repair, Medical Specialties, Southampton University General Hospital, Southampton, UK

Abstract

Asthma is a disease of usually reversible airways obstruction that has prominent inflammation of the mucosa as an underlying feature. It is a disease of varying severity, with some individuals suffering from only occasional symptoms and others having daily symptoms and requiring large doses of inhaled and oral corticosteroids. Although the different forms of asthma have some common pathological features, the extent of change varies from mild to severe forms. The only cell type that has been widely correlated with disease severity has been the eosinophils, but it is evident that altered function and responsiveness of T cells to steroids plays a key role as a determinant of asthma severity. More recently, attention has focused on restructuring of the airways, which results in, or possibly develops in parallel with, inflammation. This restructuring consists of changes throughout the bronchial wall, beginning with changes in the epithelium, increased collagen deposition, increased microvasculature, hypertrophy and hyperplasia of submucosal glands, goblet metaplasia within the epithelium, hypertrophy and hyperplasia of smooth muscle and, finally, poorly defined changes in the adventitia. Novel treatments have been, and continue to be, developed to target specific components of inflammation and remodeling. However, to date, none has lead to a marked improvement in disease control and further efforts are needed to better understand the mechanisms of asthma, in particular those that are not responsive to corticosteroids.

Correspondence: Dr R Djukanovic, Division of Inflammation and Repair, Medical Specialties, Level D, Center Block, Southampton University General Hospital, Southampton SO16 6YD, UK. Email: rd1@soton.ac.uk

Received 23 August 2001.

Key words: airway remodeling, asthma, eosinophils, inflammation, mast cells, T cells.

Introduction

The use of bronchoscopy as a research tool has markedly improved our understanding of the pathology of asthma. Airway inflammation is a feature of all forms of this disease, irrespective of whether it is related to atopy,[1–5] occupation[6] or has no known cause (often referred to as intrinsic or non-atopic asthma).[7] Varying degrees of airway inflammation are seen across the range of asthma severity and the characteristics of the cellular infiltrate vary markedly between mild and severe forms. Following initial studies of mild asthmatics,[1–4] there has been an increasing effort to improve the understanding of the pathology of asthma and its functional implication in moderate and severe forms. The need to do this has become particularly evident with the growing appreciation of the relative ineffectiveness of the available anti-inflammatory drugs used in the treatment of this disease.

Eosinophils and mast cells as effector cells in asthma: how important are they?

A number of cell types participate in the complex cell network that typifies asthma. Of these, the cell that has been associated longest with the disease and, indeed, with many other forms of atopic disease has been the eosinophil. Initially, eosinophils were believed to be beneficial by controlling allergic inflammation through their ability to degrade histamine and stop its release, in addition to inactivating leukotrienes and platelet activating factor (PAF). Subsequently, fairly abundant information was generated to implicate these cells as prominent effector cells and the cells were thus seen as

being responsible for many of the inflammatory responses in both asthma and allergic rhinitis.[8] The combination of studies using induced sputum and bronchoalveolar lavage (BAL),[5,9–11] to study the products of cells residing in the airway lining fluid, and bronchial biopsy,[1,12] to characterize the cellular infiltrate in the epithelium and the deeper submucosal layer, has invariably shown increased numbers of eosinophils in asthma throughout the bronchus. Many, although not all, have shown an association with dieases severity and bronchial hyperresponsiveness.[11,13] This has led to the notion that asthma is a form of eosinophilic bronchitis.

The number of eosinophils in BAL is relatively low. Eosinophils rarely exceed 5–10% of the total cell count due to dilution of the recovered cells from the bronchi by cells washed out from the alveoli, which contain few eosinophils and numerous macrophages.[14,15] Eosinophil counts are much higher in induced sputum,[9–11] presumably because this technique predominantly samples the proximal airways. Eosinophils are also more abundant in the mucosa.[1,12] Comparison of BAL and sputum eosinophil counts on the one hand and mucosal counts on the other shows poor correlation between these two compartments, possibly due to quantitative and/or qualitative differences between the mechanisms responsible for the accumulation and survival of these cells.

Even the very mild, intermittent forms of asthma, requiring only occasional use of bronchodilators, are characterized by prominent sputum eosinophilia.[11] In biopsies, studies have shown that eosinophil counts are also raised in atopic non-asthmatics suffering from allergic rhinitis.[2] This suggests that a low-grade degree of eosinophilic bronchial inflammation is a feature of atopy in general and that the degree of mucosal eosinophilia may determine the likelihood of functional impairment of the airways. However, the physiological relevance of subclinical eosinophilia is unclear because these individuals are unlikely to develop any sequalae that would lead to restructuring of the airways and reduced lung function. It is also important to note that there are diseases, such as Crohn's disease, where raised eosinophil counts are seen in sputum but are not associated with either respiratory problems or hyperresponsiveness.[16]

In asthma, eosinophils are in an activated state and secrete increased amounts of arginine-rich proteins, such as eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and major basic protein (MBP), which can detected in BAL and induced sputum.[11,15,17,18] All these proteins are stored in the granule matrix, except for MBP, which is contained within the crystalline core that is identified as an electron-dense crystal structure by the electron microscope.[8] In contrast with the healthy state, in which sputum ECP levels rarely exceed 20 ng/mL, in asthma, concentrations as high as 650 ng/mL can be detected.[11] Exposure to allergen in the laboratory leads to further elevations in ECP during the late-phase asthmatic response.[17] These basic proteins are toxic to the epithelium;[19] together with the pressure exerted by mucosal edema, this is thought to lead to epithelial damage. Consistent with raised levels of ECP and MBP in BAL fluid of asthmatic patients,[15] electron microscopic investigation of the eosinophil granule ultrastructure shows dissolution of both the granule matrix and the crystal core. This is seen as marked heterogeneity in the appearance of eosinophil granules, with swelling of the granules, and dissolution of the both the core and matrix.[1] As further evidence of eosinophil degranulation, immunohistochemical analysis shows prominent deposits of both ECP and MBP in areas of epithelial damage of asthmatic lungs.[20] In addition, it is now appreciated that eosinophils also produce an array of cytokines and chemokines. Some of these (interleukin (IL)-3, IL-5 and granulocyte–macrophage colony stimulating factor (GM-CSF)) act in an autocrine manner to promote eosinophil maturation, activation and survival.[8] Others, such as IL-16 and RANTES, promote recruitment of eosinophils and T cells. Regulatory cytokines IL-2, IL-4 and IL-10 conceivably contribute to the control of T cell activation, proliferation and cytokine generation. Thus, in addition to causing immediate damage to the epithelium, bronchoconstriction, vasodilatation and mucus hypersecretion, eosinophils have their own capability to promote chronic inflammation.

The mechanisms leading to accumulation of eosinophils in the airways have been a topic of great interest. Eosinophil chemotactic activity can be readily demonstrated in induced sputum.[11] A number of studies using BAL conducted in the mid-1980s have shown that stimulation with allergen using inhalation challenge leads to increased infiltration and degranulation of eosinophils in the lumen, especially in subjects who develop so-called dual (early and late)-phase asthmatic responses.[17,21,22] These observations have now been confirmed in biopsy studies.[23] Allergen exposure during the pollen season leads to an increased release of chemokines.[24] The influx of eosinophils is associated with increased expression of intercellular adhesion molecule (ICAM)-1 and E-selectin[23] on mucosal post-capillary venules and an

early accumulation of neutrophils and lymphocytes, all of which points to a complex upregulation of inflammatory processes involving a host of chemotactic factors and adhesion molecules. Several chemotactic factors have been identified, including PAF,[25] leukotrienes[26] and chemokines, such as eotaxin,[27] RANTES[24] and monocyte chemotactic protein (MCP).[28]

When considering the role of eosinophils in chronic disease, it has been of particular interest to study these cells in severe disease. The first study gave somewhat surprising and contradictory results: although some patients with severe asthma treated with oral corticosteroids had raised eosinophils in bronchial biopsies, the majority did not.[29] Similar observations were made in another study.[30] Subsequent careful characterization of a larger number of patients found two groups that were felt to be different in respect to their pathology.[31] One group had raised eosinophils whereas the other did not; patients with eosinophils had evidence of mast cell activation, increased T cell infiltration and raised collaged deposition, all pointing to a role of persistent eosinophilia as either a marker or determinant of severe asthma.

The final proof of the importance of a cell or mediator comes with the development of specific drugs that inhibit their effects. Recent clinical trials involving either IL-12[32] or anti-IL-5 antibodies,[33] both of which have been effective at almost totally depleting the airways of eosinophils, have been surprisingly negative. This has placed an important question mark about the role of eosinophils in asthma. In neither of these studies did treatment with IL-12 or anti-IL-5 antibody ablate the late-phase asthmatic response. This was very much in opposition to the central role that has been attributed to eosinophils as effector cells in allergen-provoked asthma. Ongoing clinical trials are looking at disease activity in the natural setting using two different anti-IL-5 antibodies. If these manage to deplete the blood and airways of eosinophils without improving the clinical symptoms of asthma, the studies will further undermine the position for eosinophil as a key player in asthma.

The other cell that has been viewed as being key in allergic responses has been the mast cell. Mast cells have long been associated with allergic diseases by way of their ability to respond specifically to allergens cross-linking IgE molecules bound to the cell surface via FcεRI receptors. In non-asthmatic subjects, mast cells recovered by BAL constitute a minority (0.04–0.6%) of the total nucleated cells; in asthmatics, these numbers can be increased as much as fivefold.[34,35] However, even with such an increase, mast cells still constitute only a minority of the inflammatory cells in the lumen. Whereas mast cell numbers are not necessarily raised in asthma,[1,36] examination by electron microscopy shows extensive changes in their granules, which have been associated with mast cell activation.[1] In support of ongoing mast cell mediator secretion, levels of mast cell-derived mediators are raised in BAL and induced sputum in asthmatics.[11,37–40] As with eosinophils, these changes are seen even in the mildest forms of the disease. Allergen challenge *in vivo* leads to mast cell activation and release of histamine, which can be detected in BAL.[38,41] This is associated with a rise in the 134 kDa neutral protease tryptase,[38] a mediator found almost uniquely in mast cells. Other mast cell mediators that are important as effectors of bronchoconstriction seen during the early asthmatic response (EAR) are prostaglandin (PG) D_2 and the sulfidopeptide leukotrienes. Mast cells are a major source of sulphidopetide leukotrienes, which also increase vascular permeability and are between 100- and 1000-fold more potent than histamine and methacholine as contractile agonists. The past few years have seen a particular interest in leukotrienes, with the development of potent and selective antagonists of either the 5-lipoxygenase enzyme or the leukotriene (LT) D_4 receptor, which have been shown to be effective in abrogating the airway responses to exercise or allergen challenge[42] and improving clinical symptoms.[43] Their efficacy may be related to disease severity in view of the limited evidence that expression of the 5-lipoxygenase enzyme is normal in the airway mucosa of mild asthmatics, but is raised in those with more severe disease.[44,45]

Tryptase has recently generated significant interest, both as an important mediator in asthma and as a therapeutic target. In comparison with control subjects, in whom tryptase is either undetectable or at very low levels (< 2 ng/mL), concentrations as high as 40 ng/mL can be detected in the induced sputum of asthmatics.[11] However, the range of concentrations of tryptase in sputum from asthmatics is wide, suggesting that the extent of mast cell involvement may vary, although there appears to be no relationship with disease severity. Tryptase has been shown to enhance airway smooth muscle responsiveness in dogs.[46] It also degrades bronchodilator neuropeptides, such as the vasoactive intestinal peptide (VIP),[47] and cleaves complement components, to form anaphylatoxins,[48] and kininogen, to yield bradykinin and lysyl-bradykinin.[49] Tryptase also contributes to increased microvascular leakage in the mucosa,[50] which, together with the capacity to chemoattract and activate eosinophils[51]

and enhance the expression of ICAM-1 on epithelial cells,[52] serves to promote mucosal eosinophilia.

Of particular interest is the effect of tryptase on ICAM-1 expression and it can be speculated that similar effects contribute to increased expression of this adhesion molecule on a number of other cell types (T cells, eosinophils, endothelial cells).[11,23,53] A close correlation can be demonstrated between the levels of tryptase and soluble (s) ICAM-1 in induced sputum,[11] which is in keeping with the described tryptase-induced upregulation of ICAM-1 and IL-8 in epithelial cells.[52] These observations have pointed to tryptase as a major mediator of allergic reactions and have prompted the development of therapeutic agents that specifically target tryptase. One of these, APC366, a specific tryptase antagonist, has been tested in a sheep model of allergic airway responses and has been shown to significantly attenuate the allergic response in sheep.[54] More recently, it has been shown that premedication of asthmatic volunteers with nebulized APC366 partially, but significantly, reduces the magnitude of the allergen-induced late asthmatic response (LAR).[55]

The importance of mast cell products is still uncertain. A positive correlation found between airway responsiveness and the relative numbers of mast cells recovered by BAL and sputum levels of histamine implies a role for mast cells in perpetuating this pathophysiologic abnormality.[11,14,34] This observation has been extended in studies that have shown that increased spontaneous histamine release by BAL mast cells is seen only in those subjects with increased airway responsiveness[15] and that the concentration of histamine in the cell-free supernatant of BAL is related to the level of histamine responsiveness.[41] However, it is well appreciated that cromolyns, known for their mast cell stabilizing properties, are poor at controlling asthma. In addition, treatment with short-acting β_2-adrenergic receptor agonists, which are even more potent inhibitors of mast cell mediator release, is ineffective at controlling inflammation. However, some recent studies with long-acting β_2-adrenergic receptor agonists have shown anti-inflammatory properties of these drugs;[56] however, whether this is entirely through inhibition of mast cells or some other, as yet unknown factors, remains to be seen.

Mast cells are now also recognized for their capacity to produce cytokines, the actions of which are of relevance to the initiation and maintenance of allergic responses, including IL-3, IL-4, IL-5, IL-6, GM-CSF and tumor necrosis factor (TNF)-α.[57] Importantly, IgE-mediated triggering of mast cells leads not only to secretion of stored cytokines, but also results in their *de novo* generation.[58] To what extent mast cells contribute to the overall generation of cytokines in asthma is uncertain. Staining of adjacent 2 μm tissue sections embedded into glycol methacrylate (GMA) resin localizes the majority of IL-4 protein to mast cells and a lesser proportion to eosinophils, but fails to detect IL-4 in T cells.[58] This is in contrast with studies using *in situ* hybridization to detect mRNA transcripts for IL-4 and other T helper 2 (Th2)-type cytokines, which show that the majority of cells positive for IL-4 mRNA are T cells.[4] One interpretation is that there is a relatively high turn-over of IL-4 produced by T cells that, unlike mast cells, do not have a granular storage capacity and use this cytokine as an autocrine growth factor, promoting the development of Th2-type $CD4^+$ T cells. An important consideration is the fact that only a minority of T cells in the airways are allergen specific, in contrast with mast cells, which all bear IgE and, thus, can be stimulated directly by allergen.

It can be speculated that activation of IgE-bearing mast cells and release of IL-4 may promote the differentiation of Th0-type T cells into Th2-type T cells. However, it remains to be seen whether this happens in the mucosa or regional lymphoid tissue. The use of antibodies for immunohistochemical staining, which distinguishes between stored and secreted IL-4, has been helpful in demonstrating the ability of mast cells to generate cytokines as part of allergic inflammation.[58] The finding of an increased proportion of mast cells displaying peripheral ring staining for IL-4 protein with antibody against the secreted form in stable asthma and further increases in this staining pattern in bronchial biopsies of pollen-sensitive asthmatics during the pollen season[59] point to allergen-induced activation of mast cells to secrete this central cytokine.

The central role of T cells in asthma

For an allergen (or, indeed, any antigen) to initiate an inflammatory response, it needs to be presented by an antigen-presenting cell (APC). Initially, this requires allergen uptake, its processing (partial degradation) within the APC, physical association with class II major histocompatibility complex (MHC) molecules and exteriorization of the antigen/MHC class II complex on the surface of the APC. Thereafter follows an interaction between the complex and the antigen receptor on the T lymphocyte (TCR) and between a number of accessory cell surface

molecules (including lymphocyte function-associated antigen (LFA)-1 and ICAM-1, CD40 and its ligand CD40L, and CD28 and B7-1/B7-2) present on cells that both recognize each other specifically and participate in the process of cellular costimulation. In addition, cytokines are produced that contribute to the overall response. The interaction between APC and T cells during antigen presentation may determine not only the extent of T cell activation, but also the differentiated phenotype of the T cell.

Antigen presentation in the lungs is believed to be conducted mainly by dendritic cells,[60] which are considered by some researchers as the only true professional APC.[61] In both animal and human airways, dendritic cells, which express high levels of MHC class II, are most prominent within the airway epithelium, where they form an interdigitating network.[62] The density of the intraepithelial dendritic cell population is maintained in a steady state by a balance between the recruitment of cells from the blood and their egress to local secondary lymphoid tissues where antigen presentation is believed to occur. However, in response to a range of stimuli, including inhaled viruses and soluble antigens, the density of intraepithelial dendritic cells increases rapidly, but transiently.[63] Thus, even stable asthmatics can be seen to have raised numbers of CD1α^+ dendritic cells in the airways mucosa.[64] It is of relevance to asthma therapy that the number of dendritic cells can be reduced by treatment with inhaled corticosteroids.[65]

It is now widely recognized that T cells are able to influence the function of mast cells, eosinophils, epithelial cells and fibroblasts, and these interactions are a major focus of interest in asthma research. Unlike T cells of non-allergic subjects, those of allergic patients with asthma, atopic dermatitis and perennial rhinitis become activated and proliferate when cultured in the presence of antigen *Der*P1 of *Dermatophagoides pteronyssinus*, with the degree of T cell proliferation correlating with specific IgE levels.[66] In both BAL[67] and the mucosa[3] of patients with atopic asthma, T lymphocytes are in an increased state of activation, with a greater proportion of cells expressing the activation markers IL-2 receptor (IL-2R; CD25) and the type II histocompatibility antigen HLA-DR on their surface than non-atopic control subjects. In the mucosa, the number of activated T cells has been shown to correlate with the number of eosinophils and the degree of bronchial responsiveness to methacholine.[68] In exacerbations of asthma, increased T lymphocyte activity has also been noted in peripheral blood, as judged by the percentage of CD4$^+$ T cells expressing IL-2R, HLA-DR and the very late antigen (VLA)-1.[69,70] Decreased expression of these activation markers occurs in parallel with clinical improvement, which could be attributed to corticosteroid therapy given to treat exacerbations of asthma.

In severe corticosteroid-dependent asthma, there is a further increase in activated T lymphocytes expressing IL-2R; this correlates with disease activity, as shown by high variation in peak expiratory flow (PEF).[29] Importantly, this is seen despite the use of high doses of inhaled and oral corticosteroids, suggesting a degree of corticosteroid resistance. It is now recognized that asthmatics vary in their responses to corticosteroids. Furthermore, these patients have evidence of raised IL-5 release into the mucosa, but this is not necessarily associated with increased eosinophil counts.[29] While the majority of patients respond to regular corticosteroid therapy, a subset of patients have poorly controlled asthma, even when treated with high doses of oral corticosteroids. In principle, all patients who are not fully controlled with moderate doses of corticosteroids and have persistent inflammation[13] can be seen as having a degree of corticosteroid unresponsiveness. Unresponsiveness to corticosteroids can be demonstrated in the laboratory by studying the suppressive effects of dexamethasone on T lymphocyte proliferation induced by the mitogen, phytohemagglutinin. True corticosteroid resistance, where no suppressive effect is seen at all, is rare but relative resistance to doses that are normally effective is not uncommon and presents a problem in clinical practice. Corticosteroid-resistant asthma can be defined by the failure to improve baseline morning prebronchodilator forced expiratory volume in 1 s (FEV_1) by greater than 15% predicted following 7–14 days of 40 mg oral prednisolone (20 mg twice daily or its equivalent).[71] Although some patients may respond to higher doses of prednisolone given for longer periods, such doses are undesirable because they can be associated with marked side effects.

Most of the research into the role of T cells in asthma has focused on the proinflammatory role of CD4$^+$ T cells. More recently, there has been increasing evidence that CD8$^+$ T cells may also have a role in asthma pathogenesis. It is now widely appreciated that purified CD8$^+$ T cells possess the capacity to produce a wide range of cytokines and chemokines that, in some instances (interferon (IFN)-γ, GM-CSF, macrophage inflammatory protein (MIP)-1α, IL-16, and RANTES) exceeds that of CD4$^+$ T cells. Distinct cytokine-secreting subsets of CD8$^+$ T cells

(Tc), similar to their $CD4^+$ Th1 and Th2-type counterparts, have now been identified and designated Tc1 cells, which secrete predominantly IL-2 and IFN-γ, and Tc2, which secrete IL-4 and IL-5. Circulating $CD8^+$ T cells from asthmatic individuals have increased intracellular concentrations of IL-4 compared with cells from non-atopic healthy control subjects,[72] although the mechanisms of activation of these cells and the release of IL-4 and its contribution to the overall allergic response remain unknown. $CD8^+$ T cells may play an important role in response to virus infection, which can influence the generation of cytokines by these cells.[73] This may be of importance to both mild and, in particular, severe exacerbations of asthma in which increased numbers of $CD8^+$ T cells accumulate in the airway mucosa.[74]

Other cells that may determine asthma severity

In an attempt to elucidate the mechanisms that determine asthma severity, a number of researchers have studied the events that occur during nocturnal asthma. While this form of asthma usually reflects poor disease control, genetic studies suggest that a Gly16 polymorphism of the β_2-adrenergic receptor, which may be responsible for downregulation of receptor numbers, is more frequent in nocturnal asthma, a form of asthma that is believed to reflect more severe disease. Thus, genes may help determine the extent of disease severity. Bronchoscopic studies conducted at night have shown an influx of eosinophils into the airways and their release of ECP as demonstrated by BAL.[75] Of particular interest is the finding that prominent changes occur in the lung parenchyma, which, so far, has not been thought to be involved in asthma.[76] Quantification of the cellular infiltrate shows an accumulation of eosinophils in the alveolar tissue in the early hours of the morning that is associated with increased expression of Th2-type cytokines.

However, the other cell type that is now being considered as a candidate in more severe forms of asthma is the neutrophil.[13] As disease progresses, neutrophil counts are also seen to increase compared with healthy control subjects and patients with mild asthma, with several-fold increases in both BAL and transbronchial biopsies in the very severe forms of corticosteroid-dependent asthma.[30] Neutrophils have so far not been thought to be important in asthma and have been viewed as being more characteristic of bronchitis. Increased airway neutrophilia is seen in asthma exacerbations[77] and in asthma deaths.[78] Because both of these have been linked with infections, neutrophilia has not been considered to be a feature of the chronic allergen-driven component of the pathogenesis of asthma. The mechanisms involved in recruiting and activating neutrophils remain to be elucidated, but initial studies point to raised LTB_4,[79] detected in BAL and raised tissue levels of free IL-8 in the bronchial mucosa[80] in severe forms of asthma as being possibly implicated by attracting and activating neutrophils.

If neutrophils contribute to the disordered airway function, it is unclear which mechanisms are important. The levels of myeloperoxidase (MPO) are not raised in induced sputum in asthma, although both neutrophil counts and MPO levels correlate with the levels of airway hyperresponsiveness,[81] suggesting that they are a marker of some other neutrophil function that contributes to this pathophysiologic abnormality. It has been suggested that treatment with corticosteroids may be partly responsible for increased neutrophil numbers and function.[82–84] However, short-term treatment with inhaled corticosteroids does not change neutrophil numbers in sputum (R Djukanovic, unpubl. obs.). It may be that longer-term corticosteroids may have an effect, although this remains to be demonstrated.

At the extreme end of the spectrum of asthma severity are patients who die during an asthma attack. At post-mortem, their lungs are invariably hyperinflated and remain so upon opening of the chest wall, reflecting extensive bronchial obstruction. The presence of overwhelming bronchospasm in the absence of mucus plugging is probably rare, as suggested in a review of a large number of cases of fatal asthma.[85,86] Abundant mucus plugs account for areas of atelectasis, which can lead to segmental and even lobar collapse following reabsorption of air distal to the site of bronchial obstruction. This points to hypersecretion by the hyperplastic submucosal glands and, possibly, goblet cells within the epithelium. Mucus plugging, leading to partial lung collapse, can also be noted occasionally in living asthmatics, especially in those suffering from allergic bronchopulmonary aspergillosis. The extent of mucus plugging in fatal asthma has been estimated to result in more than 50% occlusion of the peripheral airways.[87] The pathophysiologic implications of mucus plugging in severe asthma resulting in death are significant and the presence of physical obstruction to airflow offers an explanation for the ineffectiveness of bronchodilator drugs.

When scrutinizing the individual data from most studies of patients with asthma, it is apparent that there is large

variation in cell counts and mediators. Even when allowing for methodologic problems of sampling, one cannot ignore the observation that many patients with clinically mild disease have abundant inflammation; others with severe disease have little inflammation. Thus, it is clear that there is a component of airway pathology that is needed before disease can develop; this same component may indeed determine the severity of the disease. It is now increasingly believed that this airway restructuring, usually referred to as airway remodeling, is that component.

The process of airways restructuring in asthma

Extensive morphological changes occur in asthmatic airways as a consequence of chronic inflammation. The existence of these changes has been long known in cases of severe asthma,[88] but is now also seen on mild forms of the disease[89] and even in atopic individuals without clinical signs of asthma.[2] The entire depth of the airway wall is affected and the functional changes involve all the structural cells and the extracellular matrix.

The airway epithelium forms the first line of defence in the lungs by providing integrity and protecting the airways from environmental insults. It is composed of columnar ciliated, secretory and basal cells. The epithelial cells are organized into a stratified layer, with the columnar cells resting on basal cells that are bound to the basement membrane.[90] Any loss of this protective barrier is likely to have significant implications for the deeper mucosal layers by causing leakage, either through the grossly damaged epithelial layer or via breaks in the intercellular tight junctions.

The interpretation of epithelial damage in biopsies obtained by bronchoscopy has been controversial because of possible artefactual, bronchoscopically induced damage. Nonetheless, studies that have used rigid bronchoscopy and large biopsy forceps, which are less likely to cause artefactual damage than those used in fibreoptic bronchoscopy, have demonstrated widespread damage and shedding of the epithelium in asthma. Studies of bronchoscopic biopsies and BAL suggest an increase in the extent of epithelial sloughing as an integral part of the asthmatic process.[15,36,91] These findings are consistent with epithelial clumps seen in sputum and described in the early literature as Creola bodies and which have been reported to be increased in asthma, especially during acute exacerbations.[92,93]

The mechanisms leading to epithelial damage have not been fully elucidated. They probably involve a combination of mediators that are toxic to the epithelium, such as the arginine-rich eosinophil-derived cationic proteins and proteolytic enzymes,[19,94] which results in a fault-line between the columnar and basal cells,[95] and upward pressure from the edema of the submucosa. The damage inflicted on the epithelial layer is usually not due to cytoxicity, but appears to result from weakening of the junctional adhesion structures.[95] This results in a more selective loss of columnar cells and relative preservation of the basal cell layer. As a consequence, an increased proportion of columnar cells are seen in clumps of two or more cells.

Within the spectrum of airway diseases, damage of the bronchial epithelium appears to be unique to asthma and is not seen in chronic bronchitis, where squamous cell metaplasia develops. While epithelial damage is associated with attempts at repair, as suggested by increased expression of the CD44 adhesion molecules in areas of regeneration,[96,97] this seems to be unable to lead to complete restitution.[98] Whether this is due to repeat injury or impaired repair mechanisms is unclear. Damage to the epithelium and the resulting repair cause a dysregulation of epithelial cell function, with the production of mediators that can act on inflammatory as well as structural cells.[98] Normally, communication between the epithelium and fibroblasts occurs during morphogenesis of the lung, which results in branching of the airways. This interaction is referred to as the epithelial–mesenchymal trophic unit, which appears to be highly abnormal in asthma.[98]

The full implications of epithelial damage for asthma pathogenesis are not known and are a focus of intensive research. Theoretically, epithelial shedding should remove the protective barrier that prevents the allergens and microorganisms from damaging the deeper layers and, at the same time, reveal the nerve endings. In keeping with this notion, studies have shown that the degree of epithelial cell loss correlates with the measured level of airway hyperresponsiveness.[15,36,91] In addition, the epithelium participates in the secretion of IgA to protect against microorganisms but, if anything, the levels of IgA in sputum appear to be raised in asthma. The loss of epithelial integrity results in increased permeability to macromolecules. The epithelium also inhibits the action of kinins via neutral endopeptidase effects and has antioxidant enzymes that counter the effects of environmental oxidants, such as ozone and nitrogen dioxide.[99]

Through their capacity to generate endothelins and lipoxygenase products of arachidonic acid, epithelial cells can contribute to smooth muscle tone, which is countered by the production of nitric oxide (NO), which acts as a bronchodilator. However, an array of the mediators produced by the epithelium are proinflammatory (PGE_2, NO, 15-hydroxyeicosatetraenoic acid (HETE), endothelin, IL-1β, IL-6, IL-11, leukemia inhibitory factor (LIF), GM-CSF, IL-16, and IL-18, IL-8, MIP-1α, MCP-3, RANTES and eotaxin).[98] Although, theoretically, sloughing of the layer of activated cells may act as a means of downregulating inflammation, whether this is of physiologic relevance is unclear.

As part of the response to chronic injury, the reticular layer of the basement membrane undergoes extensive changes. Interstitial collagens type III and V, products of myofibroblasts located beneath the true basement membrane upon which the epithelial cells rest, are deposited to form a dense and thickened layer.[100] The extent of collagen deposition correlates with the increase in the number of myofibroblasts.[100] Studies have shown that both the superficial[100] as well as the deeper layers[100] of the submucosa of asthmatic airways contain significantly more collagen than is normal.

The increased production and deposition of collagen involves a number of growth factors and cytokines, such as transforming growth factor (TGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), TNF-α, IL-4 and endothelin, in association with mast cell mediators tryptase and histamine. Several growth factors may be released from inflammatory cells in the airways, such as macrophages and eosinophils, but also by structural cells, such as the airway epithelial and endothelial cells and fibroblasts. Platelet-derived growth factor is released from many different cells in the airways and consists of two peptide chains, with A-A, B-B or A-B dimers being secreted by different cells. These dimers interact with α (AA) or β (AB or BB) receptors. Macrophages release PDGF on activation.[101] Platelet-derived growth factor may activate fibroblasts to proliferate and secrete collagen and may also stimulate proliferation of airway smooth muscle,[102] which is mediated via the β receptor. The epithelium has now been shown to be an important source of factors that promote fibrosis, including PDGF, TGF-β and insulin-like growth factor (IGF)-1.[103,104] Indeed, there is now talk of the epithelial–mesenchymal trohoic unit being abnormal in asthma.[105] During normal embryogenesis, the epithelium and fibroblasts play a central role in lung development through opposing forces that make the airways grow (by way of epithelial proliferation) and bifurcate. This unit is believed to become dormant after completion of lung growth but, in disease, appears to be reactivated.

Supernatants from cultured human airway epithelial cells stimulate the proliferation of human lung fibroblasts and this activity appears to reside predominantly in IGF-1.[104] Transforming growth factor-β comprises a family of growth-modulating cytokines that have an important influence on the turnover of matrix proteins.[106] They may either inhibit or stimulate proliferation of fibroblasts, depending on the presence of other cytokines. Lung fibroblasts themselves may be a source of TGF-β,[107] but it is also secreted by inflammatory cells, including eosinophils and airway smooth muscle cells.[103]

However, the relative importance of these factors to airway remodeling and their cellular source is far from clear. A recent study has localized the major part of TGF-β to eosinophils.[108] The increased number of eosinophils has been shown correlate with reduced FEV_1 and the latter correlated with the extent of subepithelial fibrosis, pointing to a possible cause and effect relationship.[108]

In parallel with collagen deposition, there are changes in the other components of the extracellular matrix that indicate destruction of the elastic support. Furthermore, the appearance of α2 laminin chains, which are normally seen during morphogenesis, points to an increased turnover of the tissue matrix.[109] Immunohistochemical and electron microscopic analysis of elastin fibres in the bronchial mucosa shows evidence of lysis and fragmentation,[110] even though the total content of elastic fibres appears not to be different when comparing asthmatic and normal lungs.[111] Degradation of elastase could result from activation of macrophages and eosinophils and their release of elastase and other metalloproteases.[112–115] This change does not appear to be related to the use of corticosteroids, which are known to inhibit the generation of elastic fibres.[116] The pathophysiologic consequences of elastic fibre degradation are difficult to assess because the changes cannot be readily quantified and correlated with pulmonary function. The loss of elastic fibres is likely to make the airway less distensible.[117] Whether similar changes occur in the bronchial adventitia and the parenchyma, where there is now evidence of inflammatory cell involvement,[76] is uncertain, but the combination of airway and parenchymal changes could account for the loss of elastic recoil in asthma.[118]

As a further component of airway restructuring due to chronic inflammation and possibly repeated bronchoconstriction, the airways smooth muscle layer undergoes hypertrophy and hyperplasia.[88,119] In addition, prominent hyperplasia of mucus glands in the airway wall can be seen in cases of fatal asthma.[120] Finally, recent studies show an increase in the number of blood vessels in the mucosa.[121] Several cytokines and growth factors have been shown to stimulate angiogenesis, including TNF-α, IL-1 and bFGF.[103] Recently, substance P has also been found to have potent angiogenic effects.[122] The structural changes in airway smooth muscle may also result from stimulation by PDGF and endothelin released from activated inflammatory cells in the airways.[102,123,124] Even mediators such as histamine are able to stimulate growth in airway smooth muscle cells, as measured by the increase in *c-fos* expression.[125]

Another feature of asthmatic remodeling that has attracted recent interest has been the increased number of blood vessels, which, in the mucosa, are of the postcapillary venule type. These have been found both in mild[121] and severe[126] asthma. Although these vessels may not express increased amounts of adhesion molecules, the increased surface area probably provides greater interaction between circulating cells and the endothelium, thus increasing cell accumulation in the mucosa.[126] Initial studies suggest that treatment with corticosteroids can reduce the number of blood vessels.[127]

How, exactly, the remodeling changes impact on airway physiology is unclear. The changes observed under the microscope find confirmation in studies using computed tomography, which is able to demonstrate abnormalities of the airway wall in a significant proportion of asthmatics.[128,129] In some studies, the changes can be related to the duration and severity of asthma.[130] Therefore, it seems logical that airway remodeling should be a target for asthma treatment and, indeed, a number of studies have shown an increased rate of decline in lung function.[131–133] However, it remains unclear which patients are at risk of developing structural changes that are of physiologic relevance and there are no cellular or physiologic predictors for such a development. Nevertheless, long-term follow up of asthmatic patients treated with either β_2-adrenergic receptor agonists alone or with β_2-adrenergic receptor agonists and inhaled corticosteroids has shown a clear benefit of anti-inflammatory treatment in reducing the risk of loss of airways function.[134,135]

Mechanical removal of epithelial cells *in vitro* results in increased responsiveness of airway smooth muscle to several spasmogens[136] and suggests that epithelial cells may release a relaxant factor analogous to endothelial-derived relaxant factor in blood vessels. Thus, damage of the epithelium would remove the protective effect of this relaxant factor, leading to exaggerated bronchoconstrictor responses. The nature of this putative relaxant factor, termed EpDRF, is not certain, although it is not an eicosanoid or NO. Its existence has been demonstrated in superfusion experiments,[137] but the effects of epithelial removal on contractile responses to spasmogens is small and the relevance of this phenomenon is not certain, particularly *in vivo*, when the labile factor would have to diffuse across the submucosa and its blood supply to reach airway smooth muscle. Perhaps this factor may have a more important role as a vasodilator regulating airway blood flow.

Loss of epithelial integrity may result in loss of mediators that serve to counter the actions of bronchoconstrictor agents. Epithelial cells strongly express neuroendopeptidase (NEP),[138] which is an important enzyme in degrading various bronchoconstrictor peptides, such as tachykinins, bradykinin and endothelin-1. Mechanical removal of airway epithelium greatly potentiates the bronchoconstrictor effect of tachykinins[99] and bradykinin,[137] an effect that is mimicked by the NEP inhibitor phosphoramidon. Several factors that lead to exacerbations of asthma appear to downregulate the function of epithelial NEP, including virus infections, oxidants and toluene diisocyanate.[139]

Epithelial damage will also expose sensory nerve endings, which may be activated by inflammatory mediators, leading to inflammation via an axon reflex mechanism.[140] Epithelial cells themselves may release several inflammatory mediators, such as 15-lipoxygenase products, which are chemotactic for inflammatory cells, PGE_2, which may protect against bronchoconstriction[141,142] or may sensitize sensory nerves endings, endothelin-1 and NO.[143,144]

Concluding remarks

With the abundance of information on the pathology of asthma that we now possess, there is no doubt that we have greatly improved our understanding of the pathogenesis of this disease. However, we are no closer to identifying the main factors that cause asthma and sustain the chronic inflammation. Indeed, we do not know whether the mechanisms involved in initiating the disease and maintaining it are the same. Thus, elucidation

of early events during the development of asthma are crucial if efforts are to result in preventative measures that may alter the susceptibility of atopic individuals to develop clinical asthma. Another main challenge is to identify which cell types, signal transduction mechanisms and nuclear transcription factors are the main players in what is undoubtedly a complex cellular network. It is almost certain that targeting and neutralizing a single mediator or cell type is not going to arrest the asthmatic process. Finally, further attempts have to be made to improve our classification of asthma, which should be based not only on descriptive pathology, but also on common intracellular defects, so as to be able to target more specifically the disease process.

References

1 Djukanovic R, Wilson JW, Britten KM *et al*. Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am. J. Respir. Crit. Care Med*. 1990; **142**: 863–71.

2 Djukanovic R, Lai CK, Wilson JW *et al*. Bronchial mucosal manifestations of atopy: A comparison of markers of inflammation between atopic asthmatics, atopic nonasthmatics and healthy controls. *Eur. Respir. J.* 1992; **5**: 538–44.

3 Azzawi M, Bradley B, Jeffery PK *et al*. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am. Rev. Respir. Dis*. 1990; **142**: 1407–13.

4 Robinson DS, Hamid Q, Ying S *et al*. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med*. 1992; **326**: 298–304.

5 Bousquet J, Chanez P, Lacoste JY *et al*. Eosinophilic inflammation in asthma. *N. Engl. J. Med*. 1990; **323**: 1033–9.

6 Saetta M, Di Stefano A, Maestrelli P *et al*. Airway mucosal inflammation in occupational asthma induced by toluene diisocyanate. *Am. Rev. Respir. Dis*. 1992; **145**: 160–8.

7 Bentley AM, Menz G, Storz C *et al*. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Relationship to symptoms and bronchial responsiveness. *Am. Rev. Respir. Dis*. 1992; **146**: 500–6.

8 Djukanovic R, Roche WR, Wilson JW *et al*. Mucosal inflammation in asthma. *Am. Rev. Respir. Dis*. 1990; **142**: 434–57.

9 Pin I, Gibson PG, Kolendowicz R *et al*. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992; **47**: 25–9.

10 Fahy JV, Liu J, Wong H, Boushey HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. *Am. Rev. Respir. Dis*. 1993; **147**: 1126–31.

11 Louis R, Shute J, Biagi S *et al*. Cell infiltration, ICAM-1 expression, and eosinophil chemotactic activity in asthmatic sputum. *Am. J. Respir. Crit. Care Med*. 1997; **155**: 466–72.

12 Azzawi M, Bradley B, Jeffery PJ *et al*. Identification of activated T-lymphocytes and eosinophils in bronchial biopsies in stable atopic asthmatics. *Am. Rev. Respir. Dis*. 1990; **142**: 1407–13.

13 Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. *Am. J. Respir. Crit. Care Med*. 2000; **161**: 9–16.

14 Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am. Rev. Respir. Dis*. 1987; **136**: 379–83.

15 Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma: Relationship to bronchial hyperreactivity. *Am. Rev. Respir. Dis*. 1988; **137**: 62–9.

16 Louis E, Louis R, Shute J *et al*. Bronchial eosinophilic infiltration in Crohn's disease in the absence of pulmonary disease. *Clin. Exp. Allergy* 1999; **29**: 660–6.

17 De Monchy JGR, Kauffman HF, Venge P *et al*. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am. Rev. Respir. Dis*. 1985; **131**: 373–6.

18 in't Veen JC, de Gouw HW, Smits HH *et al*. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. *Eur. Respir. J.* 1996; **9**: 2441–7.

19 Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin. Proc*. 1981; **56**: 345–53.

20 Filley WV, Holley KE, Kephart GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet* 1982; **ii**: 11–16.

21 Metzger WJ, Zavala D, Richerson HB *et al*. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. Description of the model and local airway inflammation. *Am. Rev. Respir. Dis*. 1987; **135**: 433–40.

22 Metzger WJ, Richerson HB, Worden K, Monick M, Hunninghake GW. Bronchoalveolar lavage of allergic asthmatic patients following allergen bronchoprovocation. *Chest* 1986; **89**: 477–83.

23 Montefort S, Gratziou C, Goulding D *et al*. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte–endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic airways. *J. Immunol*. 1994; **93**: 1411–21.

24 Lampinen M, Rak S, Venge P. The role of interleukin-5, interleukin-8 and RANTES in the chemotactic attraction of eosinophils to the allergic lung. *Clin. Exp. Allergy* 1999; **29**: 314–22.

25 Wardlaw AJ, Moqbel R, Cromwell O, Kay AB. Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J. Clin. Invest*. 1986; **78**: 1701–6.

26 Muñoz NM, Douglas I, Mayer D, Herrnreiter A, Zhu X, Leff AR. Eosinophil chemotaxis inhibited by 5-lipoxygenase blockade and leukotriene receptor antagonism. *Am. J. Respir. Crit. Care Med*. 1997; **155**: 1398–403.

27 Garcia-Zepeda EA, Rothenberg ME, Ownbey RT, Celestin J, Leder P, Luster AD. Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat. Med*. 1996; **2**: 449–56.

28 Stellato C, Collins P, Ponath PD *et al*. Production of the novel C-C chemokine MCP-4 by airway cells and comparison of its biological activity to other C-C chemokines. *J. Clin. Invest*. 1997; **99**: 926–36.

29 Vrugt B, Wilson S, Underwood J *et al*. Mucosal inflammation in severe glucocorticoid-dependent asthma. *Eur. Respir. J*. 1999; **13**: 1245–52.

30 Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma: Persistent inflammation associated with high dose glucocorticoids. *Am. J. Respir. Crit. Care Med*. 1997; **156**: 737–43.

31 Wenzel SE, Schwartz LB, Langmack EL *et al*. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am. J. Respir. Crit. Care Med*. 1999; **160**: 1001–8.

32 Bryan SA, O'Connor BJ, Matti S *et al*. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; **356**: 2149–53.

33 Leckie MJ, ten Brinke A, Khan J *et al*. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; **356**: 2144–8.

34 Flint KC, Leung KB, Hudspith BN, Brostoff J, Pearce FL, Johnson NM. Bronchoalveolar mast cells in extrinsic asthma. A mechanism for the initiation of antigen specific bronchoconstriction. *BMJ* 1985; **291**: 923–6.

35 Tomioka M, Ida S, Shindoh Y, Ishihara T, Takishima T. Mast cells in bronchoalveolar lumen of patients with bronchial asthma. *Am. Rev. Respir. Dis*. 1984; **129**: 1000–5.

36 Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am. Rev. Respir. Dis*. 1989; **139**: 806–17.

37 Wenzel SE, Westcott JY, Smith HR, Larsen GL. Spectrum of prostanoid release after bronchoalveolar allergen challenge in atopic asthmatics and in control groups. An alteration in the ratio of bronchoconstrictive to bronchoprotective mediators. *Am. Rev. Respir. Dis*. 1989; **139**: 450–7.

38 Wenzel SE, Fowler AAD, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. *In vivo* release of histamine and tryptase in atopic subjects with and without asthma. *Am. Rev. Respir. Dis*. 1988; **137**: 1002–8.

39 Broide DH, Gleich GJ, Cuomo AJ *et al*. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J. Allergy Clin. Immunol*. 1991; **88**: 637–48.

40 Lam S, Chan H, LeRiche JC, Chan-Yeung M, Salari H. Release of leukotrienes in patients with bronchial asthma. *J. Allergy Clin. Immunol*. 1988; **81**: 711–17.

41 Casale TB, Wood D, Richerson HB, Zehr B, Zavala D, Hunninghake GW. Direct evidence of a role for mast cells in the pathogenesis of antigen-induced bronchoconstriction. *J. Clin. Invest*. 1987; **80**: 1507–11.

42 Finnerty JP, Wood-Baker R, Thomson H, Holgate ST. Role of leukotrienes in exercise-induced asthma: Inhibitory effect of ICI 204219, a potent leukotriene D_4 receptor antagonist. *Am. Rev. Respir. Dis*. 1992; **145**: 746–9.

43 Spector SL, Smith LJ, Glass M, Group AAT. Effects of 6 weeks of therapy with oral doses of ICI 204,219, a leukotriene D_4 receptor antagonist, in subjects with bronchial asthma. *Am. J. Respir. Crit. Care Med*. 1994; **150**: 618–23.

44 Bradding P, Redington AE, Djukanovic R, Conrad DJ, Holgate ST. 15-Lipoxygenase immunoreactivity in normal and in asthmatic airways. *Am. J. Respir. Crit. Care Med*. 1995; **151**: 1201–4.

45 Shannon VR, Chanez P, Bousquet J, Holtzman MJ. Histochemical evidence for induction of arachidonate 15-lipoxygenase in airway disease. *Am. Rev. Respir. Dis*. 1993; **147**: 1024–8.

46 Sekizawa K, Caughey GH, Lazarus SC, Gold WM, Nadel JA. Mast cell tryptase causes airway smooth muscle hyperresponsiveness in dogs. *J. Clin. Invest*. 1989; **83**: 175–9.

47 Barnes PJ. Airway neuropeptides and asthma. *Trends Pharmacol. Sci*. 1987; **8**: 24–7.

48 Schwartz LB, Kawahara MS, Hugli TE, Vik D, Fearon DT, Austen KF. Generation of C3a anaphylatoxin from human C3 by human mast cell tryptase. *J. Immunol*. 1983; **130**: 1891–5.

49 Proud D, Siekierski ES, Bailey GS. Identification of human lung mast cell kininogenase as tryptase and relevance of tryptase kininogenase activity. *Biochem. Pharmacol*. 1988; **37**: 1473–80.

50 Walls AF, He S, Teran L, Holgate ST. Mast cell proteases as mediators of vascular leakage and cell accumulation. *J. Allergy Clin. Immunol*. 1993; **91**: A256 (Abstract).

51 Jung K, Shute J, Cairns J, Park H, Church MK, Walls A. Human mast cells tryptase. A mediator of eosinophil chemotaxis and degranulation. *Am. J. Respir. Crit. Care Med*. 1995; **151**: A530 (Abstract).

52 Cairns JA, Walls AF. Mast cell tryptase is a mitogen for epithelial cells. Stimulation of IL-8 production and intercellular adhesion molecule-1 expression. *J. Immunol*. 1996; **156**: 275–83.

53 Hansel TT, Braunstein JB, Walker C *et al*. Sputum eosinophils from asthmatics express ICAM-1 and HLA-DR. *Clin. Exp. Immunol*. 1991; **86**: 271–7.

54 Molinari JF, Moore WR, Clark J, Tanaka R, Butterfield JH, Abraham WM. Role of tryptase in immediate cutaneous responses in allergic sheep. *J. Appl. Physiol*. 1995; **79**: 1966–70.

55 Krishna MT, Chauhan AJ, Little L *et al*. Effect of inhaled APC 366 on allergen-induced bronchoconstriction and airway hyperresponsiveness to histamine in atopic asthmatics. *Am. J. Respir. Crit. Care Med*. 1998; **157**: A456 (Abstract).

56 Wallin A, Sandstrom T, Soderberg M *et al*. The effects of regular inhaled formoterol, budesonide, and placebo on mucosal inflammation and clinical indices in mild asthma. *Am. J. Respir. Crit. Care Med*. 1999; **159**: 79–86.

57 Bradding P, Roberts JA, Britten KM *et al*. Interleukin-4, -5, and -6 and tumor necrosis factor-α in normal and asthmatic airways: Evidence for the human mast cell as a source of these cytokines. *Am. J. Respir. Cell Mol. Biol*. 1994; **10**: 471–80.

58 Okayama Y, Petit-Frere C, Kassel O *et al*. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J. Immunol*. 1995; **155**: 1796–808.

59 Djukanovic R, Feather I, Gratziou C *et al*. Effect of natural allergen exposure during the grass pollen season on airways inflammatory cells and asthma symptoms. *Thorax* 1996; **51**: 575–81.

60 Semper AE, Hartley JA. Dendritic cells in the lung: What is their relevance to asthma? *Clin. Exp. Allergy* 1996; **26**: 485–90.

61 Matzinger P. Tolerance, danger, and the extended family. *Annu. Rev. Immunol*. 1994; **12**: 991–1045.

62 Holt PG, Schon-Hegrad MA, Phillips MJ, McMenamin PG. Ia-positive dendritic cells form a tightly meshed network within the human airway epithelium. *Clin. Exp. Allergy* 1989; **19**: 597–601.

63 Fokkens WJ, Vroom TM, Rijntjes E, Mulder PG. Fluctuation of the number of CD-1 (T6)-positive dendritic cells, presumably Langerhans cells, in the nasal mucosa of patients with an isolated grass-pollen allergy before, during, and after the grass-pollen season. *J. Allergy Clin. Immunol*. 1989; **84**: 39–43.

64 Tunon-De-Lara JM, Redington AE, Bradding P *et al*. Dendritic cells in normal and asthmatic airways: Expression of the alpha subunit of the high affinity immunoglobulin E receptor (Fc epsilon RI-alpha). *Clin. Exp. Allergy* 1996; **26**: 648–55.

65 Moller GM, Overbeek SE, Van Helden-Meeuwsen CG *et al*. Increased numbers of dendritic cells in the bronchial mucosa of atopic asthmatic patients: Down-regulation by inhaled corticosteroids. *Clin. Exp. Allergy* 1996; **26**: 517–24.

66 Rawle FC, Mitchell EB, Platts-Mills TA. T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, antigen P1. comparison of patients with asthma, atopic dermatitis, and perennial rhinitis. *J. Immunol*. 1984; **133**: 195–201.

67 Wilson JW, Djukanovic R, Howarth PH, Holgate ST. Lymphocyte activation in bronchoalveolar lavage and peripheral blood in atopic asthma. *Am. Rev. Respir. Dis*. 1992; **145**: 958–60.

68 Bradley BL, Azzawi M, Jacobson M *et al*. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: Comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J. Allergy Clin. Immunol*. 1991; **88**: 661–74.

69 Corrigan CJ, Kay AB. CD4 T-lymphocyte activation in acute severe asthma. Relationship to disease severity and atopic status. *Am. Rev. Respir. Dis*. 1990; **141**: 970–7.

70 Corrigan CJ, Hartnell A, Kay AB. T lymphocyte activation in acute severe asthma. *Lancet* 1988; **i**: 1129–32.

71 Durham SR, Smurthwaite L, Gould HJ. Local IgE production. *Am. J. Rhinol*. 2000; **14**: 305–7.

72 Stanciu LA, Shute J, Promwong C, Holgate ST, Djukanovic R. Increased levels of IL-4 in $CD8^+$ T cells in atopic asthma. *J. Allergy Clin. Immunol*. 1997; **100**: 373–8.

73 Coyle AJ, Erard F, Bertrand C, Walti S, Pircher H, Le Gros G. Virus-specific CD8+ cells can switch to interleukin 5 production and induce airway eosinophilia. *J. Exp. Med*. 1995; **181**: 1229–33.

74 Faul JL, Tormey VJ, Leonard C *et al*. Lung immunopathology in cases of sudden asthma death. *Eur. Respir. J*. 1997; **10**: 301–7.

75 Mackay TW, Wallace WA, Howie SE *et al*. Role of inflammation in nocturnal asthma. *Thorax* 1994; **49**: 257–62.

76 Kraft M, Djukanovic R, Wilson S, Holgate ST, Martin RJ. Alveolar tissue inflammation in asthma. *Am. J. Respir. Crit. Care Med*. 1996; **154**: 1505–10.

77 Turner MO, Hussack P, Sears MR, Dolovich J, Hargreave FE. Exacerbations of asthma without sputum eosinophilia. *Thorax* 1995; **50**: 1057–61.

78 Sur S, Crotty TB, Kephart GM *et al*. Sudden-onset fatal asthma. A distinct entity with few eosinophils and relatively more neutrophils in the airway submucosa? *Am. Rev. Respir. Dis*. 1993; **148**: 713–19.

79 Vrugt B, Djukanovic R, Bron A, Aalbers R. New insights into the pathogenesis of severe corticosteroid-dependent asthma. *J. Allergy Clin. Immunol*. 1996; **98**: S22–6.

80 Shute JK, Vrugt B, Lindley IJ *et al*. Free and complexed interleukin-8 in blood and bronchial mucosa in asthma. *Am. J. Respir. Crit. Care Med*. 1997; **155**: 1877–83.

81 Louis R, Holgate ST, Djukanovic R. Induced sputum in asthma. Comparison of asymptomatic vs. symptomatic and steroid naive vs. steroid patients. *Eur. Respir. J*. 1996; **9**: A13 (Abstract).

82 Chanez P, Paradis L, Vignola AM *et al*. Changes in bronchial inflammation of steroid (GCs) dependent asthmatics. *Am. J. Respir. Cell Mol. Biol*. 1996; **153**: A212 (Abstract).

83 Cox G. Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. *J. Immunol*. 1995; **154**: 4719–25.

84 Schleimer RP, Freeland HS, Peters SP, Brown KE, Derse CP. An assessment of the effects of glucocorticoids on degranulation, chemotaxis, binding to vascular endothelium and formation of leukotriene B4 by purified human neutrophils. *J. Pharmacol. Exp. Ther*. 1989; **250**: 598–605.

85 Messer JW, Peter GA, Bennett WA. Causes of death and pathologic findings in 304 cases of bronchial asthma. *Dis. Chest* 1960; **38**: 616–24.

86 Reid LM. The presence or absence of bronchial mucus in fatal asthma. *J. Allergy Clin. Immunol.* 1987; **80**: 415–16.

87 Saetta M, Di Stefano A, Rosina C, Thiene G, Fabbri LM. Quantitative structural analysis of peripheral airways and arteries in sudden fatal asthma. *Am. Rev. Respir. Dis.* 1991; **143**: 138–43.

88 Dunnill MS. The pathology of asthma with special reference to changes in the bronchial mucosa. *J. Clin. Pathol.* 1960; **13**: 27–33.

89 Vignola AM, Kips J, Bousquet J. Tissue remodeling as a feature of persistent asthma. *J. Allergy Clin. Immunol.* 2000; **105**: 1041–53.

90 Montefort S, Djukanovic R, Holgate ST, Roche WR. Ciliated cell damage in the bronchial epithelium of asthmatics and non-asthmatics. *Clin. Exp. Allergy* 1993; **23**: 185–9.

91 Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am. J. Respir. Crit. Care Med.* 1989; **140**: 1745–53.

92 Motojima S, Kushima A, Ogata H, Tateishi K, Fukuda T, Makino S. [Relationship between presence of Creola bodies and airway hyperresponsiveness in patients with bronchial asthma.] *Arerugi* 1990; **39**: 377–83.

93 Ogata H, Motojima S, Fukuda T, Makino S. [Creola bodies and the eosinophil cationic protein in sputum in acute asthmatic attacks with respect to their clinical significance.] *Arerugi* 1990; **39**: 1567–75.

94 Redington AE, Polosa R, Walls AF, Howarth PH, Holgate ST. Role of mast cells and basophils in asthma. *Chem. Immunol.* 1995; **62**: 22–59.

95 Montefort S, Roberts JA, Beasley R, Holgate ST, Roche WR. The site of disruption of the bronchial epithelium in asthmatic and non-asthmatic subjects. *Thorax* 1992; **47**: 499–503.

96 Lackie PM, Baker JE, Gunthert U, Holgate ST. Expression of CD44 isoforms is increased in the airway epithelium of asthmatic subjects. *Am. J. Respir. Cell Mol. Biol.* 1997; **16**: 14–22.

97 Peroni DG, Djukanovic R, Bradding P *et al*. Expression of CD44 and integrins in bronchial mucosa of normal and mildly asthmatic subjects. *Eur. Respir. J.* 1996; **9**: 2236–42.

98 Holgate ST, Lackie P, Wilson S, Roche W, Davies D. Bronchial epithelium as a key regulator of airway allergen sensitization and remodeling in asthma. *Am. J. Respir. Cell Mol. Biol.* 2000; **162** (Suppl.): S113–7.

99 Frossard N, Rhoden KJ, Barnes PJ. Influence of epithelium on guinea pig airway responses to tachykinins: Role of endopeptidase and cyclooxygenase. *J. Pharmacol. Exp. Ther.* 1989; **248**: 292–8.

100 Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; **i**: 520–4.

101 Haynes AR, Shaw RJ. Dexamethasone-induced increase in platelet-derived growth factor (B) mRNA in human alveolar macrophages and myelomonocytic HL60 macrophage-like cells. *Am. J. Respir. Cell Mol. Biol.* 1992; **7**: 198–206.

102 Hirst SJ, Barnes PJ, Twort CH. Quantifying proliferation of cultured human and rabbit airway smooth muscle cells in response to serum and platelet-derived growth factor. *Am. J. Respir. Cell Mol. Biol.* 1992; **7**: 574–81.

103 Kelley J. Cytokines of the lung. *Am. Rev. Respir. Dis.* 1990; **141**: 765–88.

104 Cambrey AD, Kwon OJ, McAnulty RJ *et al*. Release of fibroblast proliferative activity from cultured human airway epithelial cells: A role for insulin-like growth factor 1 (IGF-1). *Am. Rev. Respir. Dis.* 1993; **147**: 272 (Abstract).

105 Holgate ST, Lackie PM, Davies DE, Roche WR, Walls AF. The bronchial epithelium as a key regulator of airway inflammation and remodelling in asthma. *Clin. Exp. Allergy* 1999; **29**: 90–5.

106 Moses HL, Yang EY, Pietenpol JA. TGF-beta stimulation and inhibition of cell proliferation: New mechanistic insights. *Cell* 1990; **63**: 245–7.

107 Kelley J, Fabisiak JP, Hawes K, Absher M. Cytokine signaling in lung: Transforming growth factor-beta secretion by lung fibroblasts. *Am. J. Physiol.* 1991; **260**: L123–8.

108 Minshall EM, Leung DY, Martin RJ *et al*. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am. J. Respir. Cell Mol. Biol.* 1997; **17**: 326–33.

109 Altraja A, Laitinen A, Virtanen I *et al*. Expression of laminins in the airways in various types of asthmatic patients: A morphometric study. *Am. J. Respir. Cell Mol. Biol.* 1996; **15**: 482–8.

110 Bousquet J, Lacoste JY, Chanez P, Vic P, Godard P, Michel FB. Bronchial elastic fibers in normal subjects and asthmatic patients. *Am. J. Respir. Cell Mol. Biol.* 1996; **153**: 1648–54.

111 Godfrey R, Majumdar S, Adelroth E, Johansson S, Jeffrey P. Airway and lung parenchymal content of elastic fibre is not reduced in asthma. *Am. Rev. Respir. Dis.* 1992; **145**: 463 (Abstract).

112 Rodriguez RJ, White RR, Senior RM, Levine EA. Elastase release from human alveolar macrophages. Comparison between smokers and nonsmokers. *Science* 1977; **198**: 313–14.

113 Senior RM, Connolly NL, Cury JD, Welgus HG, Campbell EJ. Elastin degradation by human alveolar macrophages. A prominent role of metalloproteinase activity. *Am. Rev. Respir. Dis.* 1989; **139**: 1251–6.

114 Lungarella G, Menegazzi R, Gardi C *et al*. Identification of elastase in human eosinophils: Immunolocalization, isolation, and partial characterization. *Arch. Biochem. Biophys.* 1992; **292**: 128–35.

115 Shapiro SD, Kobayashi DK, Ley TJ. Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. *J. Biol. Chem.* 1993; **268**: 23 824–9.

116 Kahari VM. Dexamethasone suppresses elastin gene expression in human skin fibroblasts in culture. *Biochem. Biophys. Res. Commun.* 1994; **201**: 1189–96.
117 Wilson JW, Li X, Pain MC. The lack of distensibility of asthmatic airways. *Am. Rev. Respir. Dis.* 1993; **148**: 806–9.
118 McCarthy DS, Sigurdson M. Lung elastic recoil and reduced airflow in clinically stable asthma. *Thorax* 1980; **35**: 298–302.
119 Hossain S, Heard BE. Hyperplasia of bronchial muscle in chronic bronchitis. *J. Pathol.* 1970; **101**: 171–84.
120 Takizawa T, Thurlbeck WM. Muscle and mucous gland size in the major bronchi of patients with chronic bronchitis, asthma, and asthmatic bronchitis. *Am. Rev. Respir. Dis.* 1971; **104**: 331–6.
121 Li X, Wilson JW. Increased vascularity of the bronchial mucosa in mild asthma. *Am. J. Respir. Crit. Care Med.* 1997; **156**: 229–33.
122 Fan TP, Hu DE, Guard S, Gresham GA, Watling KJ. Stimulation of angiogenesis by substance P and interleukin-1 in the rat and its inhibition by NK1 or interleukin-1 receptor antagonists. *Br. J. Pharmacol.* 1993; **110**: 43–9.
123 Komuro I, Kurihara H, Sugiyama T, Yoshizumi M, Takaku F, Yazaki Y. Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. *FEBS Lett.* 1988; **238**: 249–52.
124 Stewart AG, Tomlinson PR, Wilson J. Airway wall remodelling in asthma. A novel target for the development of anti-asthma drugs. *Trends Pharmacol. Sci.* 1993; **14**: 275–9.
125 Panettieri RA, Yadvish PA, Kelly AM, Rubinstein NA, Kotlikoff MI. Histamine stimulates proliferation of airway smooth muscle and induces c-fos expression. *Am. J. Physiol.* 1990; **259**: 365–71.
126 Vrugt B, Wilson S, Bron A, Holgate ST, Djukanovic R, Aalbers R. Bronchial angiogenesis in severe glucocorticoid-dependent asthma. *Eur. Respir. J.* 2000; **15**: 1014–21.
127 Orsida BE, Li X, Hickey B, Thien F, Wilson JW, Walters EH. Vascularity in asthmatic airways: Relation to inhaled steroid dose. *Thorax* 1999; **54**: 289–95.
128 Lynch DA, Newell JD, Tschomper BA, Cink TM, Newman LS, Bethel R. Uncomplicated asthma in adults. Comparison of CT appearance of the lungs in asthmatic and healthy subjects. *Radiology* 1993; **188**: 829–33.
129 Paganin F, Trussard V, Seneterre E *et al*. Chest radiography and high resolution computed tomography of the lungs in asthma. *Am. Rev. Respir. Dis.* 1992; **146**: 1084–7.
130 Boulet L, Belanger M, Carrier G. Airway responsiveness and bronchial-wall thickness in asthma with or without fixed airflow obstruction. *Am. J. Respir. Crit. Care Med.* 1995; **152**: 865–71.
131 Schachter EN, Doyle CA, Beck GJ. A prospective study of asthma in a rural community. *Chest* 1984; **85**: 623–30.
132 Ulrik CS, Lange P. Decline of lung function in adults with bronchial asthma. *Am. J. Respir. Crit. Care Med.* 1994; **150**: 629–34.
133 Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. *N. Engl. J. Med.* 1998; **339**: 1194–200.
134 Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a beta 2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: A randomized, double-blind, parallel-group controlled trial. *J. Allergy Clin. Immunol.* 1992; **90**: 32–42.
135 Haahtela T, Jarvinen M, Kava T *et al*. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N. Engl. J. Med.* 1994; **331**: 700–5.
136 Cuss FM, Barnes PJ. Airway smooth muscle and disease workshop: Epithelial mediators. *Am. Rev. Respir. Dis.* 1987; **136** (Suppl.): S32–5.
137 Bramley AM, Samhoun MN, Piper PJ. The role of the epithelium in modulating the responses of guinea-pig trachea induced by bradykinin *in vitro*. *Br. J. Pharmacol.* 1990; **99**: 762–6.
138 Johnson AR, Ashton J, Schulz WW, Erdos EG. Neutral metalloendopeptidase in human lung tissue and cultured cells. *Am. Rev. Respir. Dis.* 1985; **132**: 564–8.
139 Nadel JA. Neutral endopeptidase modulates neurogenic inflammation. *Eur. Respir. J.* 1991; **4**: 745–54.
140 Barnes PJ. Asthma as an axon reflex. *Lancet* 1986; **i**: 242–4.
141 Pavord ID, Wong CS, Williams J, Tattersfield AE. Effect of inhaled prostaglandin E2 on allergen-induced asthma. *Am. Rev. Respir. Dis.* 1993; **148**: 87–90.
142 Pavord ID, Tattersfield AE. Bronchoprotective role for endogenous prostaglandin E2. *Lancet* 1995; **345**: 436–8.
143 Black PN, Ghatei MA, Takahashi K *et al*. Formation of endothelin by cultured airway epithelial cells. *FEBS Lett.* 1989; **255**: 129–32.
144 Robbins RA, Hamel FG, Floreani AA *et al*. Bovine bronchial epithelial cells metabolize L-arginine to L-citrulline. Possible role of nitric oxide synthase. *Life Sci.* 1993; **52**: 709–16.