THE RISING TRENDS IN ASTHMA
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THE RISING TRENDS IN ASTHMA
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*Symposium on The rising trends in asthma, held at the Ciba Foundation on 11–13 June 1996*

This symposium is based on a proposal made by Stephen T. Holgate

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Introduction

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One of the most exciting prospects of this symposium is the opportunity to bring together people from different countries and different disciplines within the field of asthma. In particular, it's a pleasure to have epidemiologists, clinicians, pathophysiologists and geneticists sharing mutual experiences, exploring new ways of thinking about asthma and generating novel ideas about an 'old' disease.

I am particularly keen that we do not focus on drawing any specific conclusions too early in the symposium because they are often difficult to distil during the discussion periods. However, I hope we will be able to arrive at some principles relating to the possible causes of any rise in asthma trends that we will wish to pursue in the future.

In 1860 Henry Hyde Salter published a treatise on asthma, which was remarkably insightful considering that there was very little knowledge of pathophysiology of the disease at that time (Salter 1860). In this treatise he referred to narrowing of the airways in asthma as being due to smooth muscle contraction and that this was intimately involved in the mechanisms of episodic breathlessness in asthma. He also had ideas about the factors leading to airway narrowing in other forms of obstructive diseases that broadly fall into the category of chronic obstructive pulmonary disease, a debate that still continues.

The concept emerging from Salter's writings, and from two other Ciba Foundation symposia in 1959 and 1971 (Ciba Foundation guest symposium 1959, Ciba Foundation study group 1971), was that asthma was predominantly a disease of airway smooth muscle, which was altered so that it contracted too easily and too much. Following on from this concept, industry has responded by creating drugs such as the β2-agonists and cholinergic antagonists that relax the contracted airway smooth muscle and relieve the obstruction. However, thinking of asthma purely in terms of smooth muscle contraction hasn't been a very constructive exercise because somehow we have to explain why the smooth muscle around the airways contracts too easily and too much.

One reason why there has been so much interest in asthma is because of the view that over the last two decades it has been increasing in prevalence and possibly in severity. I am hoping that at the end of this symposium we will be able to make some clear statements about these trends in different parts of the world and come to some clear conclusions on the epidemiology of the disease. One can identify a range of markers that might be indicative of a rising trend in asthma: for example, between 1980 and
1993 in the UK there has been an increased prescribing of β2-agonists, inhaled corticosteroids and other anti-inflammatory drugs. I'm confident that we will be able to tease out whether this is being driven by an increased awareness of asthma by patients and doctors, and changes in the way asthma health care is delivered, or whether it is truly reflective of a change in the prevalence of asthma. We are also familiar with epidemics of asthma that have occurred in association with an increase in mortality. For example, in Barcelona asthma has been associated with the release of soybean allergens into the air around the storage silos of the harbour when soybean is off-loaded from ships (Synek et al 1996). An increased mortality has also been described in relation to possible adverse effects of treatment. For example, in New Zealand an association has been reported between increased asthma mortality and the prescribing of a particular type of β2-agonist (Crane et al 1989). This has fuelled an important debate on the consequences of relying too heavily on β2-agonists for relief of asthma symptoms (Beasley et al 1997, this volume).

Objective data on the prevalence of asthma in different countries have also been collected using questionnaires that have been well validated. These have been used on whole populations as well as on well-described subgroups of individuals over time. For example, the Finnish study of conscripts has demonstrated that there has been an increase in both the prevalence and severity of asthma (Haahtela et al 1990). There is currently a view that any increase in the trends of asthma is accounted for by a parallel rise in allergy expressed in multiple organs (Lewis et al 1996). However, it appears that asthma has increased to a greater extent than other allergic manifestations, suggesting that other forces are operating in the lower airways.

Moving on to the causes of asthma, although we will hear about the importance of genetic factors in increasing the risk of acquiring asthma through interactions with the environment, most of this symposium will focus on the environment itself. This also includes the intrauterine environment because gestation is a period when the fetus is being programmed through the materno-fetal relationship influencing the developing immune system and lungs. The fact that the induction of asthma is so closely linked to aeroallergen exposure indicates the importance of this environmental aspect, especially in the first two to three years of life. Discussion relating to asthma will focus on the types of allergen and their marked geographical differences. House dust mites, cats, pollens and fungi in different parts of the world are all associated with specific sensitization of the lower respiratory tract and the induction of asthma. It would be helpful if a consensus were to emerge about the relative role of allergens, and if there are some that are more important than others. This would help in the design of allergen avoidance primary and secondary intervention studies.

Over the last half century there have also been substantial changes to our domestic and working environments. Allergens are important in our domestic environment, and we will probably hear about the increase in allergen load that has arisen as a consequence of sealing homes for air conditioning and heating. Poor ventilation of houses and work place environments leads not only to high concentrations of sensitizing allergens
INTRODUCTION

accumulating, but also to other pollutants such as combustion emissions and tobacco smoke, which could serve as adjuvants in the sensitization processes.

The public are presented with a very different view of the rising trends in asthma. Largely because of promotion by the lay press, they believe that external ambient pollutants, and especially emissions from motor vehicles, play a key role. However, there is little supporting evidence for this—at least in terms of new cases of asthma, rather than promoting attacks of asthma in those who already have the disease (COMEAP 1995). Studies in former East and West Germany have suggested that air pollution from industrial sources, as opposed to motor vehicles, may even be protective (von Mutius et al. 1994). Cigarette smoking in pregnancy and the influence of inhalation of cigarette smoke by non-smokers are important causes of respiratory morbidity and may well contribute to the acquisition of asthma.

The role of viruses is presently a paradox and I'm not sure whether we will resolve the issues at this symposium. One fascinating observation is that children from one-child families seem to have more asthma and allergic disease than children from multiple sibling families (Strachan 1989). One explanation is that viruses have a protective role early in life by creating a stimulus that tilts the immune response away from the development of allergy and asthma. In contrast, other viruses such as the respiratory syncitial virus, and other infective organisms, such as Chlamydia pneumoniae or Micoplasma pneumoniae, might drive an asthmatic-type immunological response rather than protecting against it. A consideration of the roles of infection early in life will be particularly relevant, as will be the role of infection in exacerbating asthma. Although there is a strong emphasis on the role of allergens in triggering attacks of asthma, an important role of respiratory viruses in asthma morbidity is being increasingly recognized.

Much of this symposium will focus on the early life origins of asthma. There has been a change in the way we look at asthma, in that the infant and child are becoming the focus of novel exciting studies, which are not only epidemiological but also based on pathogenesis. By identifying possible genes that are important at increasing risks, by looking at vulnerable periods in life (especially early in life) and by identifying factors which through different mechanisms may be driving the immunological processes (amongst others) that lead to asthma, we will gain a better insight with which to make informed decisions regarding intervention studies. The crucial issues are whether early life interventions are truly effective, how to achieve interventions, what sort of interventions are most effective and what will be acceptable to patients. I would like to encourage you all to be as frank and as open as possible in your discussions so that we can expose some of the more controversial areas that will help take forward concepts in the origins and prevention of asthma.

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Asthma: a dynamic disease of inflammation and repair

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Abstract. It is now widely accepted that asthma in its varied forms is an inflammatory disorder of the airways in which mediator release from activated mast cells and eosinophils plays a major role. T lymphocytes take a primary role in orchestrating these processes through their capacity to generate a range of cytokines of the interleukin 4 gene cluster encoded on the long arm of chromosome 5. Additional cytokines derived from mast cells and eosinophils also play a key role, especially tumour necrosis factor α, which is responsible for initiating the up-regulation of vascular adhesion molecules involved in the recruitment of eosinophils and other inflammatory cells from the circulation. The importance of C-X-C and C-C chemokines as local chemoattractants and activating stimuli is also recognized. In addition to releasing an array of pharmacologically active autacoids, the inflammatory response in asthma results in the generation of proteolytic activities from mast cells (tryptase, chymase), eosinophils (MMP-9) and the epithelium itself (MMP-2, MMP-9), which exert tissue--destructive and cell-signalling effects. The epithelium is also highly activated, as evidenced by the up-regulation of cytokine production, inducible enzymes and soluble mediators. Increased surface expression of the epithelial isoform of CD44(9v) and subepithelial proliferation of myofibroblasts are indicative of a simultaneous active repair process and the laying down of new interstitial collagens. Together, inflammatory and repair processes create the complex phenotype that characterizes asthma and its progression.

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In his classical treatise on asthma, published in 1860, Salter describes asthma as 'Paroxysmal dyspnoea of a peculiar character, generally periodic with healthy respiration between attacks.' (Salter 1860) He illustrates this in his book as being caused by contraction of airway smooth muscle, and separates it mechanistically from 'bronchial catarrh', 'recent' and 'old' bronchitis. Although he accepted 'nervous asthma', Salter made the important observation that there were other causes that precipitated attacks, including animal emanations, impure air, hay fever and foods. Among the many subjects covered in his book was a classification of asthma, which used the term 'intrinsic', and a description of cells characteristic of asthmatic sputum which Paul Ehrlich was later to identify as eosinophils (Ehrlich 1879). With this
historical background, it is of interest to see how much progress into asthma mechanisms has been achieved in the 100 years to follow.

There have been two previous meetings on asthma held under the auspices of the Ciba Foundation. These occurred in 1958 and 1971 and they focused on trying to agree a definition of various forms of airway obstruction (Ciba Foundation guest symposium 1959, Ciba Foundation study group 1971). From the conclusions drawn from the 1971 publication, it is clear that the participants were frustrated by the lack of knowledge on the underlying mechanisms of asthma and chronic obstructive pulmonary disease available at that time and a plea was made for further research. The clinical description of asthma that emerged from the 1959 publication of the symposium and later endorsed by the American Thoracic Society was a disease characterized by wide variation over short periods of time in resistance to flow in the airways of the lung. It is of interest to note that this description is almost identical to that of Salter's almost 100 years earlier.

The last decade has witnessed a dramatic increase in our understanding of the cellular and molecular basis of asthma, fuelled largely by the advent of fibreoptic bronchoscopy and its use to obtain airway surface fluid and mucosal biopsies. Since even in the mildest form of the disease airway inflammation was present, the World Health Organization/National Heart, Lung and Blood Institute (WHO/NHLBI) combined working group described asthma as:

A chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils and T lymphocytes. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and/or early in the morning. These symptoms are usually associated with widespread but variable airflow limitation reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli (National Institutes of Health 1995)

By attaching primacy to airway inflammation, with disordered airway function following on from this, there emerged two important new principles concerning asthma. Firstly, the disease is a chronic (often lifelong) disorder which, like other chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease, fluctuates in severity over time, sometimes with prolonged remissions. Secondly, therapy should be targeted towards preventing and/or reversing the inflammatory processes using appropriate environmental and pharmacological strategies. These principles have formed the basis of the national and international guidelines on asthma management, such as those produced by the WHO/NHLBI (National Institutes of Health 1995). Since airway inflammation is fundamental to modern thinking about asthma, it is worth reflecting on some of the common principles that underpin its importance to disease pathogenesis.
In all forms of asthma there appears to be a common set of local events leading to eosinophil-mediated airway injury. Irrespective of the underlying cause of asthma, much of the symptomatology results from the release of a wide range of biologically active molecules (mediators) with effects on airway smooth muscle, the microvasculature and nerves. For convenience these mediators can be divided into those released from primary effector cells and those secondary to the stimulation of other airway cells. The mast cell and eosinophil are thought to be the most important sources of autacoid mediators (i.e. those that have an effect distant from their site of release) although other cells, including monocytes and platelets, may contribute to the mediator pool in chronic forms of the disease. It seems reasonable to consider mast cells and eosinophils first and then those cells that are considered to orchestrate the inflammatory response.

**Mast cells**

Mast cells have long been known to play a key role in allergic tissue responses.

1. They have a key role in allergic tissue responses by releasing bronchoconstrictors, such as histamine, prostaglandin D₂ (PGD₂) and leukotriene C₄ (LTC₄).
2. They are derived from bone marrow precursor mononuclear cells in the tissue locality where they eventually reside.
3. The differentiation/maturation of mucosal mast cells requires mast cell growth factors, such as stem cell factor (c-kit ligand) produced by mesenchymal cells including fibroblasts.
4. There are two types of mast cell, differentiated on the basis of their neutral protease content: one found predominantly at mucosal sites containing tryptase alone (MC₃T); and the other also containing chymase and carboxypeptidase A in connective tissue (MC₃TC).
5. The MC₃T cell is thought to be the more important in asthma.
6. Full maturation requires additional factors, some of which are produced by lymphocytes.

Increased concentrations of mast cell mediators are recovered from the airways of asthmatics by bronchoalveolar lavage (BAL). In the presence of specific allergen, mast cells in asthma are activated for mediator secretion by the cross linkage of IgE bound to high affinity receptors expressed on the cell surface. This sets into train a series of membrane and intracellular biochemical events that culminate in the release of intracellular calcium from microsomal stores, and the activation of protein kinases that initiate non-cytotoxic degranulation and the activation of phospholipases for the generation of the newly formed mediators (prostanoids, leukotrienes and platelet-activating factor). Once activated, mediator secretion occurs rapidly and explains the acute bronchoconstriction characteristic of acute allergen exposure.
The mast cell in asthma is also sensitive to other stimuli. It is widely thought that asthma provoked by exercise occurs through mast cell activation triggered by the hypertonic airway lining fluid (Makker & Holgate 1994). Hypertonicity results from increased water loss from the airway to condition the inspired air to body temperature and full humidity. In asthmatic but not in normal airways mast cells will respond to other stimuli including inhaled hypotonic aerosols, adenosine and, in susceptible subjects, non-steroidal, anti-inflammatory drugs.

Allergen provocation has frequently been used as a laboratory model of asthma, not so much because of the early bronchoconstrictor response, but because many patients also experience a second wave of bronchoconstriction, referred to as the late asthmatic reaction (LAR) (Pepys 1973). In being accompanied by an increase in airway responsiveness to a wide variety of stimuli, such as histamine, methacholine and cold air, the LAR is a particularly interesting model which is considered to be closer to clinical asthma than the early reaction. During the LAR, measurements of BAL and bronchial mucosal biopsy at different time points during evolution and recovery (2–24 h) shows that:

1. it is dependent on the recruitment of leukocytes from the microvasculature (neutrophils at 1–6 h, eosinophils at 3–24 h);
2. neutrophils and eosinophils are recruited following the sequential up-regulation of molecules that enhance adhesion of leukocytes to vascular endothelial cells; and
3. endothelial adhesion molecules interact with specific receptors on the surface of passing leukocytes, resulting initially in rolling of the cells along the inside of the capillary walls, followed by tethering and cell activation.

Recent work suggests that mast cells are also an important source of cytokines — such as interleukin (IL)-4, IL-5, IL-6, IL-8 and granulocyte macrophage colony-stimulating factor (GM-CSF) (Bradding et al 1993) — that are important in the up-regulation of vascular adhesion molecules and the subsequent recruitment and activation of eosinophils (Casale et al 1996). With IgE triggering, these cells not only secrete preformed cytokines along with the more traditional mast cell products, but also produce newly formed cytokines which, in the case of IL-5 and tumour necrosis factor α (TNF-α), may persist for up to 72 h post-challenge (Okayama et al 1995, 1997). Acting in concert with other mast cell products, including the neutral protease tryptase, these cytokines most likely account for a major component of the LAR (Montefort et al 1994a). At the later time points the recruitment and activation of T helper (Th2)-type cells become increasingly important (Robinson et al 1993a). These mechanisms help explain the marked attenuation of antigen-induced late phase inflammatory responses in sensitized mice that are genetically mast cell deficient (Galli & Costa 1995).

**Eosinophils**

Although eosinophils at one time were thought to be protective in the allergic inflammatory response, they are now considered to be the cells that mediate much of
the pathology and disordered airway function which characterizes atopic and non-atopic asthma (Gleich 1996).

Of the proteins secreted by eosinophils, major basic protein (MBP) (Frigas et al. 1980) and eosinophil cationic protein (ECP) (Okayama et al. 1995) seem particularly active in rendering the epithelium more fragile and unstable. Eosinophil-derived neurotoxin destroys ribonucleic acid, while eosinophil peroxidase is a prodigious generator of active free radicals (Ayars et al. 1989). The mechanisms through which recruited eosinophils lead to epithelial damage in asthma are probably multiple. In addition to the direct cytotoxic effects of granule proteins on epithelial integrity, these cells also cause a non-cytotoxic loss of adhesion between the columnar (suprabasal) and basal cells of the epithelium (Montefort et al. 1992a), probably due to a targeted attack on desmosomes and tight junctions, the adhesion structures that are largely responsible for maintaining epithelial integrity (Montefort et al. 1992b). Explant and chamber studies indicate that eosinophils must enter into cell–cell contact with epithelial cells, probably involving an interaction between intercellular adhesion molecule (ICAM)-1 expressed on the surface of the eosinophil cell and a complementary ligand, called lymphocyte function antigen (LFA)-1, on the surface of the eosinophil (Herbert et al. 1993). Chemokine production by epithelial cells also seems important in directing transepithelial leukocyte migration (Calaral & Casale 1996). In addition to releasing arginine-rich proteins with the capacity to neutralize heparin and heparan cell matrix proteoglycans, eosinophils also release a 92 kDa metalloprotease (MMP-9) with broad activity against proteins of the intercellular matrix, including epithelial adhesion proteins.

Although IL-5 plays a key role in eosinophil recruitment (Egan et al. 1996), the mechanisms responsible for activating eosinophils in asthma are not well understood. Although they express a wide range of cell surface receptors, the precise process by which the eosinophil mediates tissue damage in asthma remains speculative. Priming of eosinophils for mediator secretion is important and results in a reduction in the threshold at which the cell responds to a range of activating stimuli. Cytokines such as IL-5 and GM-CSF seem particularly active in this regard and also prolong eosinophil survival by inhibiting programmed cell death (apoptosis) (Robinson et al. 1993b, Wooley et al. 1996). Chemokines are also important in eosinophil chemoattraction and priming, although they do not prolong eosinophil survival. A compelling hypothesis which is emerging is that IL-5, released from the airway cells into the circulation, recruits a population of eosinophils from the bone marrow which, when attracted into the lung, are especially susceptible to the chemoattractant properties of chemokines which then serve to direct their migration within the airways (Denburg 1996). Recent work also suggests that chemokines can induce eosinophils to generate their own cytokines, specifically IL-3, IL-5 and GM-CSF, which further prolong cell survival in an autocrine fashion.

Orchestration of airway inflammation: the role of T lymphocytes

Although it appears that all forms of asthma are characterized by mast cell- and eosinophil-mediated inflammation, it has become increasingly clear that the mucosal
immune system, and particularly the T lymphocyte, plays a key role in orchestrating this inflammatory response (Anderson & Coyle 1994). Most asthma in later childhood and in adults occurs in association with atopy, which is defined as the predisposition to generate IgE against common environmental (usually airborne) allergens. Of particular relevance are allergens derived from domestic dust mites (*Dermatophagoides* sp), proteins from domestic pets (especially cats), cockroaches, fungal antigens and pollens. To initiate the synthesis of allergen-specific IgE, the subject must first become sensitized, which involves the participation of cells that recognize, process and in turn present allergens to the T cell effector arm of the immune system. For domestic allergens this may occur early in life (Sporik et al 1990) and it possibly starts prenatally (Warner et al 1994, 1996), whereas occupational asthma of later onset is initiated by exposure to the offending agent(s) in the work place. In those genetically at risk of developing allergic diseases such as asthma and eczema circulating allergen-specific T cells can be detected at birth. These cells are thought to take up residence in the specific organ and begin to express the disease phenotype over the following six to 12 months. Such findings indicate that the mother is able to present antigens to her offspring via the placenta; for occupational antigens, initial exposure is likely to occur through the lung. Additional factors may also be important in this initial sensitization, including the capacity of the fetus to generate IL-4, the cytokine responsible for the maturation of the T cell population associated with allergy (to become the so-called Th2 type) and the isotype switching of B cells from IgM to IgE synthesis. During development the amnion is a particularly important source of IL-4, and secretion into the amniotic fluid may be an important determinant of prenatal programming. Once sensitized, the level of allergen exposure during the first year or so of life is a major determinant of the later development of asthma.

**Amplification of the inflammatory response**

Allergen-specific responses occur locally in the airways through the recruitment of a network of professional antigen-presenting cells called dendritic cells. These processes are summarized below.

1. Dendritic cells develop from mononuclear cell precursors in the presence of IL-3, stem cell factor and GM-CSF.
2. Dendritic cells present antigen peptides to naive T cells in local lymphoid tissue (thereby sensitizing them).
3. The clonal expansion of naive T cells directed towards the Th2 phenotype occurs in the presence of IL-4.
4. Antigen presentation involves: intracellular processing of the allergen to peptides; presentation to the T cell receptor of peptides in the groove of major histocompatibility complex class II (HLA) molecules on the surface of the presenting cells; and engagement of a number of accessory adhesion molecules.
A DISEASE OF INFLAMMATION AND REPAIR

Once committed and having returned to the airway mucosa, allergen-specific Th2-like cells respond to challenge with the generation of a number of cytokines encoded in the IL-4 cluster on the long arm of chromosome 5. The switching of B lymphocytes from IgM to IgE synthesis requires a cognate interaction with the T cells, contact between accessory molecules and the presence of specific cytokines (IL-4 or IL-13).

Once sensitized, the asthmatic airway will respond to further allergen exposure by rapid recruitment and expansion of the T cell population with elaboration of cytokines encoded in the IL-4 gene cluster (Robinson et al 1993). Molecular-based techniques, including in situ hybridization, reverse transcriptase (RT)-PCR and direct cytokine measurement from T cell clones, indicate the importance of this cell type in generating cytokines that support the inflammatory response in asthma.

In occupational asthma and in late onset 'intrinsic' (non-allergic) asthma, CD8+ T cells are also an important source of IL-4 and IL-5. Virus infection, an important cause of asthma exacerbations, also leads to expansion of both CD4+ and CD8+ T cells exhibiting the Th2-like phenotype (Fraenkel et al 1995, Coyle et al 1995).

Mechanisms of asthma severity and chronicity

There is overwhelming evidence to indicate that airway inflammation underlies the pathophysiology of asthma; however, its relationship to disease severity is less clear. Although the presence of eosinophils in the sputum, a persistent BAL fluid and blood eosinophilia with increased circulating levels of eosinophil granule proteins broadly relate to disease severity, these measures are too variable to provide clinically useful markers to predict the level of airway inflammation.

The selective recruitment of cells from the microvasculature with accompanying activation and enhanced survival underlies the ongoing inflammation in severe and chronic disease. Of considerable significance is the observation that asthma can be transferred to a non-asthmatic recipient by lung transplantation, even in the presence of sufficient immunosuppression to prevent lung rejection (Corris & Dart 1993). Since transplantation of normal lung into a previously asthmatic recipient fails to initiate asthma, it is likely that local factors in the lung, possibly immunological, are important in maintaining the chronic asthma phenotype.

Corticosteroids are highly effective anti-asthma drugs that act to reduce the inflammatory response. However, there are many patients in whom only partial relief is achieved, even when these drugs are taken by inhalation (and orally) at high doses. In a well-defined population of 'corticosteroid-resistant' asthmatics with preservation of β2-agonist bronchodilatation, abnormalities of circulating monocyte and T cell cytokine function have been described (Barnes & Adcock 1995). However, such patients represent only a minority of the 'difficult to control' asthmatics and the mechanisms underpinning corticosteroid refractoriness are probably multiple.
The majority of asthma occurs in association with atopy, the predisposition to generate IgE to common environmental allergens through a Th2 cell-dependent mechanism. In severe disease ongoing allergen-specific IgE production provides a rationale for allergen avoidance and high altitude treatment. However, environmental interventions have no effect on non-allergic asthma and many patients with severe atopic disease only partially or fail to respond (Djukanović et al 1995). Irrespective of atopy, powerful epidemiological studies have linked the total serum IgE to the presence of asthma and the level to disease progression (Burrows et al 1989). Moreover, there have been several long-term studies demonstrating that poorly controlled asthma progresses to an increasingly irreversible disorder (Peat et al 1987). Although airway inflammation has recently become accepted as an obligatory feature of asthma, increasingly, as with other chronic inflammatory diseases, tissue remodelling must now be considered as an integral component of the asthma phenotype.

It follows that the chronicity of asthma results from the dysregulation of cytokine networks leading to persistent inflammation, in structurally altered airways, which becomes refractory to treatment. Responsibility for disease progression does not lie with any single cellular element but embraces T and B cells, mast cells, eosinophils, endothelial cells, epithelial cells and myofibroblasts, which act co-operatively and also with the structural elements of the airway, including smooth muscle, matrix, microvasculature and nerves, leading to the variable phenotype characteristic of severe disease. This integrated view of asthma as a chronic disease of ongoing inflammation and repair leads us to incriminate a number of effector cells.

T cell activation and expression of mRNA for cytokines is a common feature of all types of asthma. In mild disease the level of T cell involvement in the airways is relatively low. The mast cell, with its capacity to respond to allergen provocation with mediator and cytokine release, alone is capable of recruiting and activating neutrophils and eosinophils. This might help explain why cromone-like drugs are more efficacious at the milder end of the asthma spectrum and in children with early disease (Barnes et al 1995). By contrast, a biopsy and lavage study of patients with severe asthma poorly controlled with corticosteroids reveals a vigorous ongoing T cell- and eosinophil-mediated inflammatory response in the absence of any known provoking factors (Djukanović et al 1995). Although in atopic asthmatics 24 h after allergen exposure there occurs a marked increase in IL-5 transcription in relation to T cell recruitment and activation (which supports the Th2 hypothesis) (Robinson et al 1993a,b, Shute et al 1995), in asthmatics of widely differing disease severity cloning studies have shown that airway T cells lavaged from the surface of the airways exhibit considerable heterogeneity of cytokine expression (Bodey et al 1997). At baseline, T cells from atopic asthmatic airways show strong expression of mRNA for IL-13, GM-CSF, γ-interferon (IFN-γ) and TNF-α; whereas upon allergen exposure there occurs a cytokine shift in favour of IL-3, IL-4, IL-5 and IL-13, and away from IFN-γ and TNF-α. Those factors that are responsible for the continuous T cell-mediated inflammation at baseline may be different from those brought into play on exposure.
to allergen. To explore this further, we have used a technique to evaluate the cytokine protein production by airway T cells stimulated with ionomycin and 12-O-tetradecanoylphorbol 13-acetate (TPA), in which monensin is used to inhibit Golgi-mediated cytokine transport, allowing flow cytometry to be used to quantify the population of CD3+ cells expressing a particular cytokine (Jung et al 1993). Even in clinically active asthma, 60% of asthmatic BAL T cells produced IFN-γ and/or IL-2, whereas only 2-4% of the cells accumulated IL-4 or IL-5, an observation that was not found in blood T cells obtained from the same patients (Krug et al 1996). It is becoming clear that, while a Th2-like response explains the induction and some of the inflammatory components of asthma (especially that associated with allergen exposure), little is known about T cell responses influencing disease chronicity in asthma and how they may escape corticosteroid suppression.

The role of IgE in chronic asthma

Although the ability of IgE to bind to mast cells and to mediate antigen-induced degranulation is clear, its role in maintaining chronic asthma is not fully understood. However, patients with chronic disease have raised levels of allergenspecific IgE in serum, and IgE, particularly in complex form, is capable of mediating the release of a range of cytokines via FcεRI, and FcεRII (CD23). Since both high and low affinity IgE receptors are up-regulated on eosinophils, mast cells, macrophages and dendritic cells in asthma, the opportunities for IgE to contribute to local inflammatory processes are numerous (Humbert et al 1996, Tunan-de-Lara et al 1996).

The question raised is whether the IgE is specific for allergens, or whether the spectrum of recognition widens during disease progression to include viral antigens and autoantigens, as recently described in chronic urticaria (Hide et al 1993). Past analysis of IgE specificities has been limited to serological investigation of mixed IgE but the new technology will allow the investigation of individual IgE molecules. It is also feasible to compare the molecular range of IgE found at the local sites of inflammation to that in the blood. Methods for amplifying the variable region genes used to encode IgE have been developed and already reveal an unexpected asymmetric usage of immunoglobulin VH genes in patients with asthma (Snow et al 1995). In the inflammatory environment, where there may be local release of cytokines possibly exacerbated by viral infection, it is conceivable that autoantigens may be released. IgE antibodies could therefore be generated against allergens, viral antigens or autoantigens. The high levels of IgE characteristic of chronic asthma could also induce autoantibodies against IgE itself (Shakib et al 1994). These could have an additional role in inflammation, either by cross-linking IgE on the mast cell surface or by generating immune complexes that stimulate mononuclear phagocytes to release cytokines.
Leukocyte recruitment and the role of the microvasculature

Exposure of the asthmatic airway to a wide variety of environmental stimuli results in the recruitment of leukocytes to the airways involving a well co-ordinated and dynamic sequence of events in which several cell adhesion molecules and chemotactic cytokines play a role (Lawrence & Springer 1991). In vitro lines of evidence predict a multistep model involving: (1) initial low affinity selectin molecule-dependent 'vascular rolling' (margination); (2) leukocyte activation by endothelial-derived chemoattractants (e.g. IL-8, monocyte chemotactic peptide [MCP]-1); and (3) a transition to β-integrin-dependent high affinity leukocyte adherence cytokine-mediated up-regulation of the Ig superfamily of adhesion proteins, ICAMs and vascular cell adhesion molecules (VCAMs); followed by (4) transendothelial migration involving combinations of cell adhesion molecules. 

Within 6 h of segmental allergen challenge of sensitized asthmatic airways, there occurs marked endothelial up-regulation of E-selectin and ICAM-1 accompanied by an influx of LFA-1+ leukocytes comprising neutrophils and eosinophils (Montefort et al 1994a, Pilewski & Albeda 1995). By 24 h there is a marked increase in activated T cells and eosinophils present in BAL with variable expression of VCAM-1, an adhesion molecule not normally constitutively expressed. Because leukocyte recruitment occurs so rapidly, the first step most probably involves up-regulation of P-selectin (by histamine) and E-selectin (by TNF-α) released from activated mast cells which mediate rolling through a lectin interaction with the ligand sialyl Lewis X on the leukocyte surface (Bochner et al 1994). The IgE-dependent secretion of newly formed TNF-α increases ICAM-1 expression (Okayama et al 1997), while its interaction with mast cell-derived IL-4 induces and stabilizes VCAM-1 expression (Galli & Costa 1995). These cell adhesion molecules interact with the integrins LFA-1 and very late antigen (VLA)-4 to recruit T cells and eosinophils selectively. Immunohistochemical studies on biopsies from severe asthmatics have revealed a marked up-regulation of ICAM-1 and VCAM-1 in the absence of allergen exposure and while they are taking high doses of corticosteroids. Thus, in severe asthma there is continued expression of endothelial cell adhesion molecules to promote ongoing leukocyte recruitment and activation. Such a mechanism would explain the finding of elevated circulating and BAL levels of soluble cell adhesion molecules in symptomatic asthma (Montefort et al 1994b).

The expression of E-selectin, ICAM-1 and VCAM-1 is controlled by the nuclear transcription factor NFκB, a p50/p65 heterodimer of which both subunits contain the 300 amino acid NFκB/rel/dorsal (NRD) domain. The N-terminal end of the NRD domain is involved in specific binding to DNA, whereas the C-terminal end contains the nuclear localization signal (NLS), a cluster of positively charged amino acids necessary for translocation of NFκB across the nuclear membrane (Manning et al 1994). NFκB binds to the decameric DNA sequence 5'-GGANNCCTC-3' found in the promoters of a number of genes that are up-regulated in inflammation, especially in those that encode the cell adhesion molecules and specific cytokines (IL-2, IL-6, IL-8,
members of the IL-4 gene cluster and TNF-α). Within the E-selectin promoter there are three closely spaced binding sites for NFκB clustered within a 40 bp segment and two additional regulatory elements, NF-ELAM-1 and NF-ELAM-2 (nuclear factors for the endothelial leukocyte adhesion molecule). Following cytokine exposure, all three NFκB sites are essential for maximal promoter activity. The promoter for the human ICAM-1 gene contains binding sites for Sp1, AP-1, AP-2, AP-3, NFκB and a putative silencer, whereas NFκB alone mediates VCAM-1 expression.

A range of factors have been shown to initiate activation of NFκB, including TNF-α, IL-1, IL-2, leukotriene B₄ (LTB₄) and viruses. Endothelial cells express a cytoplasmic inhibitor of NFκB activity, IκBα, the over expression of which inhibits E-selectin and VCAM-1 transcription. IκBα binds selectively to NFκB heterodimers and prevents its nuclear uptake by binding to the NLS (Manning et al 1994). A pathway of NFκB activation has been proposed that sequentially involves phosphorylation of IκBα, followed by its specific chymotryptic proteolysis, which reveals the previously masked NLS site and nuclear translocation signal (Baeurle & Henkel 1994). The activation is only transient as NFκB is also able to induce IκBα mRNA transcription resulting in re-accumulation of IκBα and its functional inhibition of cytoplasmic NFκB. Reactive oxygen intermediates serve as second messengers of NFκB activation, redox changes leading to activation of chymotryptic IκBα protease through modification of intracellular serpins (Manning et al 1994, Baeurle 1991). These intracellular events provide unique opportunities to investigate NFκB activation in severe asthma as it relates to increased cell adhesion molecules and cytokine expression, and to investigate pharmacological intervention with potential therapeutic significance. Activation of NFκB also provides one explanation for how air pollutant oxidants (O₃, NOₓ, particulates, cigarette smoke) and respiratory viruses may exacerbate asthma by enhancing ongoing inflammatory pathways.

The bronchial epithelium

The bronchial epithelium has been viewed traditionally as a passive barrier that serves as a target for the inflammatory response. However, it is also an important source of inflammatory mediators including arachidonic acid products, endothelin-1, nitric oxide (NO) and cytokines which, along with altered adhesion molecule expression, participate directly in inflammatory cell recruitment and activation.

Arachidonic acid metabolism

The epithelium is a major source of 15-HETE (15-hydroxy-6,8,11,14-eicosatetraenioc acid) and, although expression of immunoreactive 15-lipoxygenase is unaltered (Bradding et al 1995), others have shown increased enzyme activity in severe disease (Shannon et al 1993). Although 15-HETE and 15-dihydroxy acids exhibit some mediator functions, more active oxidate products of arachidonic acid are the prostaglandins PGE₂ and PGF₂α with their opposite actions in bronchial smooth
muscle. The epithelial expression of the inducible form of cyclooxygenase (Cox2) is enhanced over the constitutive form (Cox1), a change that is suppressed by corticosteroid treatment in parallel with their clinical efficacy (Springall et al 1995). Over expression of Cox2, NO and ET-1 molecules under the control of NFkB persists in asthma poorly controlled with corticosteroids.

**Endothelin**

Human endothelin comprises three structurally distinct 21 amino acid peptides, ET-1, ET-2 and ET-3, encoded on separate genes. In addition to its potent vasoconstricting property, ET-1 is a powerful spasmogen of airway smooth muscle mediated through the ETB receptor subtype (Mattoli et al 1990). ET-1 is also a mitogen for airway smooth muscle and in fibroblasts it is a chemotactant, is mitogenic and provides an activating signal for collagen synthesis, again mediated through ETB receptors (Springall et al 1991). ET-1 induces collagenase production and is important in myofibroblast-mediated contraction of granulation tissue.

Human bronchial epithelial cells cultured from the airways of asthmatics secrete increased amounts of ET-1, which is sensitive to inhibition with corticosteroids (Mattoli et al 1990). ET-1 immunoreactivity *in vivo* is also increased in the epithelium (Springall et al 1991). In BAL, levels of ET-1 are increased in proportion to the resting level of airflow obstruction (Redington et al 1995a). The importance of ET-1 as a novel bronchoconstrictor is revealed by its capacity to reduce FEV1 (forced expiratory volume in one second) by \( \geq 20\% \) of baseline at inhaled concentrations of \( 10^{-10} - 10^{-12} \text{ M} \). With effective corticosteroid treatment of asthma, both lavage ET-1 levels and ET-1 expression in the epithelium return to those found in normal subjects.

**Nitric oxide**

NO is a short-lived, highly soluble free radical that plays a major role in cell-cell communication. It is generated from L-arginine by NO synthase which exists in both constitutive and inducible isoforms (iNOS). Both enzymes require NADPH as a cofactor and are inhibited by L-arginine analogues such as NG-nitro-L-arginine (L-NNA) and NG-monomethyl-L-arginine (L-NMMA). The inducible form is also selectively inhibited by aminoguanidine (Barnes & Liew 1995), and it immunolocalizes strongly to the epithelium in bronchial biopsies from asthmatics but only rarely in those from normal controls (Hamid et al 1993). Consistent with enhanced NO generation in airway mucosal inflammation is the increased NO detected in exhaled air active asthma and rhinitis (Alving et al 1993, Kharitonov et al 1994). *In vitro* iNOS is induced in response to IFN-\( \gamma \), IL-1\( \beta \) and TNF-\( \alpha \) and it is inhibited by corticosteroids (Barnes & Liew 1995). In corticosteroid-responsive asthma iNOS in the epithelium is down-regulated and associated with a reduction in exhaled NO. Whilst NO produced by iNOS is a powerful vasodilator at nanomolar
concentrations, it is also cytotoxic because it forms peroxynitrate and hydroxyl radicals and nitrosylating key mitochondrial enzymes. In severe asthma the epithelium is likely to be a major source of toxic levels of NO and as such may provide a novel surrogate marker of disease activity and response to treatment (Barnes 1995).

Cytokines

Human bronchial epithelial cells in vitro constitutively synthesize and release IL-1β, IL-6, IL-8 and GM-CSF, with greatly enhanced production occurring on exposure to IL-1β or TNF-α. Enhanced release of these cytokines has also been reported in asthmatic epithelial cells in vitro. Application of immunohistochemistry shows that the bronchial epithelium in asthma is a particularly rich source of IL-1β, IL-8 and GM-CSF.

IL-8, a member of the α(C-x-C) chemokine family, is particularly important in the expression of chronic and severe disease, although it is usually regarded as a neutrophil chemoattractant. IL-8 can induce human eosinophils from asthmatic subjects to secrete IL-3, IL-5 and GM-CSF. In a nasal polyp explant model of chronic mucosal inflammation, IL-8 and GM-CSF are the dominant cytokines produced (Park et al 1995).

In bronchial biopsies from asthmatics and in the peripheral circulation IL-8 binds strongly to IgA (Shute et al 1995). Despite clear immunostaining for IL-8 in the epithelium, no free IL-8 can be detected in detergent-extracted homogenates; however, IL-8-IgA complexes can be readily detected with significantly more being presented in allergic asthmatics. In patients with chronic severe asthma, both free and complexed forms of IL-8 are present in mucosal tissue, serum and sputum. Thus, although IL-8 can be formed by many cells in asthma, the bronchial epithelium seems to be the major site of production and concentration of this chemokine. Of particular interest is the finding that IL-8 co-localizes with secretory IgA in the epithelium. Secretory but not serum IgA is able to up-regulate the eosinophil chemotactic response to IL-8, reaching optimum at 10⁻¹⁰ M; whereas, under the same conditions, the neutrophil response is inhibited (Shute et al 1995). IL-8 is also known to complex carbohydrate residues of proteoglycans and glycoproteins, resulting in greatly enhanced eosinophil-specific properties. These include the secretory piece of IgA containing up to 20% N-linked oligosaccharides and proteoglycans, such as the granule products of mast cells and eosinophils, and CD44, which is expressed in greater amounts in the asthmatic epithelium (Peroni et al 1997, Lackie et al 1997). We hypothesize that IL-8 binding regulates the activity of and changes the target cell specificity for this cytokine, rendering it a potent attractant and activator of eosinophils.

Recently, a range of other chemokines belonging to the C-C class have been shown to be generated by human bronchial epithelial cells including RANTES (acronym for regulated on activation, normal T expressed and presumably secreted), MCP-1, macrophage inflammatory protein (MIP)-1α (Berkmann et al 1996) and a recently
Bronchial epithelium

Tight junction
Desmosome

Hemi-desmosome
Basal cells

Damage
Creola body

Barrier loss: Macromolecular permeability +++

First response

Interim repair

Differentiation