

Mediator and cytokine mechanisms in asthma*

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Asthma is the most common treatable chronic disease of the lung affecting all age groups; yet its treatability—despite improvements in the potency and selectivity of the drugs used and in the devices administering them—appears to be having relatively little impact on the mortality and the rising morbidity of this disease. To some extent these rising trends, which include a 3–5 fold increase in hospital admissions,¹ may reflect increased awareness, causing a diagnostic shift, or increased caution in the treatment of acute asthma;² there is mounting evidence that the true incidence of the disease may be increasing, along with that of other allergic disorders.³ As this is not due to a change in genetic factors, we are left with changes in the environment as the phenomenon underlying the rising trends.

With the clear recognition that most asthma in childhood and young adults is associated with atopy (increased synthesis of IgE against common allergens), a good start towards gaining a better understanding of the pathophysiology of asthma might be obtained by focusing on its immunological basis. It is 100 years since Olser in his classical textbook *The Principles and Practice of Medicine* (first edition published 1892)⁴ described asthma as “a special form of inflammation effecting the small bronchioles,” and yet until recently little progress has been made in relating inflammation to disordered airway function in the disease. Careful histopathological studies of airways from the lungs of patients who have died from asthma have again highlighted the importance of airway inflammation, comprising mast cell degranulation, infiltration by eosinophils and mononuclear cells, epithelial disruption, hypertrophy of airway smooth muscle, hypersecretion of mucus, and increased microvascular leakage.^{5,6} Because this material has been obtained from patients who had died from asthma, however, these findings could not be related to events in life.

Immunopathology of mild to moderate asthma

The availability of fiberoptic bronchoscopy, enabling lavage and tissue samples to be obtained from the airway of patients with both allergic and non-allergic asthma, has reaffirmed the view that airway inflammation underlies disordered airway function even in mild disease.^{7–9} The changes observed within small biopsy specimens obtained from sub-

carinae are entirely in line with those described in fatal asthma, though less striking. When monoclonal antibodies are used to identify individual cell types mast cells and eosinophils are seen to be the prominent effector cells, with their capacity to secrete an array of preformed and newly generated mediators of inflammation.^{10,11}

Although mast cells appear not to be increased in submucosa of patients with mild to moderate asthma careful electron microscopical studies, undertaken by Laitinen and coworkers,¹² have shown an increase in the number of these cells present in the bronchial epithelium, which in other studies is paralleled by a 3–5 fold increase in the number of mast cells obtained from the airways by lavage.^{13,14} In normal airways the number of mast cells increases towards the periphery of the lung, a region not accessible to endobronchial biopsy. In a recent immunohistochemical study of lungs from patients who have died from asthma we have shown a substantial increase in mast cells, particularly outside the airway smooth muscle. This is a new and potentially important finding, which draws the mast cell firmly back into the debate on the role of different inflammatory cells in this disease. Electron microscopy applied to mucosal biopsy specimens from patients with mild to moderate asthma shows ultrastructural evidence for mast cell degranulation,^{7,10,14} a feature confirmed by the 5–15 fold increase in the concentration of both preformed and newly generated mediators present in lavage fluid in asthma (fig 1). Most of the mast cells observed in the airways of normal and asthmatic subjects contain tryptase as their major neutral protease, suggesting dependence on factors such as interleukin (IL)-3, IL-4, and IL-10 secreted by T lymphocyte for their maturation and survival.¹⁵

Bronchial biopsy and lavage have placed the eosinophil at the forefront of the effector leucocytes of asthma.^{8,9,11,12} These cells are seen to infiltrate the full thickness of the airway wall. In addition to containing the arginine rich basic proteins (major basic protein, cationic protein, eosinophil derived neurotoxin, eosinophil peroxidase), which impart the characteristic eosin staining to the granules, eosinophils when suitably activated have the capacity to generate large amounts of the sulphidopeptide leukotriene (LT) C₄,¹⁶ a constituent of slow reacting substance of

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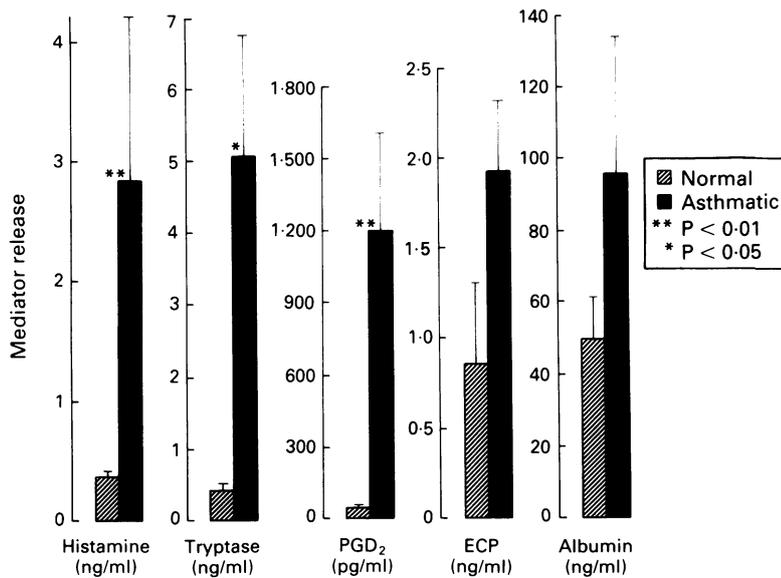
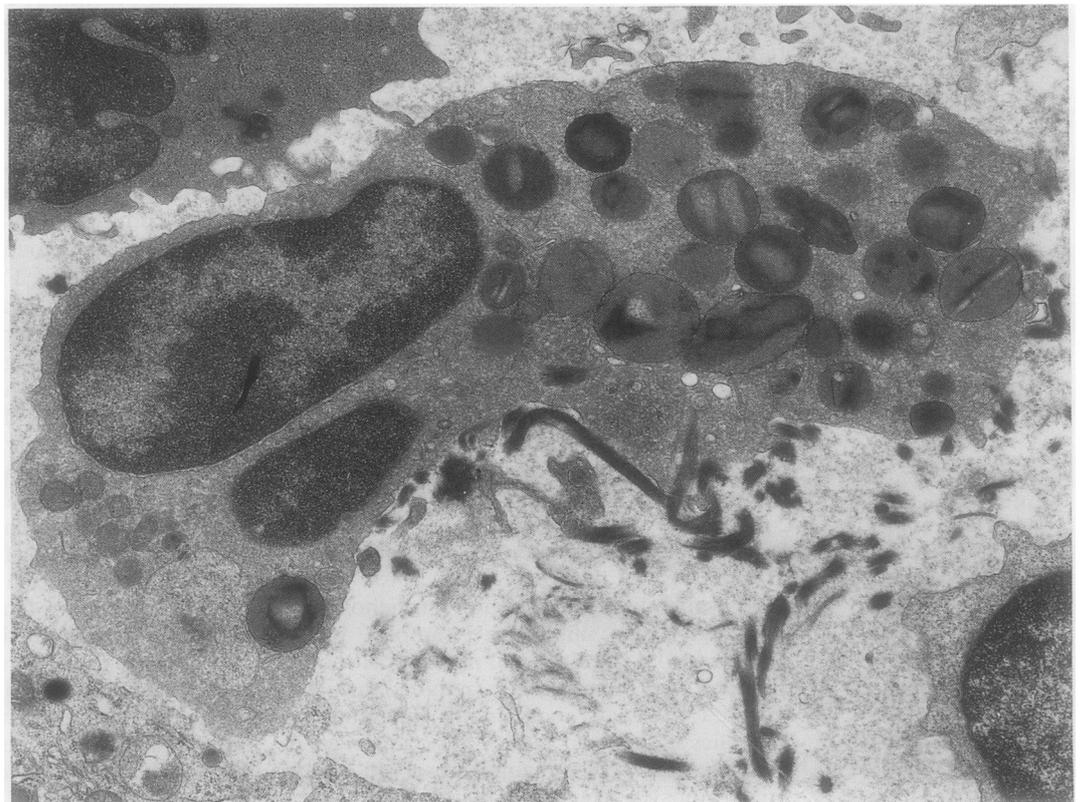


Figure 1 Mediator concentrations in bronchoalveolar lavage fluid in matched normal and asthmatic subjects. ECP—eosinophil cationic protein; PGD₂—prostaglandin D₂.

anaphylaxis. Although there is some evidence that eosinophils may develop from precursors in the airways,¹⁷ most of these cells are recruited into the airways from the bronchial circulation after a specific interaction with adhesion molecules expressed on the endothelium. Like mast cells, eosinophils observed in the airway wall have the ultrastructural features of classical and piecemeal degranulation, together with loss of the electron density of the core of the eosinophil granules containing major basic protein¹⁸ (fig 2). Eosinophil mediator secretion is confirmed by lavage studies showing increased concentrations of the granule derived, arginine rich proteins.¹⁹

Figure 2 Transmission electron micrograph of an eosinophil in the bronchial submucosa of a symptomatic patient with allergic asthma. Note the reversed electron density of the granule crystalloids containing major basic protein (MBP).



Other inflammatory cells, including neutrophils, platelet, monocytes and macrophages, may contribute to the mediator pool of asthma; but their precise roles have yet to be clearly defined.

The availability of lavage and biopsy tissue from the airways of patients with asthma has provided convincing evidence that T cells have an important orchestrating role in the inflammatory response. Both in lavage fluid²⁰ and in tissue section²¹ a proportion of the T lymphocytes show increased expression of activation markers, such as the interleukin 2 receptor (CD25) and the major histocompatibility class II molecule (HLA-DR). These lymphocytes also have phenotypic characteristics that incriminate them directly in the inflammatory response in asthma. In cells recovered by lavage²² and in bronchial biopsy material²³ activated T cells express messenger RNA for the cytokines known to play a part in the recruitment and activation of mast cells and eosinophils and include IL-3, IL-4, IL-5, and granulocyte-macrophage colony stimulating factor (GM-CSF). In order to understand how these cells interact within the asthmatic airway it is instructive to define how the sensitive asthmatic airway responds to inhaled allergen.

Allergen exposure as an important risk factor of asthma

There is now overwhelming evidence to indicate an important genetic component in the development of allergy, though controversy surrounds the mode of inheritance.²⁴ On the basis of extended family studies and sib pair analysis, Hopkin and colleagues have presented evidence for the existence of a dominant mode

of inheritance of atopy with a specific gene on the short arm of chromosome 11 close to the centromere (11q13).^{25,26} More recently they have shown that linkage to 11q13 is expressed predominantly through the mother, for which they propose the hypothesis of paternal genomic imprinting,²⁷ which would result in non-expression of genes derived from the father. A gene imprinted in this way would remain inactive through the life of an individual, though when passed on through a female to an offspring it could result in disease. An alternative explanation for the maternal influence over IgE regulation in the neonate is the intrauterine environment, as shown for the development of adult chronic airway disease.²⁸ Others have suggested a more complex mode for the inheritance of atopy, via multiple genes, and have failed to show genetic linkage to chromosome 11.²⁹⁻³¹

Irrespective of the genetic factors underlying the predisposition to allergic inflammation, all are agreed that environmental exposure to an offending allergen is crucial for the expression of the gene or genes, and in the case of asthma this usually means inhaled aeroallergens.³² In temperate climates the allergens that have been most definitely incriminated as pathogenetic in asthma are those derived from the house dust mite—in particular the neutral proteases *Der p* I (a cysteine protease), *Der p* II (lysozyme), and *Der p* III (serine protease) from the gastrointestinal tract of *Dermatophagoides pteronyssinus*—and the allergens derived from cat proteins—in particular *Fel d* I, located in cat skin and salivary gland.³³ In atopic individuals who are genetically at risk the level of allergen exposure seems to be an important determinant of whether or not asthma is clinically expressed.³⁴ There is increasing evidence to suggest that the first year of life, while the mucosal immune system is still developing, is a particularly vulnerable time, in which sensitisation to inhaled allergens occurs.³⁴⁻³⁶ Moreover, sensitisation of the respiratory tract appears to be favoured when allergen is presented in association with adjuvant factors, which include inhaled irritants (for example, “passive” smoke) and viral respiratory tract infections, and with prematurity³⁷ (fig 3).

The proteolytic nature of many of the inhaled allergens favours their penetration through the protective bronchial epithelium to the mucosal immune system.³⁸ IgE antibodies directed to common aeroallergens may be detectable within the first few months of life, indicating systemic sensitisation,^{34,35} though we do not yet know for certain whether the respiratory tract can be sensitised in the absence of a systemic IgE response.

Irrespective of the source of IgE, this immunoglobulin is able to bind to specific receptors on the surface of mast cells (Fcε R1) and eosinophils (Fcε R2), providing an important trigger mechanism for the release of inflammatory mediators. Careful *in vitro* studies have shown that switching of B lymphocytes from IgG and IgM synthesis to secreting allergen specific IgE requires a cognate interaction with allergen sensitised T cells and the presence of a specific cytokine, interleukin-4 (IL-4).³⁹ The subtype of T lymphocyte capable of generating IL-4 (along with IL-3, IL-5, IL-6, IL-10, and GM-CSF) has been designated T_H2, to distinguish it from the T cells that take part in the delayed hypersensitivity reaction (T_H1) with their preferential generation of IFN-γ, IL-2, and lymphotoxin (TNF-β)⁴⁰ (fig 4). There exists a reciprocal relationship between T_H1 and T_H2 cells, differentiation and survival of T_H2 cells and the synthesis of IgE by B cells being stimulated by IL-4 and inhibited by IFN-γ from T_H1 cells, whereas the survival and proliferation of T_H1 cells requires IL-2 and is inhibited by IL-10 derived from T_H2 cells.

Allergen provocation

The sensitised airways of asthmatic patients respond to inhaled allergen to produce an early phase of bronchoconstriction, reaching a maximum 15–20 minutes after challenge and recovering over the following hour. This is followed by a late phase response starting at around 2–4 hours, reaching a maximum at 6–8 hours, and recovering over the following 24 hours. Interest in the responses increased when it was shown that there was a parallel increase in airway responsiveness to agents such as methacholine and histamine, which persisted for several days after resolution of the late

Figure 3 Schematic representation of early and later life events leading to airway allergen sensitisation and asthma.

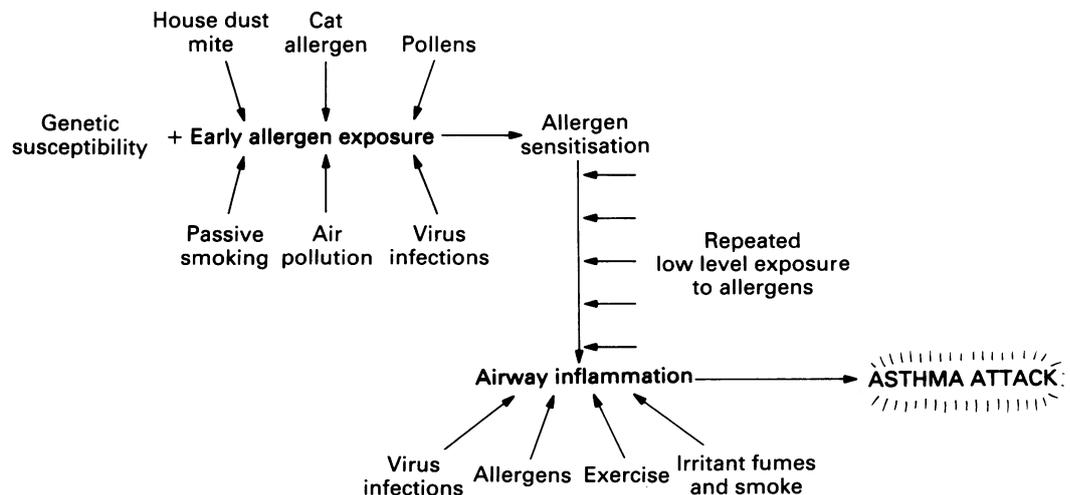
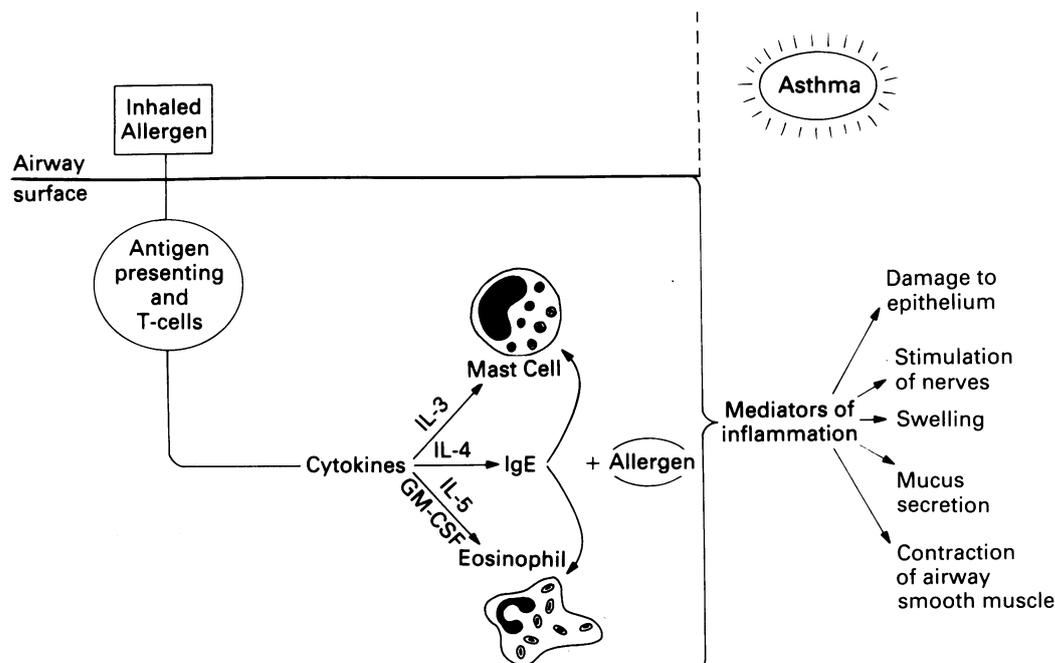


Figure 4 Role of antigen presenting cells, T lymphocytes, and cytokines in the inflammatory response of asthma.



phase obstructive response. The observation that, when administered before allergen challenge, sodium cromoglycate and nedocromil sodium could inhibit all three components, whereas inhaled corticosteroids inhibited the late phase response and acquired bronchial hyperresponsiveness and β_2 agonists only the early response, further increased interest in allergen provocation as a dynamic model of airway inflammation in asthma.

Through measurement of mediators in bronchoalveolar lavage fluid, blood, and urine a convincing case has been made for secretion of mediators from activated mast cells as underlying the early asthmatic response. More definitive evidence for the part played by mast cells has come from studies using specific mediator receptor antagonists (H_1 histamine, LTD_4 and prostaglandin (PG) D_2 , thromboxane (TX) A_2) (cyclooxygenase, 5-lipoxygenase), and inhibitors that have been shown to attenuate the early asthmatic reaction when provoked either with allergen or with exercise (reviewed in ref 41). The mediators most likely to be responsible for acute airway narrowing in these reactions, in order of their potency, are LTC_4 and LTD_4 , PGD_2 (and its metabolite $9\alpha,11\beta$ - PGF_2), and histamine. In the case of exercise induced asthma mast cells in the asthmatic airway appear to be "primed" to respond to hypertonic stimuli to release both preformed and newly generated mediators. Direct vision of the airways after local challenge with allergen or hypertonic saline shows a rapid reduction in airway calibre that is easily reversible with a β_2 agonist. The most likely cause for this reduction in calibre is contraction of the underlying airway smooth muscle, which contains receptors for mast cell derived histamine, PGD_2 , TXA_2 , LTC_4 , and LTD_4 .

The efficiency of LTD_4 antagonists in inhibiting the allergen induced late phase res-

ponse suggests a further important role for this mediator.⁴² Both in animal models and more recently in human asthma the late phase reaction has been shown to occur in association with an influx into the airways of neutrophils followed by eosinophils. Bronchial biopsy specimens taken 4–6 hours after local allergen challenge show a substantial increase in neutrophils and eosinophils within the submucosa and epithelium, with cells seen to be adhering to and migrating through the microvasculature. These changes occur in parallel with an increased expression of specific adhesion molecules expressed on postcapillary venular endothelial cells, specifically ELAM-1 and ICAM-1, and with an influx into the airway of cells expressing the ligand complementary to ICAM-1, the integrin LFA-1 (Cd11a/Cd18), which is expressed on most leucocytes.⁴³

The factors responsible for the allergen induced upregulation of ELAM-1 and ICAM-1 on endothelial cells include the cytokines IL-1, $TNF\alpha$, and interferon (IFN - γ).⁴⁴ Twenty four hours after challenge a third adhesion molecule belonging to the immunoglobulin superfamily, called VCAM-1, is expressed in greater numbers within the airway microvasculature.⁴⁵ VCAM-1 interacts with a specific integrin ligand expressed on lymphocytes and eosinophils into the airway. The capacity of IL-4 to upregulate VCAM-1 expression on endothelial cells⁴⁷ provides an important link with the early events following allergen challenge. Although it is most unlikely that, when exposed to allergen, T_H2 lymphocytes can generate sufficient IL-4 to upregulate VCAM-1 expression, we have recently shown that in biopsy specimens from patients with asthma preformed IL-4 can be localised to mast cells.⁴⁸ Further experiments have shown that cross linkage of mast cell bound IgE releases both $TNF\alpha$ and IL-4 in large quantities without the necessity to synthesise new protein. This provides an

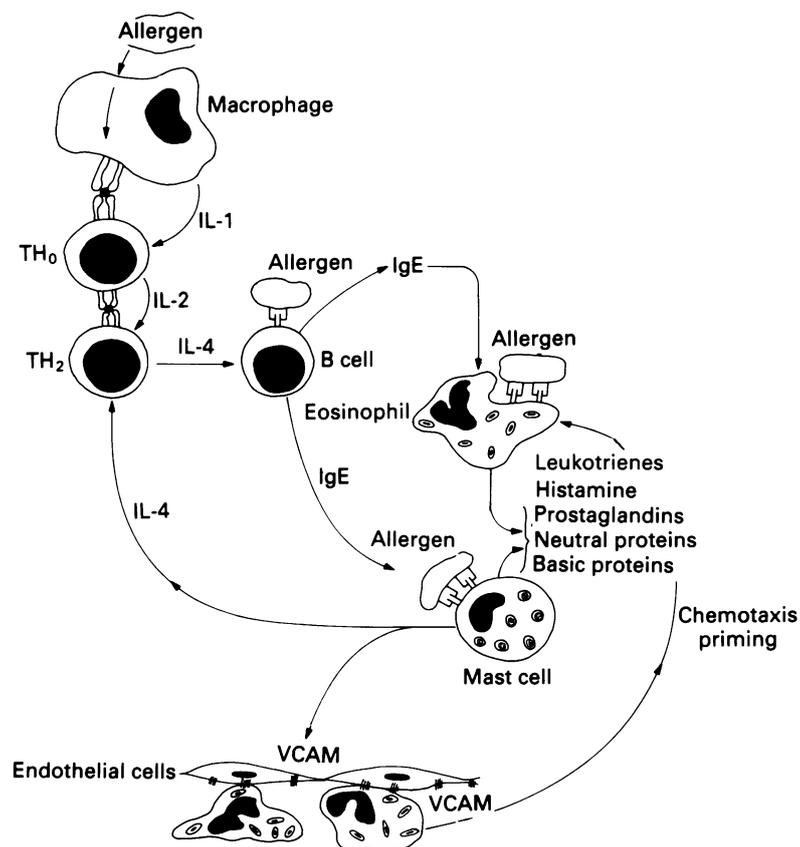


Figure 5 Augmenting role of the mast cell and interleukin-4 in the allergic airway inflammation.

important link between mast cell activation, upregulation of adhesion molecules, and the subsequent T cell and eosinophil recruitment that occurs in the late phase response. These observations indicate a novel role for the mast cell as an early instigator of allergen provoked inflammatory events (fig 5). The fact that T_H2 cells also depend on IL-4 for their survival and proliferation⁴⁹ indicates a further role for mast cells in augmenting and prolonging T cell dependent inflammation.

T lymphocytes may also have an important role in initiating early inflammatory events in the airway. We have shown that within 15 minutes of allergen challenge a subset of T cells is lost from the airway lumen, presumably owing to their migration into the bronchial epithelium.⁵⁰ Within this epithelium there are specific macrophage like cells (dendritic cells), which have a key role in presenting antigen to T cells⁵¹ (figs 4 and 5). How the T cell population within the bronchial epithelium communicates with immune cells within the airway wall is not known, however. Possibly the main function of dendritic cells is to initiate the inflammatory cascade based on mast cells and eosinophils at the interface between the environment and the internal milieu.

Targets for the inflammatory response

Although mediators derived from mast cells and eosinophils clearly have potent effects on airway smooth muscle and the microvasculature, these seem unlikely to be the primary

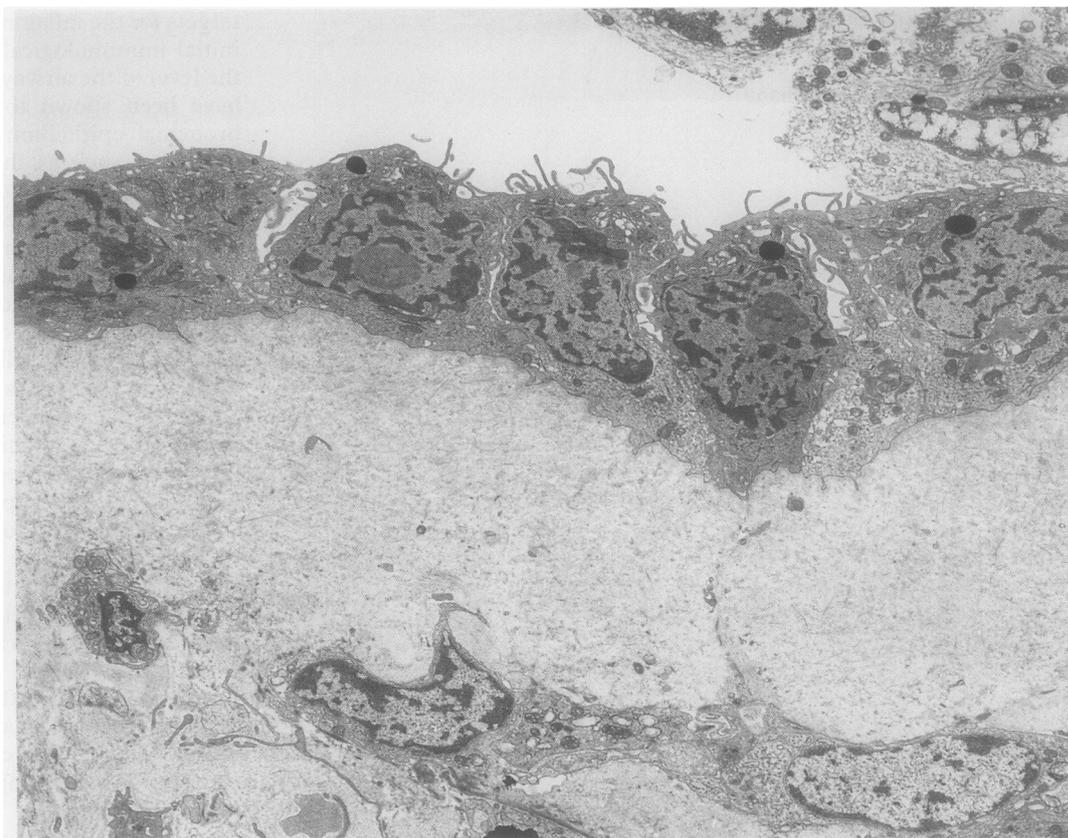
targets for the inflammatory response when the initial immunological confrontation occurs at the level of the airway epithelium. Eosinophils have been shown to selectively damage the bronchial epithelium through the release of mediators such as the arginine rich proteins and active radicals of oxygen.⁵² We have shown that in mild to moderate asthma there is selective loss of columnar epithelial cells from their attachment to basal cells.^{53 54} The integrity of the pseudostratified bronchial epithelium is maintained through desmosomal adhesion of adjacent columnar cells and between these and basal cells, which are themselves attached firmly to the basement membrane via hemidesmosomes. Loosening of these links probably accounts for the epithelial fragility that has been so frequently reported in asthma.^{18 54} The precise mechanisms for this have not been fully worked out, but one possibility is a cognate interaction between the eosinophil leucocyte and the epithelial cell with the induction of metalloendoproteases and nitric oxide synthase by epithelial cells, which either directly (proteases) or indirectly (nitric oxide and active radicals) degrade matrix proteins found in desmosomes.⁵⁵ Mast cell tryptase also has the capacity for disrupting these points of attachment, providing a further function for intraepithelial mast cells. The net result of these changes is a breakdown in the permeability barrier of the bronchial epithelium, an abnormality that has now been shown clearly in acute asthma.⁵⁶ Loss of the permeability barrier would enable tissue damaging molecules to pass from the airway lumen into the airway wall unimpeded, and provide further stimuli for the development of chronic inflammation.

Damage to the epithelium might explain why "thickening of the basement membrane" is a characteristic pathological feature of asthma (fig 6). We have shown that this material consists of interstitial collagens (types I, III, and V) laid down by a special group of mesenchymal cells that proliferate under the bronchial epithelium, having characteristics of both smooth muscle cells and fibroblasts (myofibroblasts).⁵⁷ Subepithelial fibrosis may be interpreted as an attempt by the airway to seal a leaking epithelium, but by doing so it brings additional sources of proinflammatory factors, such as GM-CSF and *c-kit* ligand, which are capable of sustaining eosinophils and mast cells respectively.

Clinical interpretation of viewing asthma as an inflammatory disease

Although allergen induced airway inflammation may not underlie acute exacerbations of asthma, there is mounting evidence to suggest that they create the necessary environment within the airway, on which many other stimuli may act. Of particular interest is the upregulation of ICAM-1 in the bronchial epithelium—which, in addition to being an important adhesion molecule for inflammatory leucocytes, is also the receptor for the major type of rhinoviruses.⁵⁸ A breakdown in the epithelial

Figure 6 Transmission electron micrograph showing the epithelial disruption and deposition of collagen on the subbasement membrane in asthma.



permeability, with loss of control of the constituents of the airway lining fluid, provides the necessary prerequisite for generating a hyperosmolar environment with exercise in patients with exercise induced asthma,⁵⁹ while exposure of afferent nerve terminals within the epithelium is a stimulus for neurogenic inflammation.

Both national and international guidelines on the management of asthma emphasise the need to avoid provoking factors, such as allergens, and implement early anti-inflammatory treatment, with drugs such as topical corticosteroids, sodium cromoglycate, and nedocromil sodium, rather than relying on symptomatic treatment with bronchodilators alone. Their advice now has a basis in relation to pathogenetic mechanisms. Bronchial mucosa, eosinophils, and T cells increase in number in relation to seasonal exposure to allergens⁶⁰ and inhaled corticosteroids have been shown to have a profound effect in reducing both mast cell and eosinophil populations within the airway wall,^{61 62} presumably by their inhibitory effect on cytokine production by T cells and antigen presenting cells (J W Wilson *et al*, unpublished findings). The therapeutic efficacy of drugs such as sodium cromoglycate and nedocromil sodium might be explained, at least in part, on the basis of inhibition of release of preformed cytokines (for example IL-4, TNF α). Even β agonists, although most of their activity depends on their capacity to cause acute dilatation, may exert some inhibitory effects on infiltrating inflammatory cells, though the former activity is likely to greatly outweigh the latter.¹²

Over the last five years enormous advances have been made in understanding the

immunological and environmental mechanisms of asthma, but still many questions remain unanswered. One particular outstanding question is how and why the asthmatic airway remains chronically inflamed even in the apparent absence of allergen, and what effect this inflammation has on the behaviour of the formed elements of the airway, including nerves, mucus secreting cells, microvasculature, and airway smooth muscle. The application of modern techniques of cellular and molecular biology should provide the necessary tools to answer many of these questions in the coming decade.

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