Leading Article

MECHANISMS OF ASTHMA: IMPLICATIONS FOR TREATMENT*

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In his treatise (1868) entitled *On Asthma, Its Pathology and Treatment*, Dr Henry Hyde Salter, a physician at Charing Cross Hospital in London, described asthma as 'paroxysmal dyspnoea of a peculiar character generally periodic with healthy respiration between attacks'.[1] His astute clinical observations regarding obstruction to the airways and its reversibility relates to his personal experience as an asthma sufferer and to an analysis of the few cases of the disease that he was able to find in several London teaching hospitals at that time. Almost 100 years later a Ciba Foundation Guest Symposium was convened in an attempt to define asthma and at the end of the deliberations the participants were a little further on in describing asthma as 'a condition characterised by widespread airway narrowing varying in calibre over short periods of time either spontaneously or in response to treatment'.[2] Further consideration was given to this at a Ciba Foundation Study Group in 1971, but the conclusions of those who participated was that there was insufficient information for a clear definition to be agreed.[3]

Our general failure to understand the underlying cellular processes of asthma has been the result of the great difficulty in gaining access to airway tissue in living patients with this disease. Indeed, until this decade, most research in asthma had concentrated on the physiology and pharmacology of the airways rather than on the factors underlying their disordered function. The availability of instruments to measure airway function has provided methods for objective monitoring of asthma and has had the effect of raising physicians' awareness of the chronicity of the disease and the frequently observed disparity between symptoms and objective measures of air flow. The presence of airways hyper-responsiveness measured either directly with agents such as histamine and methacholine or indirectly with stimuli such as exercise and cold air led to the American Thoracic Society in 1962 incorporating this component into its definition of asthma.[4] The ability of the asthmatic airway to respond in an exaggerated manner to constrictor agents such as histamine has been known since 1946.[5] However, while broadly indicating the severity of disease across populations,[6] studies in which bronchial provocation has been undertaken repeatedly over prolonged periods have failed to indicate a close relationship between the level of bronchial hyper-responsiveness and disease severity.[7,8] However, the overall concept of airway hyper-responsiveness is useful since it provides a plausible mechanism to explain the paroxysmal symptomatology of asthma and its link with exogenous trigger factors. The use of other bronchial provocation tests with stimuli such as hypotonic and hypertonic saline, exercise, cold air, adenosine and its 5′-monophosphate,

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propranolol, bradykinin, sulphur dioxide and sodium metabusulphite, has broadened the concept of bronchial hyper-responsiveness to include indirect mechanisms involving the release of bronchoconstrictor mediators from nerves and inflammatory cells. There remains the important question as to *why* asthmatic airways show a propensity for becoming more easily obstructed in response to these different stimuli.

Inflammation as the basis of asthma

In his first edition of the *Principles and Practice of Medicine*, published in 1892, William Osler refers to asthma as 'a special form of inflammation of the smaller bronchioles-*bronchiolitis exudativa* (Curschmann)' which he differentiated from 'spasm of the bronchial muscles'.[9] At the turn of the century, Schmidt, Fraenkel and Ellis described the clinical and pathological features of severe asthma,[10–12] however it was not until the 1960s that Dunnill and co-workers undertook an exhaustive study of the cellular components of the airways in asthma death.[13] They highlighted the presence of excess luminal secretions, epithelial damage, hypertrophy and hyperplasia of goblets cells and submucous glands, thickening of the epithelial basement membrane region and infiltration of the airway wall with a mixture of mononuclear cells and granulocytes (especially eosinophils). Thus a clear picture began to emerge which explained the pathological processes that lead to death from asthma. However, the technology, was not available to translate these findings to asthma in life. The development of new methods to measure different aspects of lung function and the effects of bronchodilator drugs had the effect of directing physicians' attention to airway smooth muscle as a focus for the disease. The description of bronchial hyper-responsiveness further served to direct attention in asthma towards the abnormal behaviour of airway smooth muscle rather than to the underlying reasons for this dysfunction. The concept of viewing asthma as episodic bronchospasm provided a major incentive for the pharmaceutical industry to develop bronchodilator drugs. While this was happening a number of disparate observations about asthma were made. Throughout the last half century, an association was noted of a blood and sputum eosinophilia with asthma, the presence of tenacious plugs of secretions containing clumps of epithelial cells (Creola bodies) from the airways during the recovery phase of acute severe asthma, and the increasing recognition that the disease frequently occurred in association with other atopic diseases. This provided clues that asthma in life extended beyond disordered smooth muscle function. A clearer understanding of this has been slow to emerge.

A breakthrough came with the application of bronchoscopy to the study of asthmatic airways. Although the rigid bronchoscope had been used in some studies of asthma,[14] the need for anaesthesia and risks of bronchoconstriction precluded its widespread use. Fibreoptic bronchoscopy provided the way forward for obtaining lavage and mucosal tissue specimens from asthmatic airways, thereby enabling a detailed study of the cellular events in the airways of patients with mild-moderate disease. Analyses of bronchoalveolar lavage fluid indicated that asthmatic airways were subject to an inflammatory response involving eosinophils, mast cells and mononuclear cells, and that the disordered airway function was associated with the secretion of preformed and newly generated bronchoactive mediators. Fibreoptic bronchoscopy proved an invaluable technique to obtain small mucosal biopsies under direct vision for detailed histochemical

analysis.[15] Initial studies confirmed the view that, even in mild asthma, the airways were infiltrated with activated mast cells, eosinophils and T cells.[16–18] Thus, a picture emerged of asthma as a chronic inflammatory disorder which underlay the disordered airway physiology and clinical symptomatology of the disease.

Allergens as an important cause of asthma

While it had long been known that asthma could be provoked by inhaling respirable materials, the reasons for this had to await the astute observations of Dr Charles Blackley, a general practitioner in Manchester who suffered from rhinitis and asthma. In his treatise *Catarrhus Aestivus* published in 1873, he describes careful experiments which linked increased pollen counts across the spring and summer to the occurrence of his own upper and lower airway symptoms.[19] In 1880 he was the first to report the allergen-induced skin wheal on introducing pollen into the skin with a lancet.[20] Almost a century passed before Voorhorst and colleagues finally showed that the domestic house dust mite (*HDM—Dermatophagoides pteronyssinus*), which has since been the subject of much research and practical interest, was the major allergenic cause of perennial asthma.[21] It is now recognised that the faecal particles of HDM are the source of most of the allergenic activity in asthmatic airways. Seven groups of allergens have been identified with HDMs, the first four of which are known to exhibit proteolytic or other enzymic activities.[22] For example Der P1, the major allergen of *D. Pteronyssinus*, is a cysteine protease derived from the mite's gastrointestinal tract, whereas Der P2 is lysozyme and Der P3 a chymotryptic enzyme. The potent biological activities of these and other allergens might explain their ability to penetrate epithelial surfaces so easily and lead to specific sensitisation.

Genetic factors

Recent studies have emphasised the importance of early life factors in the development of dust mite and other forms of allergy related to asthma. It has long been known that asthma and allergies run in families, although the genetic basis for this has been elusive.[23] Considerable controversy still exists over the mode of inheritance of atopy and asthma, probably because multiple genes are involved and environmental factors play such an important role. Nevertheless, in Oxford, Cookson and colleagues have suggested that atopy is inherited as an autosomal dominant trait and that a genetic abnormality exists on the short arm of chromosome 11 close to the centromere (11q13).[24,25] A locus coding for the β-chain of the high affinity IgE receptor ($Fc_\varepsilon R1$) has been identified and linkage of atopy to this is manifest most strongly when the gene is inherited through the mother.[26] A specific abnormality on the intramembrane portion of the β-chain (Leu 181) of $Fc_\varepsilon R1$ also has been demonstrated.[27] At least five other groups who have studied somewhat smaller numbers of families have failed to confirm linkage of IgE hyper-responsiveness to 11q13.[27] Our own study of 131 random families also failed to show any evidence for a dominant pattern of inheritance for atopy, or linkage of log IgE to markers around the β chain of $Fc_\varepsilon R1$ and found no support for the maternal effect on this genetic trait.[28] If polymorphisms involving $Fc_\varepsilon R1\beta$ are responsible for the increased expression of atopy, then they are likely to be uncommon when compared to almost 40 per cent of the population who express specific IgE to common allergens.

The major cytokines involved in allergic disease are encoded on the long arm

of chromosome 5; IL-4, IL-13, IL-5, GM-CSF, IL-6, IL-9, IL-12, interferon regulatory factor 1 (a transcription factor which inhibits γ-IFN expression), the β_2 adrenergic and corticosteroid receptors are all relevant to asthma. Recognising this we have taken the opportunity to probe this region of the genome in our 131 families. We have found evidence for at least two major genes linked to IgE hyper-responsiveness and, using two somewhat uncommon polymorphisms of IL-9, have shown allelic association between these and log IgE ($p<0.00034$).[29] Clearly this does not tell us what the genetic abnormality is on chromosome 5, but adds to the recent report showing strong evidence for linkage to a gene related to IL-4[30] and another reporting a linkage between markers close to both IL-4 gene cluser and β_2 adrenoceptor and indices of atopy (IgE) and asthma.[31]

The genetics of atopy are further compounded by important associations between IgE responses to specific allergens and the HLA system. Thus, in addition to regulatory loci, there are significant associations between particular HLA Class II DR and DP phenotypes and allergic IgE and IgG responses to environmental allergens including ragweed, rye grass pollens and house dust mite.[32] In parallel studies, HLA-DRB1, DRB3, DRB5 and DPB1 gene products restrict the recognition of HDM allergen determinants by components of the T cell repertoire.[33] Propagation of an immune response involving allergens requires the generation of specific IgE. Selected peptides of the allergen are presented in the cleft of the MHC Class II complex to the T cell receptor (TCR). The linkage between specific sequences on the α chain of the TCR encoded on Chr 14 and the allergic phenotype[34] adds a second measure of complexity to the genetics of atopy (and asthma).

Atopy is certainly not synonymous with asthma. The reasons why the lung should be selected as the target organ for the expression of the atopic phenotype is not known, nor are those factors (genetic or otherwise) which determine the severity of the disease. One particularly interesting finding is that certain polymorphisms of the β_2-adrenoceptor are found more commonly in asthmatics with severe disease.[35]

Early life environmental influences

Irrespective of genetic factors, exposure to environmental agents is clearly of major importance in the development of asthma. Studies linking the month of birth to the development of specific allergies point to early allergen exposure as a risk factor for sensitisation in children genetically at risk. In collaboration with Tom Platts-Mills (Charlottesville, Virginia, USA) we have shown that the level of exposure to mite allergen in the first year of life determines whether or not a child born of atopic parents develops asthma and airways hyper-responsiveness by the age of 11 years.[36] Moreover, the age of onset of first wheezing in these children correlated with the level of HDM Der P1 exposure.

Maternal smoking and maternal nutrition also seem to be important factors determining the IgE status of the newborn child. Infants whose mothers smoked in pregnancy have reduced lung function and raised cord blood IgE levels when compared with babies whose mothers did not smoke.[37] Studies both in experimental animals and humans have indicated that fetal undernutrition during critical periods in early development can have lifelong effects on structure, physiology and metabolism (fetal programming).[38] In an analysis of 825 men aged 60–70 years in Hertfordshire, who had detailed anthropometric measure-

ments made at birth, both those who were symmetrically small at birth and those with a low birth weight, had a reduced FEV_1 in adult life. These data are consistent with the concept that fetal lung development is of central importance for the subsequent expression of respiratory illness.[39]

In experimental animals the thymus is one of the organs most sensitive to fetal and neonatal undernutrition. Its size and DNA content is permanently reduced by transient maternal undernutrition during pregnancy and early lactation.[40] Compared to experimental animals, the human fetus completes a greater proportion of its growth *in utero* and the effects of intrauterine growth failure are more severe.[41] Our hypothesis is that the imbalance of T helper ($CD4^+$) lymphocytes associated with IgE production to common environmental antigens may be the result of impaired thymic maturation during a critical period in fetal development. To determine whether a long term alteration in immune function and atopy may be associated with either impaired or disproportionate fetal growth, we have studied the serum IgE concentration in 280 men and women born in Preston between 1935 and 1943, whose size at birth had been measured. Compared with subjects with a normal serum IgE level, subjects with elevated IgE concentrations above 80 IU/ml had, on average, a 0·30 inch larger head circumference at birth, despite similar birth crown–heel lengths. This indicates that *in utero* those subjects with a raised IgE had experienced disproportionate growth of the head in relation to the trunk and limbs. The effect was independent of the mother's pelvic size and parity, and of adult physique, social class and smoking, and was similar in men and women. It was also independent of gestational age at birth, although the highest prevalence of a raised IgE occurred in babies with a large head circumference who were also postmature. We have now found that the same pattern of disproportionate fetal growth is related to IgE levels and positive skin prick tests in 12 year old Southampton children. Moreover, in a pilot study we found a raised cord blood eosinophil protein X (EPX) (also known as eosinophil derived neurotoxin and indicative of fetal eosinophil activation) correlates positively with head circumference at birth. This provides further evidence that disproportionate growth is associated with fetal immune and allergic inflammatory responses.

One possible explanation for our findings is that increased growth factors associated with nutrient surplus preferentially increase the Th-2 lymphocyte population (*vide infra*). In mice lymphoid irradiation leads to a dominant Th-2 response as a result of a selective reduction in the negative feedback provided by a Th-1 cell population[42,43] which suggests that Th-2 dominance may result from a greater sensitivity of Th-1 cells to adverse environmental stimuli (Fig 1). An adverse intrauterine environment with fetal undernutrition throughout gestation is associated with global impairment of thymic development and reflected in the increased susceptibility to infection of low birth weight infants.[41] The effects of fetal undernutrition late in gestation depend on the fetal growth trajectory set in the earlier stages of pregnancy. The most rapidly growing fetuses, indicated by larger head size, experience prompt slowing of growth if exposed to undernutrition, while those growing more slowly are unaffected.[41] In those on a fast growth trajectory, a 'brain-sparing' reflex[44] redistributes blood and nutrients to the brain at the expense of the trunk and limbs, resulting in a disproportionate fetus. Under such circumstances, the thymus is severly reduced in weight, the functional significance of which is unknown. Indeed, in the post-term fetus,

while most organs simply experience deceleration in growth, there is marked wasting of the thymus.[41] The reduced thymic weight found in both disproportionate and postmature fetuses parallels our observations of a higher prevalence of a raised IgE in these groups. Ecological evidence points to a strong environmental component in the aetiology of atopic diseases and our finding of an association between accelerated but disproportionate fetal growth and a raised IgE is compatible with the higher prevalence of atopy in more affluent socioeconomic groups.[45] Although the increase in head circumference at birth over recent decades parallels the rising trends in atopic diseases, there is insufficient evidence to distinguish whether or not there is a superimposed adverse stimulus late in gestation as suggested by our observations. Our hypothesis is that disproportionate fetal growth and postmaturity may be associated with atopic disease as the result of a diminished cytokine influence of Th-1 lymphocytes due to impaired thymic maturation during a critical period late in fetal development.

The implications of these findings for the prevention of allergic disease are profound. Arshad and co-workers have shown that early avoidance of dietary allergens (cow's milk and egg) and measures taken to reduce domestic mite allergen levels in the home when applied to babies born of atopic mothers had a dramatic effect in reducing the prevalence of eczema and episodic wheezing.[46] Whether the beneficial effect is sustained throughout childhood can only be answered with further follow-up studies. Thus, while not definitively proven, there is increasing evidence that intrauterine nutrition and early life exposure to allergens are critical factors in determining the level of sensitisation and the subsequent development of allergic disease. At a cellular level are the recent findings that T-cells isolated from the cord blood of babies that subsequently develop atopic dermatitis and/or asthma have greater proliferative responses to allergen stimulation with lower interferon-γ (IFN-γ) production and detectable mRNA expression of IL-4 at birth to foods (egg and milk proteins) and/or inhalant allergens.[47] Since cytokines are critically involved in the allergic tissue response (Fig 1), IL-4 being responsible for isotype switching of B cells to IgE synthesis and for the maintenance of the Th2 lymphocyte subpopulation,[48] and IFN-γ serving to oppose the actions of IL-4,[49] a possible mechanism is provided for early expression of the atopic phenotype in these at risk infants.

Adjuvant factors in sensitisation of the airways

Maternal smoking both before and after birth has been shown to be a consistent risk factor for developing respiratory disease early in life. Exposure to environmental tobacco smoke has been shown to increase IgE in adults[50] and some studies,[51] though not all,[52] have suggested that maternal smoking in pregnancy increases cord blood IgE and the subsequent risk of atopic disease. It is interesting to note that cigarette smoking later in life also elevates serum IgE and that this has a synergistic effect with the direct effects of cigarette smoking in accelerating the decline in pulmonary function with age.[53] An adjuvant effect of cigarette smoking on the development of occupational asthma related to such sensitising agents as acid anhydrides and platinum salts further indicates an important interactive effect of tobacco smoke with the development of respiratory tract allergen sensitisation.[54] The mechanism(s) responsible for this adjuvant effect may be exerted through a direct destructive effect of cigarette smoke on the bronchial

epithelium, thereby facilitating access of inhaled antigens to the mucosal immune system, or through influencing the mucosal immune system itself, by facilitating antigen presentation or by biasing T cell differentiation towards the Th2 phenotype (*vide infra*).

Other adjuvant factors implicated in the early life origins of asthma include respiratory tract virus infections particularly respiratory syncytial virus (RSV). In a mouse model, Openshaw and colleagues have shown that two virulence proteins in the virus capsid, designated F and G, are able to bias T cell development either along the Th1 pathway (F protein-driven) or the Th2 pathway (G protein-driven) with only the latter being associated with an allergic (eosinophil mediated) inflammatory response with an adverse outcome.[55] There is some evidence that infection with RSV in early life is a predisposing factor for the development of IgE hyper-responsiveness[56] and asthma but, until studies on T cells similar to the mouse studies described by Openshaw can be demonstrated in humans, this remains speculative.

Another important effect of respiratory tract viruses is to damage the bronchial epithelium, thereby augmenting the penetration of the airway mucosa by inhaled allergens. The suggestion that certain air pollutants (e.g. passive cigarette smoke and NO_2) impair the lower respiratory tract's capacity to resist virus infection, provides a link between two environmental factors that predispose the airways to becoming sensitised to specific allergens.[57] Exposure to environmental air pollutants such as ozone, sulphur dioxide and oxides of nitrogen has been shown in humans to augment allergen sensitisation of the lower respiratory tract.[58,59] Some studies indicate that at high ambient concentrations, air pollutants can lead to airway damage and enhanced responses to allergens.[60]

Cellular biology of allergen sensitisation

Atopy is the strongest identified risk factor for the development of asthma. Twelve years after the demonstration by Block and Massini in 1909 that local sensitisation to *trichophyton* could be passively transferred, Prausnitz and Küstner demonstrated the capability of serum from a sensitised individual to mediate an immediate hypersensitivity to a specific allergen of the localised recipient site of passive transfer.[61] It took a further 46 years before the Ishizakas identified this 'reaginic' activity as immunoglobulin E.[62]

The most important mechanism through which IgE determines the expression of atopy is its binding to high affinity receptors ($Fc_\varepsilon R1$) expressed on the surface of tissue mast cells and basophils and to lower affinity receptors ($Fc_\varepsilon R2$ or CD23) on macrophages, eosinophils and platelets. Cross linkage of IgE with specific allergen results in the non-cytotoxic release of an array of preformed and newly generated mediators of inflammation. For the mast cell, these include histamine, tryptase, prostaglandin $(PG)D_2$ and leukotriene $C_4(LTC_4)$ (a component of SRS-A) which, through their receptor-mediated effects on airway smooth muscle and microvasculature, are responsible for the allergen-induced early response (EAR).[63]

Understanding of the regulation of IgE synthesis by lymphocytes has increased much in the last five years. Interleukin-4 is a key cytokine involved in the isotype switching of B cells from synthesising IgM and IgG to IgE in a sequence of intracellular events and the transient generation of germline mRNA transcripts.[47] IL-4 interacts with B cells via specific cell surface receptors which

exist in both high and low affinity forms. An important accessory signal for IgE switching is provided by CD40 on T-cells signalling through CD40 ligand on B cells. Recently a second cytokine, IL-13, which exhibits 30% homology with IL-4, has also been shown to mediate IgE isotype switching through its own specific receptors but, unlike IL-4, it is also a differentiation factor for dendritic cells.[64] In the reverse direction, switching of B cells to IgE synthesis is potently inhibited by interferon-γ and -α from Th1 cells and monocyte/macrophages.[65]

It is now recognised that in human allergic disease epitopes on allergen molecules are recognised by dendritic cells and subsequently presented as fragments to T cells involving MHC Class II molecules and T cell receptors. This interaction in the presence of IL-4 results in the differentiation of T cells along the Th2 pathway with upregulation of the IL-4 gene cluster on Chromosome 5. Apart from controlling IgE synthesis, IL-4 (but not IL-13) is an obligatory cytokine for the development and maturation of the Th2 lymphocyte phenotype.[42] Interleukin-6 and a factor from fibroblasts designated stem cell factor (or *c-kit* ligand) are involved in the growth, differentiation and regulation of mast cells,[66] IL-3 is an autocrine growth factor for basophils,[66] interleukin-5 is a growth, differentiation and priming factor for eosinophils[67] while GM-CSF delays eosinophil programmed cell death (apoptosis). Together these cytokines are able to direct an inflammatory response towards that driven by IgE-dependent mast cell activation and eosinophil recruitment.

In contrast to the Th2-subtype of lymphocyte, Th1 cells develop in the presence of a different range of antigens associated with the delayed type hypersensitivity response. Thus, in diseases such as tuberculosis, sarcoidosis and leproid leprosy, antigen specific T cells (Th1 cells) generate predominantly interferon-γ, IL-2, TNFβ together with variable amounts of IL-3 and GM-CSF.[49] While antigen-specific T-cells with characteristics of both the Th1 and Th2 phenotype exist in bronchial biopsies and in bronchoalveolar lavage taken from the airways of asthmatic subjects, the dominant cytokine repertoire identified at the level of mRNA transcription using *in situ* hybridisation and reverse transcriptase polymerase chain reaction (RT-PCR) suggests dominance of the Th2-like phenotype[68,69] (Fig 1).

Cell biology of airway inflammation in asthma

Once sensitised the lower respiratory tract responds to inhaled allergens in a highly specific manner resulting in widespread airway obstruction and hyperresponsiveness. Almost 30 years ago Pepys showed that when sensitised subjects inhaled specific allergen it caused both early (5–15 minutes-EAR) and late (2–6 hours-LAR) bronchoconstrictor responses that last approximately 60 minutes and 12–24 hours respectively.[70] Later studies by Cockroft and colleagues showed that the LAR was accompanied by an acquired increase in bronchial responsiveness to such stimuli as inhaled histamine and methacholine.[71] Because hyperresponsiveness is an important component of airway dysfunction in naturally occurring asthma, these findings have attracted considerable attention as models for studying pathogenetic mechanisms.

Measurement of mediators in the peripheral blood and bronchoalveolar lavage fluid and their metabolites in urine has shown that the EAR is a mast cell-dependent response resulting from the IgE-dependent secretion of constrictor substances. Acting through specific receptors, these mediators contract airway

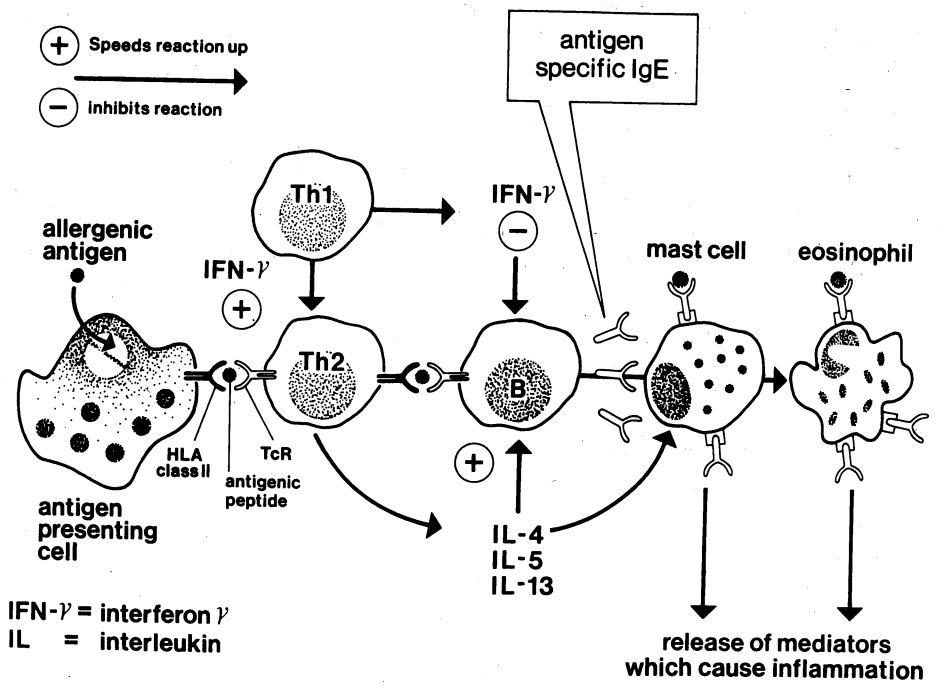

FIGURE 1

Schematic representation of the inflammatory cascade in asthma demonstrating the role of cytokines in regulating T cell, B cell, mast cell and eosinophil function.

smooth muscle, stimulate afferent neurones and increase microvascular leakage. The type of mast cell in this reaction predominantly contains tryptase as its neutral protease. Tryptase exists as a tetramer of molecular weight 130,000 daltons and constitutes 20 per cent of the total granule protein of the mucosal mast cell.[72] Amongst its biological actions, tryptase is able to produce a prolonged increase in microvascular permeability, to upregulate adhesion molecules, attract and activate eosinophils and to augment epithelial and fibroblast proliferation.[73] We have shown that tryptase has important physiological effects on tissues, including upregulation of adhesion molecules, proliferation of epithelial and endothelial cells and prolonged oedema. Histamine produces most of its airway effects via H_1 receptors which are present both on airway smooth muscle and on the microvasculature, while PGD_2 and its immediate metabolite $9\alpha 11\beta$-PGF_2 contract airways smooth muscle by interacting with thromboxane (TP1) receptors. Once released from mast cells, LTC_4 is rapidly metabolised to LTD_4 and subsequently to LTE_4, the three sulfidopeptide leukotrienes comprising the smooth muscle contractile and vasoactive properties of the biological activity previously described as slow reacting substance of anaphylaxis (SRS-A).[74]

Whereas the allergen-induced late reaction in the skin and airways has been considered to have an inflammatory basis, the underlying cellular basis for this has been controversial. For some time it has been known that during the LAR there appear in the circulation neutrophil and eosinophil chemoattractants that have been characterised physicochemically but not structurally. During the LAR, circulating eosinophils exhibit characteristics of cell activation including an

increased expression of specific cell surface markers. Cookson and co-workers demonstrated a transient decrease in the circulating eosinophil count, approximately two hours before the onset of the LAR.[75] When taken with the observation of an increased lavage eosinophilia 24 hours after challenge,[76] the findings suggested the selective recruitment of these cells into airway tissue from the microvasculature. Additional studies have confirmed that both inhalation and local allergen provocation of the airways result in an eosinophil influx into the bronchial lumen of sensitised subjects at intervals up to 24 hours post-challenge.[14]

To address the cellular mechanisms of the LAR directly we have examined bronchial mucosal biopsies 5–6 hours after either segmental allergen or saline challenge and the immunopathological changes in small mucosal biopsies.[77] At this time point there was a large influx of neutrophils, identified under the light microscope by their granule content of elastase. Under the electron microscope the neutrophils appeared to be in a highly degranulated state. Other findings included an increase in eosinophils, T-cells and, somewhat surprisingly, mast cells. Similar studies employing allergen challenge of the skin, conjunctiva and nasal mucosa in sensitised individuals have confirmed that at 4–6 hours the dominant leukocyte infiltrating the inflamed lesion is the neutrophil and not the eosinophil as previously thought. This does not exclude the eosinophil as an important cell contributing to later events of the allergen-induced inflammatory reaction. Indeed, an important role for the eosinophil in the later phase of the LAR is supported by a preferential tissue eosinophilia 24 hours after segmental allergen challenge accompanied by a strong transcription signal for the eosinophil promoting cytokine, IL-5.[77]

The mechanism(s) by which leukocytes move into the airway and become activated has attracted considerable interest. Using a panel of monoclonal antibodies to endothelial and leukocyte adhesion molecules, we have shown that six hours following allergen challenge, there occurs marked upregulation of E-selectin whose ligand on neutrophils and other leukocytes is sialyl Lewis x and intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily. One ligand for ICAM-1 is lymphocyte functional antigen-1 (LFA-1) (an integrin heterodimer CD11a-CD18) expressed on a large number of leukocytes but especially on lymphocytes, neutrophils and eosinophils.[78] Another member of the immunoglobulin superfamily, vascular cell adhesion molecule-1 (VCAM-1),[79] was expressed in the airway microvasculature at a low level but this was not increased within the time frame of six hours following allergen provocation. A positive correlation was observed between the extent of ICAM-1 expression and LFA-1^+ leukocyte infiltration, and, more specifically, between E-selectin and the increase in neutrophil numbers, suggesting an important role for these molecules in the allergic inflammatory process.

There has accumulated considerable knowledge concerning the recruitment of endothelial adhesion molecules in inflammatory responses. The initial expression of P-, L- and E-selectins which contain lectin binding regions that interact with carbohydrate ligands on leukocytes (e.g. sialyl-Lewis x) results in the rolling of leukocytes along the endothelial cell, whereas upregulation of ICAM-1 and VCAM-1 arrests the leukocytes thereby facilitating transendothelial migration.[80] An elegant study in non-human primates naturally sensitised to Ascaris antigen has shown that blocking antibodies directed to E-selectin and ICAM-1 abrogate the late airway response and acquired bronchial hyper-responsiveness with aller-

gen challenge in parallel with a reduction in neutrophils and eosinophils respective.[81]

In order to understand how allergen provocation can lead to an upregulation of endothelial leukocyte adhesion molecule expression, it is important to understand more about how these molecules are regulated. P-selectin is rapidly expressed on endothelial cells after exposure to a range of short acting mediators including histamine, platelet activating factor (PAF) and leukotrienes (LTD4, LTB4). Within one hour of autacoid exposure the expression of this molecule diminishes (probably by shedding) and is replaced by E-selectin whose expression is upregulated by cytokines especially interleukin-1, TNFα and interferon-γ.[80,81] The same cytokines are also responsible for the upregulation of ICAM-1, whereas optimal expression of VCAM-1 requires a combination of IL-1 and/or TNFα together with IL-4. Recently Bentley and co-workers have shown that 24 hours following allergen challenge VCAM-1 is upregulated on the vascular endothelium of the nasal mucosa and associated with an increased influx of leukocytes (T cells and eosinophils) bearing the integrin ligand for VCAM-1, VLA-4 ($\alpha 4\beta 6$).[82] Upregulation of VCAM-1 accounting for the continued recruitment of eosinophils following allergen challenge seems reasonable but, using standard immunohistochemistry, we have been unable to show this in the vasculature of bronchial biopsies taken 24 hours post-challenge.[78]

The cellular origin of cytokines responsible for the upregulation o vascular adhesion molecules in the short period required to initiate leukocyte recruitment following allergen provocation has been the subject of some speculation. Initially it was thought that T-cells and monocyte/macrophages were the source of these cytokines but, since these cells require at least 6 hours to generate cytokines *de novo* via transcription, it is dificult to explain the expression of E-selectin and ICAM-1 and the associated leukocyte influx that is already well established six hours ater local allergen challenge. Another possibility is the existence of a preformed source of cytokines.

Using immunohistochemistry applied to 2 μm thick sections embedded in the water soluble resin glycol methacrylate (GMA), we have shown that mucosal mast cells store IL-4, IL-5, IL-6 and TNFα.[83–85] Immunoelectron microscopy has located the cytokines to the mast cell granules (Fig 2). Following cross-linkage of IgE receptors on the surface of mast cells, the cytokines are released rapidly[83] and could provide a mechanism for the early upregulation of vascular adhesion molecules. In support of this, we have shown that dimerisation of cell bound IgE causes enhanced mast cell transcription of mRNA for IL-4, IL-5 and TNFα providing stem cell factor is also present.[84] This enhanced cytokine transcription (which for IL-5 continues for up to 48–72 hours) is accompanied by ongoing product release. Thus, by rapid release of large amounts of preformed cytokine followed by the prolonged generation of newly formed product, the mast cell has the capacity to not only initiate but also prolong the allergen induced inflammatory response. The allergen-induced release of preformed TNFα from mast cells could explain the observed upregulation of E-selectin and ICAM-1 during the LAR, IL-4 promoting the selective upregulation of VCAM-1, and IL-5 serving to promote eosinophil chemotaxis. Our most recent work has shown that human mast cells generate IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, TNFα and GM-CSF which must place this cell among the most active in its capacity to generate pleiotropic cytokines.

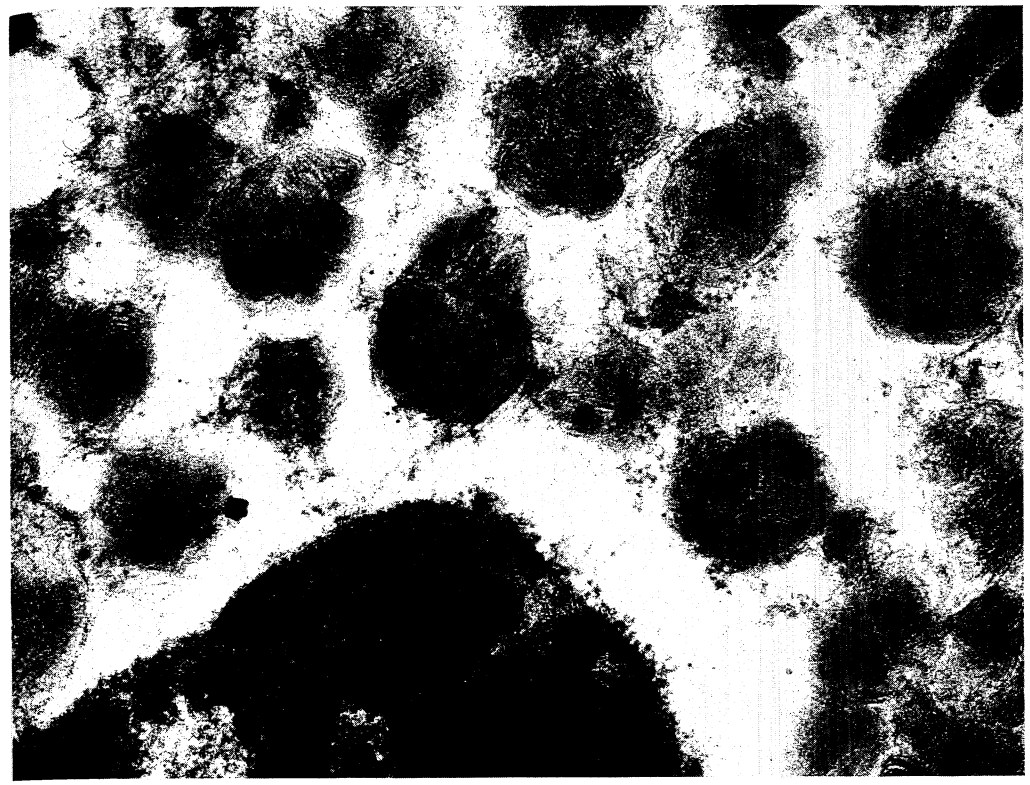

FIGURE 2

Electron micrograph showing an ultrathin section of bronchial mucosa immunohistochemically stained for Interleukin-4. Immunogold labelling is clearly visible in the mast cell granules.

Once recruited into the airways, both neutrophils and eosinophils become activated and secrete a wide array of preformed and newly generated inflammatory products. These comprise the toxic granule components of the eosinophil (major basic protein, eosinophil cationic protein and eosinophil derived neurotoxin) and a range of lipid products including prostanoids, leukotrienes and platelet activating factor (PAF). The availability of potent and selective sulphidopeptide leukotriene antagonists has provided a useful way to determine the contribution of these potent vaso- and broncho-active mediators to the EAR and LAR. The administration of LTD_4 antagonists prior to allergen provocation of sensitised airways produced marked inhibition of both the EAR and LAR and attenuation of the acquired increase in bronchial hyper-responsiveness.[86] Although PAF was at one time regarded as a prime mediator of the late phase inflammatory response and bronchial hyper-responsiveness,[86,87] investigation of the orally active PAF receptor antagonist WEB 2086 has failed to reveal any inhibitory effect on either early or late phase allergen induced airway events.[88]

A greater understanding of the mechanisms of early and late phase allergen responses has helped explain how various anti-asthma drugs might operate. Thus, sodium cromoglycate (SCG) and nedocromil sodium (NS) besides inhibiting the release of preformed and newly generated autacoids from activated mast cells, might also inhibit immunological cytokine release. These effects on the mast cell,

together with blockade of non-myelinated afferent neurones in the airways, probably result from the blockade of selective chloride channels.[89] Oral and topical corticosteroids probably reduce the late phase allergic tissue response by inhibiting cytokine gene transcription and mediated upregulation of vascular adhesion molecules.

Mucosal inflammation in clinical asthma

Bronchoalveolar lavage and more recently bronchial biopsy studies have provided a strong case for a specific type of airway inflammation underlying asthma irrespective of its cause. The dominant mediator secreting cells appear to be mast cells and eosinophils although with more severe disease monocytes, macrophages and platelet also contribute. There is convincing evidence of a key role for T cells orchestrating this inflammation through the release of multifunctional cytokines. Both in lavage and in bronchial biopsies, T-cells exhibit increased expression of the cell surface activation markers HLA-DR, CD25, LFA-1 and VLA-4, the extent of expression correlating with clinical indices of disease activity.[90,91] Activated T cells exhibit mRNA transcripts for IL-3, 4 and 5 but not IFN-γ or IL-2 indicating that they are of the Th2-like phenotype.[67,68] Further work is required on the mechanisms responsible for T cell recruitment and how they relate to the other components of the mucosal immune system at this site. The role of mucosal dendritic cells in identifying, processing and presenting specific allergens to T cells is an important area of further research to identify those factors that draw the T cell towards the Th2 phenotype (Fig 1). The finding that this process is IL-4-dependent might suggest a further role for mast cells in augmenting the inflammatory response.

The finding of activated mast cells, eosinophils and T cells within the airway wall of patients with active asthma has important clinical consequences. From the foregoing asthma may be described as:

> a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells and eosinophils. In susceptible individuals, this inflammation causes symptoms which are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment and causes an associated increase in airway responsiveness to a variety of stimuli.[29]

By laying emphasis on airway inflammation, attention is focused on the physiological and clinical consequences with implications for both diagnosis and management. It is on the basis of this and the increasing concern over the regular use of inhaled β_2 agonists that future treatment strategies are being aimed more towards preventing or inhibiting underlying airway inflammation, rather than simply treating symptoms.[93] There have now been several randomised controlled trials to show that avoidance of allergen (or in the case of some types of occupational asthma small reactive chemicals) results in clinical improvement and reduced bronchial hyper-responsiveness.[94,95] Accompanying the excellent clinical response observed with the regular use of inhaled corticosteroids, mast cell, eosinophil and T cell numbers in the asthmatic airway are all reduced in parallel with a decrease of bronchial hyper-responsiveness.[93] Regular use of inhaled sodium cromoglycate also reduces eosinophils recovered from airways mucus[96] and more recently, nedocromil sodium has been shown to decrease eosinophil numbers in mucosal biopsies from asthmatics after 16 weeks of treatment.[97] By contrast 12 weeks regular treatment with the long acting β_2 agonist, salmeterol, at a dose that

produced marked symptomatic improvement had no effect on mast cell, eosinophil or T-cell numbers either in the bronchial epithelium or submucosa nor on cell surface markers of cell activation or lavage mediator levels.[98] However, while a β_2 agonist may not reduce the background inflammation of asthma, there is evidence in animals (and more recently in humans) that it may reduce the cellular events associated with the allergen-provoked LAR.[99]

These clinical observations raise some important points about factors that may maintain the inflammatory response in asthma. While it is recognised that mucosal immune responses to inhaled allergens leading to inflammation are a key feature of extrinsic asthma, there is almost nothing known about those factors which lead to asthma where there is no obvious inducing agent i.e. intrinsic or cryptogenic asthma. When compared to extrinsic asthma the only differences appear to be in the chronicity of the disorder and possibly the presence of a greater number of activated T-cells. Both in extrinsic and intrinsic asthma, the persistence of the inflammatory response may depend upon factors other than those orchestrated by the immune system.

Epithelium as a target for the inflammatory attack in asthma

The observation by Naylor that the sputum of patients recovering from an exacerbation of asthma contained clumps of epithelial cells (Creola bodies) indicated that the bronchial epithelium was involved in the inflammatory response.[100] This has been amply confirmed in postmortem studies of asthma when, in addition to the airway lumen filled with secretions, there occur large areas where the pseudostratified ciliated epithelium is stripped to a single layer of basal cells. Elegant studies by Gleich and colleagues present a convincing case for the arginine-rich proteins of the eosinophil playing a key role in epithelial damage.[101,102] Our work leads us to believe that eosinophils require a cognate interaction with the epithelium whereupon there is release of metalloendoproteases, e.g. gelatinase and stromolysin, in parallel with an increase in epithelial permeability and detachment of columnar cells from their basal cell attachments.[103]

In patients with mild-moderate asthma, there is evidence that an important site of damage to the epithelium is localised to a plane between the columnar and basal cells, with weakening of the major adhesion structures responsible for maintaining the integrity of the epithelium, desmosomes.[104,105] These are complex structures found in large numbers at the basal-columnar cell interface but also between adjacent columnar cells, whereas the basal cells are firmly attached to the basement membrane by hemidesmosomes containing the integrin $\alpha_6\beta_4$.[106] The precise mechanisms whereby the adhesive function of desmosomes is disturbed in asthma is not known although in culture epithelial cell disaggregation with disruption of desmosomes occurs in the presence of a reduced extra-cellular Ca^{++} concentration. One possibility is that through contact with the epithelium and release of granule contents, eosinophils reduce the pericellular Ca^{++} concentration.

Epithelial disruption in asthma may be important in augmenting the inflammatory response. Integrity of the epithelium is required for adequate formation of the airway lining fluid which is rich in components which help protect the airway from noxious environmental insults such as antioxidents and immunoglobulins. Loss of ciliary function impairs mucus clearance while areas of increase

permeability allow allergens and other inflammatory stimuli to penetrate the airway wall. As a component of the epithelial damage, growth factors, such as platelet derived growth factor (PDGF), basic fibroblast growth factor and endothelin-1, are released which leads to proliferation of myofibroblasts situated just beneath the epithelial basement membrane. Myofibroblasts secrete Types I, III and V interstitial collagens which comprise the greatly expanded *lamina reticularis* of the basement membrane in asthma giving rise to the appearance of the 'thickened basement membrane' which is characteristic of the disease.[107] The number of myofibroblasts correlates well with the thickness of this collagen layer.[108]

The capacity of the epithelium to serve as a source of cytokines has aroused much interest. In both normal and asthmatic airways preformed IL-1β, IL-8, PDGF, RANTES and GM-CSF can be immunolocalised to the epithelium. In asthma there is increased production of GM-CSF and IL-8 by the epithelium and increased levels of the cytokines in fluid recovered from the airway surface by lavage. Along with IL-5, GM-CSF is an important factor which prolongs eosinophil survival by inhibiting apoptosis. There is also evidence that in asthma IL-8 is a chemo-attractant for eosinophils in addition to its well known effects on neutrophils.[109]

Other constitutive cells of the airway may contribute cytokines to the inflammatory response in asthma. Of some importance is the microvascular endothelium with its capacity to secrete GM-CSF and IL-8. Myofibroblasts may also be an important source of cytokines. In the presence of TNFα, myofibroblasts cultured from human airway epithelium generate and release substantial amounts of GM-CSF in a dose related fashion (W. Roche, personal communication). Thus the conditioned media from the cells can sustain eosinophil survival which is enhanced even further if eosinophils are in close contact with myofibroblasts. Myofibroblasts also have the capacity to help maturate and prolong the survival of mast cells, in part due to their capacity to secrete stem cell factor.

Another factor that may be important in augmenting the airway inflammatory response of asthma is the establishment of autocrine feedback pathways. Thus, in addition to mast cells surving as a source of cytokines, there is mounting evidence to incriminate eosinophils as an important source of these molecules including GM-CSF, IL-3, IL-5, TNF-α and TGF-β.[110–113] Thus, with extensive eosinophil infiltration, these cells may be a major source of pro-imflammatory cytokines and might account for the cascade of inflammatory events that lead to acute severe asthma and, occasionally, death from the disease.

While considerable progress has been made with respect to the mechanisms underlying mild asthma, the challenge for the future will be to apply modern tools of molecular and cell biology to understand more about the factors underlying severe disease. This category of patient is the one most at risk of repeated hospital admission and death and accounts for a major part of the health budget for asthma management. A concerted effort focused on the mechanisms of asthma chronicity and severity should provide a research agenda with a rich potential yield.

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REFERENCES

1 Salter HH. On asthma: Its pathology and treatment, 2nd ed. London: Churchill, 1868.
2 CIBA Foundation Guest Symposium. Terminology, definitions, classification of chronic pulmonary emphysema and related conditions. Thorax 1959; **14:** 286.
3 Porter R, Birch J (eds) Identification of asthma. In: Ciba Foundation Study Group No. 38. Edinburgh and London: Churchill Livingstone, 1971.
4 American Thoracic Society Committee on Diagnostic Standards Definitions and Classification of Chronic Bronchitis, Asthma and Pulmonary Emphysema. *Am Rev Respir Dis* 1962; **85:** 762.
5 Curry JJ. The action of histamine on the respiratory tract in normal and asthmatic subjects. *J Clin Invest* 1946; **39:** 325–39.
6 Juniper EF, Frith PA, Hargreave FE. Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. *Thorax* 1981; **36:** 575–9.
7 Joseph LK, Gregg I, Holgate ST. Does non-specific bronchial responsiveness indicate the severity of asthma? *Eur Respir J* 1990; **3:** 220–27.
8 Clough JB, Williams JD, Holgate ST. The natural history of symptoms, peak expiratory flow, and bronchial responsiveness in 7 and 8 year old children with cough and wheeze: A 12 month longitudinal study. *Am Rev Respir Dis* 1991; **143:** 755–60.
9 Osler W. The principles and practice of medicine. New York: Appleton & Co 1892, 497.
10 Schmidt A. Beitrage zur Kenntniss der Sputums inbesondere des Asthmatischen und zur Pathologie des asthma bronchiale. *Ztsch J Klin Med* 1892; **20:** 476.
11 Fraenkel A. Zur Pathologie des Bronchialasthma. *Deutsch med Wchnschr* 1990; **17:** 269.
12 Ellis AG. Pathological anatomy of bronchial asthma. *Am J Med Sci* 1908; **136:** 407.
13 Dunnill MS. The pathology of asthma with special reference to changes in the bronchial mucosa. *J Clin Pathol* 1960; **13:** 224–5.
14 Laitinen LA, Heino M, Laitinen A *et al.* Damage to the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985; **131:** 599–606.
15 Djukanović R, Wilson J, Lai C *et al.* The safety aspects of fibreoptic bronchoscopy and endobronchial biopsy in asthma. *Am Rev Respir Dis* 1991; **143:** 772–7.
16 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.
17 Djukanović R, Wilson JW, Britten KM *et al.* Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic and healthy control subjects using immunohistochemistry. *Am Rev Respir Dis* 1990; **142:** 863–71.
18 Jeffrey PK, Wardaw AJ, Nelson FC *et al.* Bronchial biopsies in asthma: an ultrastructural quantification study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989; **140:** 1745–53.
19 Blackley CH. Experimental researches on the causes and nature of catarrhus aestivus (hay fever or hay-asthma). London: Ballière Tindall and Cox, 1873.
20 Blackley CH. Hay fever: Its causes, treatment and effective prevention. Experimental Researches, 2nd ed. London: Ballière Tindall and Cox, 1880.
21 Voorhorst R, Spieksma F, Varekamp H *et al.* The house dust mite (*dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house dust allergen. *J Allergy* 1967; **39:** 325–39.
22 Stewart GA. The molecular biology of allergens. In: Holgate ST and Busse W, eds, The mechanisms of asthma and rhinitis. Oxford and Boston: Blackwells, 1994.
23 The genetics of asthma. Marsh D, Lockhart A, Holgate ST, eds. Oxford: Blackwells, 1991.
24 Cookson WOCM, Hopkin JM. Dominant inheritance of atopic immunoglobulin E responsiveness. *Lancet* 1988; **1:** 86–8.
25 Cookson WOCM, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis on chromosome 11q. *Lancet* 1989; **i:** 1292–5.
26 Sandford AJ, Shirikawa T, Moffatt MF *et al.* Localisation of atopy and β subunit of high affinity IgE receptor ($Fc_\varepsilon R1$) on chromosome 11q. *Lancet* 1993; **341:** 332–4.
27 March DG, Meyers DA. A major gene for allergy. Fact or fancy. *Nature Genetics* 1992; **2:** 252–4.

[28] Watson M, Lawrence S, Collins A, *et al.* Exclusion from proximal 11q of a common gene with megaphenic effect on atopy. *Ann Hum Genet* (submitted).
[29] Lawrence S, Watson M, Collins A, *et al.* Tests for major genes in atopy and asthma (Abstract from International Genetic Epidemiology Society) 1994.
[30] Marsh DG, Neely JD, Breazeale *et al.* Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994; **264:** 1152–6.
[31] Meyers DA, Postma DS, Panhuysen CIM *et al.* Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 1994; **23:** 464–70.
[32] Ansari AA, Friedhoff LR, Meyers DA, Bias WB, Marsh DG. Human immune responsiveness to *Lolium Perenne* pollen allergens *Lol p* III (Rye III) is associated with HLA-DR3 and DR5. *Human Immunol* 1989; **25:** 59–71.
[33] O'Hehir RE, Mach B, Berte C *et al.* Direct evidence for a functional role of HLA-DRB3 gene products in the recognition of *Dermatophagoides sp* by helper T-cell clones. *Int Immunol* 1990; **2:** 885–92.
[34] Moffatt MF, Hill MR, Cornelis F *et al.* Genetic linkage on T-cell receptor α/δ complex to specific IgE responses. *Lancet* 1994; **343:** 1597–9.
[35] Hall IP, Whealey A, Wilding P *et al.* The GLO27 β_2 adrenoceptor polymorphism is associated with lower airway reactivity in asthmatic patients. *Am J Resp Crit Care Med* [Abstract] 1995 (in press).
[36] Sporik R, Holgate S, Platts-Mills TAE, Cogswell JJ. Exposure to house dust mite allergen (*Der p1*) and the development of asthma in childhood. *N Engl J Med* 1990; **323:** 502–7.
[37] Royal College of Physicians (London). Smoking and the young, 1992, 1–23.
[38] Godfrey KM, Barker DJP, Osmond C. Disproportionate fetal growth and raised IgE concentration in adult life. *Clin Exp Allergy* 1994; **24:** 641–48.
[39] Barker DJP, Godfrey KM, Fall C *et al.* Relation of birthweight and childhood respiratory infection to adult lung function and death from chronic obstructive airway disease. *Br Med J* 1991; **303:** 671–75.
[40] Kelly FJ, Rickett GM, Hunt AN *et al.* Biochemical maturation of the guinea-pig lung and survival following premature delivery. *Int J Biochem* 1991; **23:** 467–71.
[41] Chandra RK. Interactions between early nutrition and the immune system. In: Bock, ed. The childhood environment and adult disease. Ciba Symposium 156, Chichester; John Wiley, 1991, 77–92.
[42] Ricci M, Rossi O, Bertoni M, Matucci A. Th2-like cells in the pathogenesis of airway allergic inflammation. *Clin Exp Allergy* 1993; **23:** 360–69.
[43] Mossmann TR, Cherwinski H, Bond MW *et al.* Two types of murine T helper cell clones. I. Definition according to profile of lymphokine activities. *J Immunol* 1986; **136:** 2348–57.
[44] Harding J, Lui L, Evans P *et al.* Intrauterine feeding of the growth retarded fetus: can we help? *Early Hum Devel* 1991; **29:** 193–97.
[45] Williams HC, Strachan DP, Hay RJ. Childhood eczema: a disease of the advantaged? *Br Med J* 1994; **308:** 1132–35.
[46] Arshad SH, Matthews S, Grant C, Hide DW. Effect of allergen avoidance on development of allergic disorders in infancy. *Lancet* 1993; **339:** 1493–7.
[47] Warner JA, Miles EA, Jones AC *et al.* Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic asthma? *Clin Exp Allergy* 1994; **24:** 423–30.
[48] Del Prete GF, Maggi E, Parronchi P *et al.* IL-4 is an essential co-factor for the IgE synthesis induced *in vitro* by human T cell clones and their supernatants. *J Immunol* 1988; **140:** 4193–7.
[49] Mossman TR. Cytokine secretion phenotypes of TH cells: how many subsets, how much regulation? *Res Immunol* 1991; **142:** 7–16.
[50] Tager IB. Passive smoking–bronchial responsiveness and atopy. *Am Rev Respir Dis* 1988; **138:** 507–9.
[51] Magnusson CGM. Maternal smoking influences cord serum IgE and IgD levels and increases risk of subsequent infant allergy. *J Allergy Clin Immunol* 1986; **78:** 898–904.
[52] Ownby DR, Johnson CC, Peterson EL. Maternal smoking does not influence IgE or IgD concentration. *J Allergy Clin Immunol* 1991; **88:** 555–60.
[53] Jensen EJ, Pedersen S, Schmidt E, Dahl R. Serum IgE in atopic smokers, non-smokers and recent ex-smokers: Relation to lung function, airway symptoms and atopic predisposition. *J Allergy Clin Immunol* 1992; **90:** 224–29.
[54] Venables KM, Topping MD, Howe W *et al.* Interaction of smoking and atopy in producing specific IgE antibody against a hapten protein conjugate. *Br Med J* 1985; **290:** 201–4.

[55] Alwan WH, Record FM, Openshaw PJM. Phenotypic and functional characterisation of T-cell lines specific to individual respiratory syncytial virus proteins. *J Immunol* 1993; **150:** 5211–8.
[56] Russi JC, Delfraro A, Borthagaray MD, *et al.* Evaluation of immunoglobulin E-specific antibodies and viral antigens in naso-pharyngeal secretions of children with respiratory syncytial virus infections. *J Clin Microbiol* 1993; **31:** 819–23.
[57] Rose RM, Pinkston P, Skornik WA. Altered susceptibility to viral respiratory infection during short term exposure to nitrogen dioxide. *Health Eff Inst Resp Rep* 1989; **24:** 1–24.
[58] Devalia JL, Rusznak C, Herdman *et al.* Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. *Lancet* 1994; **344:** 1668–71.
[59] Molfino NA, Wright SC, Katz J, Tarlo S, Silverman F, McClean PA *et al.* Effect of low concentration of ozone on inhaled allergen responses in asthmatic patients. *Lancet* 1991; **338:** 199–203.
[60] Holt PG, McMeramin C, Nelson D. Primary sensitisation to inhalant allergens during infancy. *Ped Allerg & Immunol* 1990; **1:** 3–13.
[61] Prausnitz C, Küstner H. Studies über Uberempfindlichkeit Centralb Bakeriol 1 *Abt Orig* 1921; **86:** 160. Trans by Prausnitz C in Gell PGH, Coombs RRA, eds, Clinical aspects of immunology. Oxford: Blackwell Scientific Publications 1962, 808–16.
[62] Ishizaka K, Ishizaka T. Identification of IgE as a carrier of reaginic activity. *J Immunol* 1967; **99:** 1187–98.
[63] Holgate ST. Mediator and cytokine mechanisms in asthma. The Altounyan Address. *Thorax* 1993; **48:** 103–110.
[64] Banchereau J, Briere F, Liu YJ, Rousset F. Molecular control of B lymphocyte growth and differentiation. *Stem Cells* 1994; **12(3):** 278–88.
[65] Snapper CM, Paul WE. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production science (Washington DC) 1987; **236:** 944–7.
[66] Schwartz LB. Differentiation of human mast cells and their involvement in asthma. In: Holgate ST, Austen KF, Lichtenstein CM, Kay AB, eds. Asthma: physiology, immunopharmacology and treatment. London: Academic Press 1993.
[67] Clutterbuck EJ, Hirst EMA, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and granulocyte macrophage colony-stimulating factor. *Blood* 1989; **73:** 1504–13.
[68] Hamid Q, Azzawi M, Ying S *et al.* Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991; **87:** 1541–6.
[69] Robinson DR, Hamid Q, Ying S *et al.* Evidence for a predominant 'Th2 type' bronchoalveolar lavage T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; **326:** 298–304.
[70] Pepys J. Disodium cromoglycate in clinical and experimental asthma. In: Austen KF, Lichtenstein CM. Asthma: physiology, immunopharmacology and treatment. London: Academic Press 1973, 279–294.
[71] Cockcroft DW, Murdock KY. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. *Thorax* 1987; **42:** 302–308.
[72] Schwartz LB, Lewis RA, Austen KF. Tryptase from human pulmonary mast cells: purification and characterisation. *J Biol Chem* 1981; **256-** 11939–11943.
[73] Walls AF, He S, Teran L *et al.* Granular site recruitment by human mast cell tryptase. *Int Arch All Immunol* 1995 (in press).
[74] Cromwell O. Inflammatory cells, mediators and asthma. *Eur J Clin Res* 1991; **2:** 29–38.
[75] Cookson WOCM, Craddock CF, Benson MK, Durham SR. Falls in peripheral eosinophil counts parallel the late asthmatic response. *Am Rev Respir Dis* 1989; **139:** 458–62.
[76] De Monchy JGR, Kauffman HF, Venge P *et al.* Bronchoalveolar lavage eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985; **131:** 373–6.
[77] Frew AJ, Teran LM, Madden J *et al.* Cellular changes 24 hours after endobronchial allergen challenge in asthma. *Int Arch Allergy Appl Immunol* 1995 (in press).
[78] Montefort S, Gratziou C, Goulding D *et al.* Bronchial biopsy evidence for leucocyte infiltration and upregulation of leucocyte-endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitised asthmatic airways. *J Clin Invest* 1994; **93:** 1411–1421.
[79] Wegner CD, Gundel RH, Reilly P *et al.* ICAM-1 in the pathogenesis of asthma. *Science* 1990; **247:** 416–8.
[80] Vonderheide RH, Springer TA. Lymphocyte adhesion through the very late antigen 4: Evidence for a novel binding site in the alternatively spliced domain of VCAM-1 and an

additional α_4 integrin counter receptor on stimulated endothelium. *J Exp Med* 1992; **175:** 1433–42.
[81] Gundell RH, Wegner CD, Torcellini CA *et al.* ELAM-1 mediates antigen-induced acute airway inflammation and late phase destruction in monkeys. *J Clin Invest* 1991; **88:** 1407–11.
[82] Bentley AM, Durham SR, Robinson DS *et al.* Expression of the endothelial leucocyte adhesion molecules ICAM-1, E-selectin and UCAM-1 in the bronchial mucosa in steady state asthma and allergen-induced asthma. [Abstract] *Thorax* 1992; **47:** 852.
[83] Bradding P, Feather IH, Howarth PH *et al.* Interleukin-4 is localised to and released by human mast cells. *J Exp Med* 1992; **176:** 1381–6.
[84] Okayama Y, Petit-Frére C, Kassel O *et al.* Expression of messenger RNA for IL-4 and IL-5 in human lung and skin mast cells in response to FCε receptor cross-linkage and the presence of stem cell factor. *J Immunol* (submitted).
[85] Bradding P, Roberts JA, Britten KM, *et al.* Interleukins (IL)-4, -5, -6 and TNF_α in normal and asthmatic airways: Evidence for the human mast cell as an important source of these cytokines. *Am Rev Respir Cell Mol Biol* 1994; **10:** 471–80.
[86] O'Hickey SP, Arm JP, Wilkinson JW, Lee TH. Mediators of hypersensitivity in bronchial asthma. *Eur J Clin Res* 1991; **2:** 29–38.
[87] Chung CF. Platelet activating factor in inflammation and pulmonary disorders. *Clin Sci* 1992; **83:** 127–38.
[88] Wilkens H, Wilkens JH, Busse S *et al.* Effects of an inhaled PAF-antagonist (WEB 2086) on allergen-induced early and late asthmatic responses and increased bronchial responsiveness to methacholine. [Abstract] *Am Rev Respir Dis* 1991; **143:** A812.
[89] Norris A. New insights on the mechanism of action of sodium cromoglycate and nedocromil sodium. *Clin Exp Allergy* 1995 (in press).
[90] Wilson JW, Djukanović R, Howarth PH, Holgate ST. Lymphocyte activation in bronchoalveolar lavage and peripheral blood in atopic asthma. *Am Rev Respir Dis* 1992; **145:** 958–90.
[91] Azzawi M, Bradley B, Jeffrey 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.
[92] National Heart, Lung and Blood Institute. International Consensus Report on Diagnosis and Management of Asthma. US Department of Health and Human Services Publication No. 92-3091, June 1992.
[93] Djukanović R, Wilson JW, Britten KM *et al.* The effect of inhaled corticosteroid on airway inflammation and symptoms of asthma. *Am Rev Respir Dis* 1992; **145:** 669–74.
[94] Platts-Mills TAE, Tovey ER, Mitchell EB *et al.* Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 1982; **2:** 675–78.
[95] Murray AB, Ferguson AC. Dust-free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. *Paediatrics* 1983; **71:** 418–22.
[96] Diaz P, Gallequillos FR, Gonzalez MC *et al.* Bronchoalveolar lavage in asthma: the effect of cromoglycate (cromolyn) on leukocyte counts in immunoglobulins and complement. *J Allergy Clin Immunol* 1984; **74:** 41–8.
[97] Trigg C, Manolitsas N, McAulay A *et al.* A pilot comparative study of the effects of inhaled nedocromil sodium and albuterol on bronchial biopsies in asthma. [Abstract] *Am Rev Respir Dis* 1993; **147:** A522.
[98] Roberts JA, Bradding P, Walls AF *et al.* The effect of salmeterol xinatoute therapy on mucosal inflammation in asthma. [Abstract] *Am Rev Respir Dis* 1992; **145:** A418.
[99] Twentyman OP, Sams VR, Holgate ST. Albuterol and nedocromil sodium affect airway and leukocyte responses to allergen. *Am Rev Respir Dis* 1993; **147:** 1425–30.
[100] Naylor B. The shedding of the mucosa of the bronchial tree in asthma. *Thorax* 1962; **17:** 69–72.
[101] Frigas E, Gleich GJ. The eosinophil and the pathophysiology of asthma. *J Allergy Clin Immunol* 1986; **77:** 527–37.
[102] Fujisawa T, Kephart GM, Gray BH, Gleich GJ. The neutrophil and chronic allergic inflammation. *Am Rev Respir Dis* 1990; 689–97.
[103] Herbert CA, Edwards D, Boot JR, Robinson C. *In vitro* modulation of the eosinophil-dependent enhancement of the permeability of the bronchial mucosa. *Br J Pharmacol* 1991; **104:** 391–8.
[104] Montefort S, Herbert CA, Robinson C, Holgate ST. The bronchial epithelium as a target for inflammatory attack. *Clin Exp Allergy* 1992; **22:** 511–20.
[105] Montefort S, Roberts JA, Beasley CR *et al.* The site of disruption in bronchial epithelium in asthmatics and non-asthmatics. *Thorax* 1992; **47:** 499–503.
[106] Jones JCR, Kurpakus MA, Cooper HM, Quaranta V. A function for the integrin $\alpha_6\beta_4$ in the hemidesmosone. *Cell Regul* 1991; **2:** 427–38.
[107] Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; **1:** 520–4.
[108] Brewster CEP, Howarth PH, Djukanović R *et al.* Myofibroblasts and sub-epithelial fibrosis in bronchial asthma. *Am Rev Respir Cell Mol Biol* 1990; **3:** 507–11.
[109] Warringa RJ, Mengelers HJJ, Raaijmakers JAM *et al.* Upregulation of formyl peptide and interleukin-8-induced eosinophil chemotaxis in patients with allergic asthma. *J Allergy Clin Immunol* 1993; **91:** 1198–205.
[110] Moqbel R, Hamid Q, Ying S *et al.* Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony stimulating factor (GM-CSF) in activated human eosinophils. *J Exp Med* 1991; **174:** 749–53.
[111] Kita H, Ohnishi T, Okubo Y *et al.* Granulocyte/macrophage colony—stimulating factor and interleukin-3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med* 1991; **174:** 745–8.
[112] Douglas JA, Bradding P, Hissey PH *et al.* Human eosinophils contain and release interleukin-5 (submitted).
[113] Costa JJ, Matossian K, Resnick MB, *et al.* Human eosinophils can express the cytokines tumour necrosis factor-α and macrophage inflammatory protein-1α. *J Clin Invest* 1993; **91:** 2673–84.