

Mediator and cellular mechanisms in asthma

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Asthma is among the commonest medical emergencies confronting the practising physician and, being highly responsive to therapeutic intervention, is one of the most rewarding diseases to treat. The high prevalence of the disease together with the usually rapid response to treatment has led to complacent attitudes in its management and too much reliance upon crisis management [1]. These attitudes are reflected in retrospective analyses of asthma deaths, from which it is clear that both patients and doctors underestimate the severity of the disease, and there is too great a reliance upon bronchodilators and inadequate use of corticosteroids; all of this can and should be avoided [2, 3]. With so many efficacious drugs available for treating asthma, it is also of considerable concern that in many countries (including the UK) the mortality and morbidity of asthma continues to increase rather than decrease [4, 5].

Historically, clinicians have regarded asthma as a disease of intermittent wheezing, precipitated by a wide variety of environmental stimuli including exercise, cold air, dust and irritant gases. This has led to the disease being considered in terms of airways smooth muscle contraction with attacks of 'bronchospasm'. With such a widely held concept of the disease it is hardly surprising that medical practice has concentrated on the use of inhaled bronchodilator β_2 agonists as a mainstay of treatment [6]. However, there is growing concern that these drugs, which produce a rapid reversal of 'bronchospasm', serve to treat symptoms alone rather than the underlying disease processes [7].

Why do the airways of asthmatic patients behave differently from those of normal subjects, and why are the therapeutic strategies used at present not having the expected effect of reducing the morbidity and mortality of this disease?

Inducing and inciting factors in asthma

The view that asthma is a disease of reversible airways obstruction gives rise to confusion over how the term

'asthma' is used and, as a consequence, how different drugs should be used to treat it. Dr Henry Hyde Salter, in his treatise on asthma published in 1860, described it as a syndrome of paroxysmal dyspnoea [8]. Despite numerous attempts, this clinical description has not been improved upon, but it is not suitable as a definition [9, 10].

The ability of a wide range of stimuli to provoke an acute attack of bronchoconstriction indicates an abnormal state of airway function, referred to as hyperresponsiveness or hyperreactivity [11]. In the laboratory a wide range of controlled stimuli to the airways can reveal hyperresponsiveness which can be quantified. Most frequently used are the inhaled smooth muscle spasmogens histamine and methacholine [12], although physical stimuli such as exercise, cold air challenge and eucapnic hyperventilation have also been adopted as laboratory indices for hyperresponsiveness [13, 14]. Increased responsiveness of the airways to a wide range of naturally occurring stimuli offers a basis for 'bronchospasm' in asthma [15, 16] and convincingly demonstrates that the disease is much more than 'symptomatic bronchial hyperresponsiveness'.

Of more concern to the clinician are the prolonged periods of airway obstruction, with or without increases in responsiveness, which punctuate the life of patients with asthma. Indeed, it is these episodes that most frequently take the patient to the medical practitioner to be initially diagnosed as asthmatic and for changes in treatment. Figure 1 demonstrates the large fluctuations that can occur over a 6 months period in a child with diagnosed asthma. Up to 20% of 7-8-year-old children experience episodes of symptomatic bronchoconstriction similar to those shown in Fig. 1. It is therefore of some concern that in up to a third of them a diagnosis of asthma has not been established and therefore appropriate treatment has not been given [17].

In most children and young adults asthma occurs in association with atopy, the genetic predisposition for immunoglobulin E synthesis against epitopes expressed on common environmental aeroallergens such as house dust mite, animal proteins, plant pollens and fungal hyphae [18, 19]. Extended family studies

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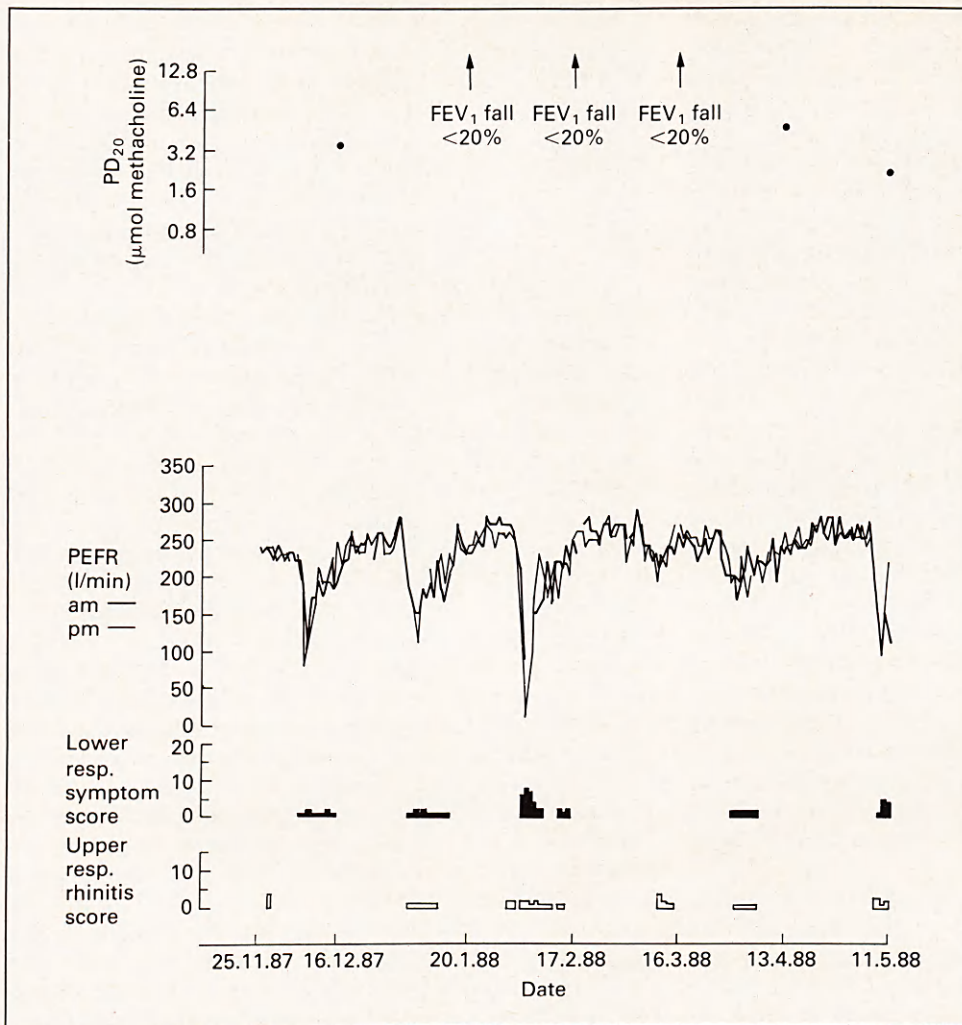


Fig. 1. Continuous 6 months clinical profile from an 8-year-old child with asthma. PD_{20} : provocative dose of inhaled methacholine causing a 20% fall in FEV_1 . PEF: peak expiratory flow rate recorded in the morning and evening. Note that the most severe and prolonged asthma episodes occur in relation to nasal symptoms and in parallel with large changes in PEF.

indicate that the high IgE response is inherited as an autosomal dominant gene [20] which has been tentatively localised to the long arm of chromosome 11 [21]. In these genetically predisposed individuals, continued exposure of the respiratory tract to inhaled aeroallergens sensitises and subsequently provokes episodes of allergic asthma such as occurs with grass pollens (seasonal asthma), house dust mite and cat dander (perennial asthma) and some occupational sensitisers, eg platinum salts [18, 22]. These environmental events are considered as inducing factors which interact with the airways to produce the abnormal asthmatic state [11].

Inflammation as the basis of asthma

In the first edition of his classic textbook *Principles and practice of medicine* (1892) William Osler refers to asthma as 'a special form of inflammation of the smaller bronchioles—*bronchiolitis exudativa*'. Pathologists have long recognised that an important factor contributing to an asthma death is the occlusion of both large and small airways with tenacious mucus mixed with serum proteins and cell debris [23, 24]. However, because of

a lack of detailed pathology in patients with day-to-day asthma, a cellular basis for disordered airway function has been difficult to obtain. Peripheral blood and sputum eosinophilia are common findings during an exacerbation of asthma [25]. Other features of the asthma expectorate during and following attacks are the presence of Charcot-Leyden crystals (eosinophil-derived lysolecithinase) and Creola bodies (clumps of ciliated epithelial cells), suggesting that both leukocyte infiltration and epithelial damage are components of disease activity.

The use of the fiberoptic bronchoscope offers new approaches for investigating airway pathology in day-to-day asthma. Bronchoalveolar lavage (BAL) and washing of isolated segments of the bronchi have yielded mast cells, eosinophils and neutrophils from the airways of mild-moderate atopic asthmatics in quantities compatible with their involvement in disease pathogenesis [26–28]. Studies of cellular function in terms of inflammatory mediator release and cell surface receptor expression incriminate these and other inflammatory cells, including T-lymphocytes and macrophages, as effectors of airway dysfunction in asthma. The increased concentrations of known spas-

mogenic and vasoactive mediators, including histamine [29], prostaglandin (PG)D₂ [30], sulphidopeptide leukotrienes (LTs) C₄, D₄ and E₄ (slow reacting substance of anaphylaxis (SRS-A)) [31] and bradykinin [32], in BAL fluid from asthmatic airways indicate that products derived from inflammatory cells and serum proteins are likely candidates for causing airways obstruction and increased responsiveness.

However, while lavage has created one opportunity for investigating airway events in asthma, interpreting the findings is complicated by lack of knowledge concerning the anatomical structures reached by the lavage fluid, exchange of fluid between compartments within the lung and airway events deep to the bronchial epithelium. For these reasons we have sought further information using biopsy of the airway mucosa at the level of the subcarinae [33]. After passing the fiberoptic bronchoscope into the lower airways under local anaesthesia and with bronchodilator cover, three to four mucosal biopsies may be obtained from the subcarinae. These 0.5–1 mm biopsies are embedded in plastic and semi-thin sections cut to study the epithelium and submucosa in detail. Figure 2 shows the characteristic morphology of the normal bronchial mucosa. This comprises a pseudostratified ciliated epithelium containing goblet cells attached to a basement membrane beneath which there is a loose stratum of connective tissue supporting the bronchial circulation and free elements of the bronchial-associated lymphoid tissue (BALT).

In mild atopic asthma, ie when individuals required only occasional use of their inhaled bronchodilator [33], the bronchial mucosa was abnormal in all cases investigated. Particularly noticeable was the presence of subepithelial mast cells in various stages of degranulation (Fig. 3) and the widespread infiltration of the airway wall with eosinophils, most of which exhibited ultrastructural features of activation and degranulation. Many eosinophils, neutrophils and mononuclear cells were present within the postcapillary venules, frequently in close contact with endothelial cells (Fig. 4).

A striking abnormality is the apparent thickening of the subepithelial basement membrane in airway sections obtained from patients who had died from asthma. A similar phenomenon was present in mucosal biopsies of the subjects with mild asthma (Fig. 5). The basement membrane itself was of normal thickness, but the thickened band consisted of dense crosslinked collagen fibrils [34]. Using a panel of monoclonal antibodies directed against epitopes of different collagen subtypes, immunohistochemical analysis of the biopsy material showed the presence of type IV collagen together with laminin and fibronectin localising to the 'true' basement membrane in tissue from both the normal controls and patients with asthma. The band of subepithelial collagen beneath this 'true' basement membrane comprised types I, III and V together with fibronectin but not laminin. These data suggest a fibroblast origin for this material rather than representing a true thickening of the basement membrane itself. This increase in connective tissue probably origi-

nates from myofibroblast cells with both collagen synthetic and contractile properties [35]. Indeed the number of myofibroblasts in the biopsies correlated closely with the extent of subepithelial thickening.

The changes seen in mucosal biopsies from patients with day-to-day asthma are similar (but less extensive) to those reported in post-mortem studies. For further progress it is necessary to make a more detailed evaluation of the cell types comprising the inflammatory response together with an assessment of their functional significance. For this purpose subjects with symptoms of asthma had a detailed assessment of their clinical disease including measurement of airways responsiveness and, for 2 weeks prior to bronchoscopy, a continuous record kept of symptoms, bronchodilator requirement and twice daily peak expiratory flow (PEF) [36]. This was a more symptomatic group of patients than in our first study, but all used inhaled bronchodilators as their only treatment. Immunohistochemistry was used to identify and quantify mast cells and eosinophils in five adjacent semi-thin sections of the epithelium and submucosa.

Mast cells were identified using a monoclonal antibody directed to human mast cell tryptase (AA-1) [37]. Tryptase is a tetrameric neutral protease (MW 234,000 daltons) which comprises approximately 60% of the total protein in the secretory granules of all human mast cells but is not present in other cells [38]. Eosinophils were identified by the presence of cationic protein within the matrix of their granules using a monoclonal antibody designated EG-2 [39]. Mast cells and eosinophils were easily identified, both in the epithelium and in the submucosa, with an avidin-biotin-enhanced immunoperoxidase technique [36]. About half of the asthma biopsies showed increased numbers of tryptase-staining mast cells both in the epithelium and in the submucosa when compared with tissue obtained from control subjects. Transmission electron microscopy of mast cells from the asthmatic but not the control biopsies displayed all the ultrastructural features of active degranulation [40], suggesting continuous mediator secretion. The number of mast cells in the epithelium correlated with the pre-bronchoscopy FEV₁, a finding that has also been reported for mast cell numbers in BAL [27, 28, 41] and their mediators in the cell-free supernatant [29]. With the knowledge that selective histamine H₁-receptor antagonists have a bronchodilator action in asthma [42], it is likely that mediator secretion from mast cells contributes towards airway narrowing in this group of disorders.

Eosinophils staining with EG-2 were also seen in large numbers both in the submucosa and in the epithelium of the asthma biopsies but not in those from the normal controls [36]. Under the electron microscope, the eosinophils exhibited granule heterogeneity both in size and electron density, strongly suggesting a heightened state of activation. Indeed, the presence of EG-2 positive material in the extracellular space supports observations made in BAL studies of asthma [27, 28], that eosinophils in this disease are



Fig. 2. Transmission electron micrograph of the bronchial epithelium as observed in subcarinae mucosal biopsy from a normal volunteer. Note the ciliated epithelial cells and mucus granule-filled goblet cells (magnification $\times 1,650$).

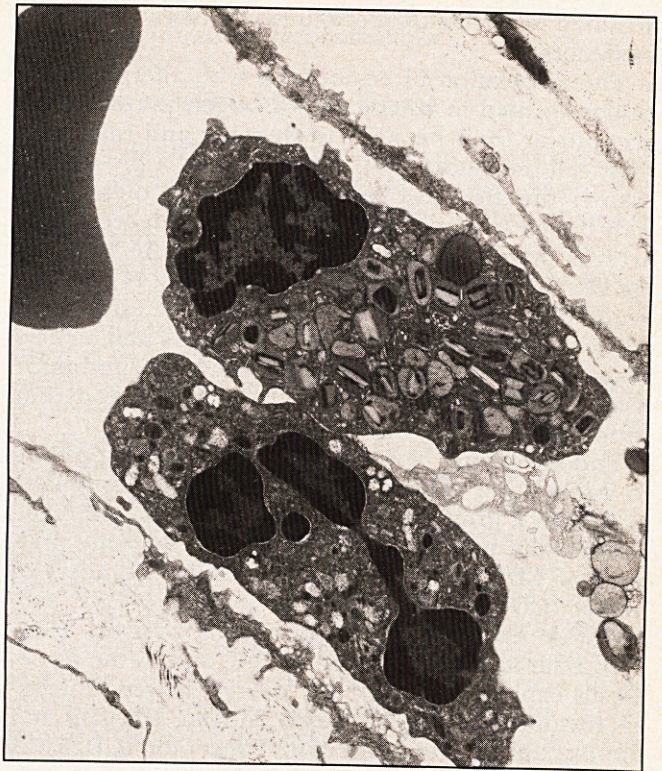


Fig. 4. Transmission electron micrograph of an eosinophil (top) and a neutrophil (bottom) lying within the lumen of a bronchial submucosal post-capillary venule. Note the marked differences in granule structure between the two cells, the related loss of the central crystalloid core (cationic MBP) from the eosinophil and the close association that these cells have to the endothelium (magnification $\times 5,500$).

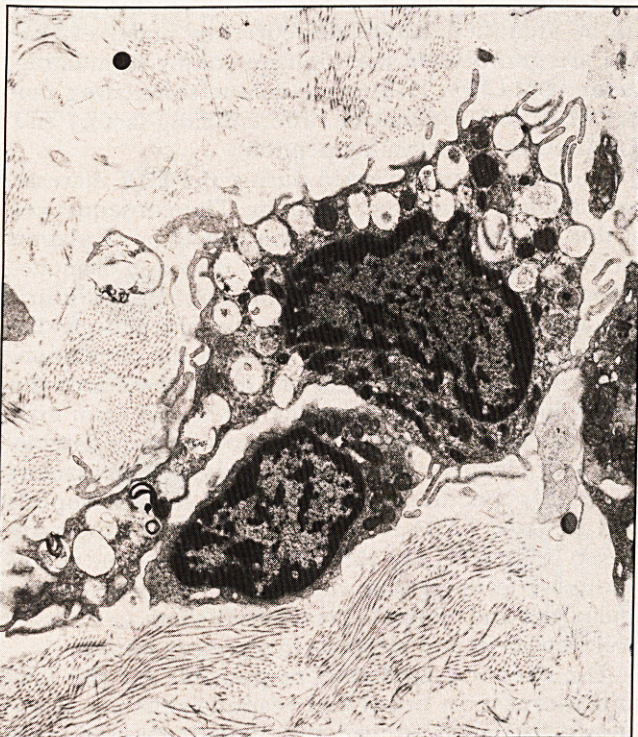


Fig. 3. Transmission electron micrograph of a mast cell embedded in collagen matrix from a patient with symptomatic atopic asthma. Morphological characteristics of degranulation are seen (magnification $\times 5,500$).



Fig. 5. Bronchial basal epithelial cells (top) attached to a normal basement membrane beneath which there is extensive deposition of crosslinked interstitial collagen; beneath this lies a small capillary (magnification $\times 1,250$).

activated to secrete their granules and newly generated mediators.

The association of eosinophils with allergic tissue responses used to be considered largely protective on account of their capacity to degrade and inactivate mast cell mediators [43]. More recently a pro-inflammatory function has been attributed to these cells, with the clear demonstration that they can secrete an array of potent mediators such as LTC₄, PAF, PGI₂, 15-HETE, O⁻ and granule cationic proteins [44]. In this group of patients no relationship could be established between mast cell or eosinophil numbers and airways responsiveness, symptom scores or bronchodilator usage, although positive correlations were apparent within these clinical variables.

Endobronchial biopsies have also made it possible to assess other cellular components in the asthmatic airway. In a group of mild-moderate atopic asthmatic subjects the number of T-cells identified by the presence of CD3 receptors and the relative numbers of helper (CD4⁺) and suppressor/cytotoxic (CD8⁺) subsets were not greater than in normal controls. However, both in cells recovered by BAL and in the tissue, T-cells expressed to a greater degree activation markers including the MHC class II antigen, HLA-DR and the β -chain of the interleukin 2 receptor (CD25). A prominent feature of the bronchial epithelium in asthma is the increased expression of HLA-DR not only on intraepithelial T-cells and antigen-handling dendritic cells but also on ciliated epithelial cells.

Role of inflammatory mediators in asthma

The ability of inhaled allergen to provoke attacks of asthma in atopic subjects has created an opportunity to dissect the cellular and mediator components of this response. As Pepys and others have shown more than 20 years ago, provocation of the airways with an extract of allergen to which the subject was sensitive provoked an early phase of bronchoconstriction, peaking at 15–20 minutes and recovering within 2 hours [45]. This early asthmatic response (EAR) is then followed over the next 4–12 hours by a late phase of airways obstruction (LAR) accompanied by an increase in bronchial responsiveness to agents such as histamine and methacholine and lasting for several days [46]. When administered prior to allergen challenge, the ability of the anti-asthma drug sodium cromoglycate to inhibit all three of these responses, and of inhaled oral corticosteroids to attenuate the LAR and hyperresponsiveness, supports the view that this experimental model of asthma has features relevant to the airway events of clinical disease [47].

Direct measurement of mediators and their metabolites in the peripheral blood, BAL and urine, together with observations using highly selective mediator receptor antagonists and synthesis inhibitors, provides convincing evidence that IgE-dependent mast cell activation is responsible for the EAR in a way analogous to the role of such factors in producing the skin wheal and flare response with allergen [48]. The mast cell

mediators which account for most of the EAR are histamine (via H1 receptors), PGD₂ (via TP1 receptors) and LTD₄ and LTE₄ (via sulphidopeptide leukotriene receptors). Mediators released secondary to mast cell activation which may also contribute include thromboxane TXA₂, PGF _{α} , PGF_{2 α} , 15-hydroxyeicosatetraenoic acid (15-HETE), bradykinin and platelet activating factor (PAF).

Bronchoalveolar lavage studies indicate that, from the onset of the EAR, there is active recruitment of leukocytes from the bronchial circulation into the airway wall [49, 50]. This appears to be a multi-step process leading to selective entrainment of eosinophils, neutrophils and T-cells, all of which have been incriminated in the pathogenesis of the LAR. Mediators released from mast cells and macrophages interact with endothelial cells to upregulate the expression of adherent molecules of the integrin family. Although the definitive nature of these molecules has yet to be defined in asthma, it is likely that they interact with the hetero-dimer CD11b/CD18 (Mac 1) which is preferentially expressed on eosinophils and neutrophils, and CD11a/CD18 (LFA-1) on T-lymphocytes and eosinophils [51]. In non-human primates there is some evidence to indicate that LFA-1 interacts with the cellular adhesion molecule-1 (ICAM-1) expressed on endothelial cells. Mediators which promote leukocyte-endothelial adherence include PAF, the sulphidopeptide leukotrienes, the dihydroxy leukotriene-LTB₄ and the cytokines-tumour necrosis factor (TNF) and interleukin (IL) 1. It is likely that other molecules which serve to augment the selectivity of this process will be identified; candidates currently under evaluation are granulocyte macrophage colony stimulating factor (GM-CSF), IL-3 (multi-colony stimulating factor), IL-4, IL-5 (eosinophil differentiating factor) and IL-8 (neutrophil activating peptide).

Other mediators released during the EAR increase post-capillary venule permeability. The consequences of this are mucosal oedema, the provision of plasma protein substrates for cleavage into vaso- and bronchoactive kinins (bradykinin, lysylbradykinin (kallidin), C5a and angiotensin) and facilitation of leukocyte diapedesis [52]. Once they have escaped the circulation, eosinophils and neutrophils are directed towards the airway lumen by chemoattractants released from mast cells, macrophages and T-cells following their interaction with allergen. Some of these chemotactic factors are highly specific, eg IL-5 and PAF for eosinophils and IL-8 and LTB₄ for neutrophils, while others are less so (C5a, GM-CSF) [53]. Moreover, the same agents also serve to upregulate cell surface receptors on the leukocytes and 'prime' (preactivate) them to increase their sensitivity and response to secretory stimuli.

The chemical mediators responsible for the LAR and increase in bronchial responsiveness are not known. Studies in animals and limited observations in humans suggest that the sulphidopeptide leukotrienes, PAF, TXA₂, kinins, reactive oxygen species and histamine may all contribute [54]. It is widely thought that most of the airway narrowing during the EAR

results from contraction of smooth muscle, although there is recent evidence to show that mucosal swelling may also be important, particularly in the more peripheral airways. While spasmogens released during LAR may also produce airway narrowing, oedema of the airway wall and hypersecretion of mucus are considered to be more important [55]. Thickening of the bronchial mucosa and swelling of the airway wall may both contribute towards the increased responsiveness observed during and after the LAR by a purely geometric effect [56, 57] and by increasing the surface area over which the distending retractile forces of the surrounding alveoli are exerted [58].

The eosinophil is a prime candidate for mediating the LAR allergen. Studies in animal models and BAL in humans demonstrate a massive influx of these cells which, once activated, secrete a wide array of inflammatory products. Their granule proteins, especially major basic protein (MBP) in the crystalloid granule core and cationic protein in the granule matrix, are cytotoxic towards bronchial epithelial cells and are also mast cell and basophil secretagogues [59]. These arginine-rich proteins also increase responsiveness of airways smooth muscle both *in vitro* and *in vivo*. For eosinophils to reach their maximum potential as mediator secreting cells, their association with endothelial cells and T-cells seems important probably by prolonging their life and enhancing their secretory capacity [60]. Four cytokines (IL-3, IL-4, IL-5 and GM-CSF) have the biological profiles to serve these functions. The ability of corticosteroids to inhibit cytokine release from activated T-lymphocytes and macrophages [61, 62] may explain why this class of drug is so

effective at suppressing the LAR and the associated increase in bronchial responsiveness.

Asthma as an immunological disease

Figure 6 illustrates in schematic form how the various cellular elements may interact to produce disordered airway function in asthma. Central to this scheme is the division of the processes into those of antigen recognition and memory (induction) and those of effector cell recruitment and mediator release. In atopic asthma there is strong evidence for the involvement of dendritic cells and macrophages in the recognition of allergen through the HLA-DR system. The presentation of this signal in conjunction with IL-1 to T-cells is the next important step which locks the subsequent immunological response into memory. It is at this stage that a number of different factors may interplay to direct the response towards an IgE–mast cell–eosinophil driven system. Genetic factors are clearly important in this, especially in the form of atopy. However, atopy alone is not a sufficient reason for developing asthma. Other genetic factors such as mediator releasability and the ability to terminate an inflammatory response efficiently may also be important. It is probable that environmental adjuvant factors such as virus infections and exposure to air pollutants also contribute [63]. Whatever the nature of these interactions, the net result is an immunological memory which, when recalled, initiates an immunological response involving IgE, mast cells and eosinophils not dissimilar to that required for parasite elimination.

Several recent discoveries appear to be crucial in

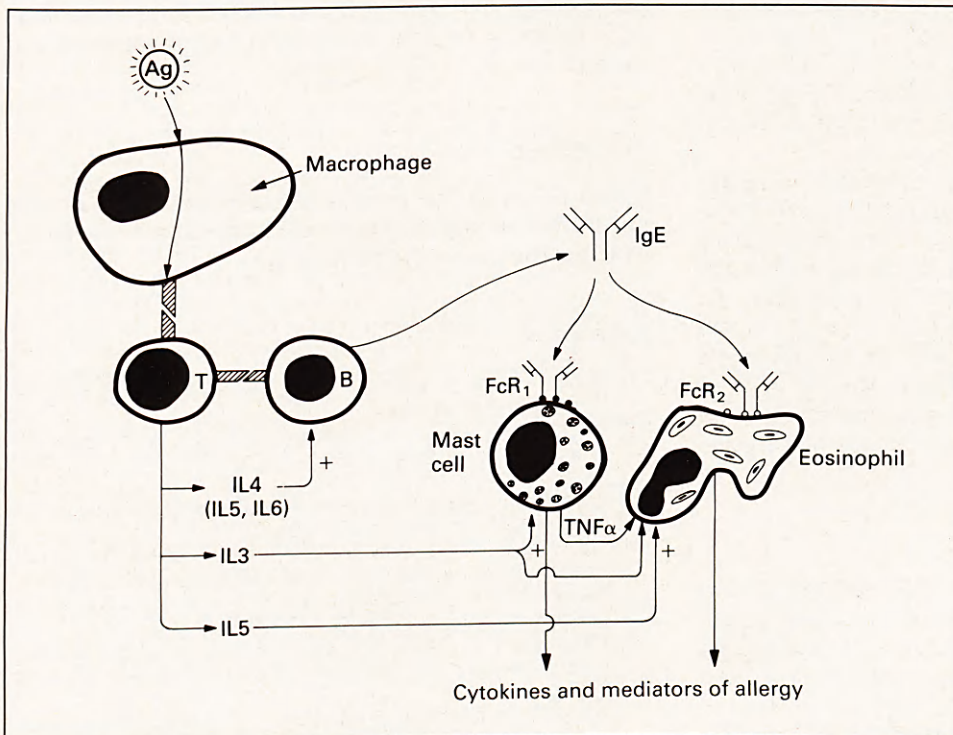


Fig. 6. Schematic representation of cell and cytokine cooperation in atopic asthma.

understanding the nature of the immunological response in human allergic diseases such as asthma. Presentation of allergen to T-lymphocytes obtained from atopic subjects leads to the preferential generation of IL-4 which, in the presence of IL-2, has the effect of switching B-cell immunoglobulin synthesis from IgM to IgE (isotope switch). Conversely, interferon- γ downregulates this process [64]. Further, IL-3 and IL-5 derived from activated T-cells (possibly of the TH-2 subtype) and other cells are growth, maturation and priming factors for mast cells. Interleukin-5 and GM-CSF derived from T-cells, macrophages, mast cells and endothelial cells are involved in the processing of eosinophils to optimise their function as mediator secreting cells [65].

The characteristic type of inflammation observed in atopic asthma makes it likely that these cytokine pathways are centrally involved in the pathogenesis of airway inflammation in this disease. Recruitment of these pathways not only provides a rational explanation of how IgE, mast cells and eosinophils interrelate, but also emphasises the importance of allergen recognition during the process of sensitisation. Corticosteroids are potent inhibitors of cytokine production. In a recently completed study we have shown that beclomethasone dipropionate administered by inhalation in a dose of 2000 $\mu\text{g}/\text{day}$ for 2 weeks and 1000 $\mu\text{g}/\text{day}$ for a further 4 weeks to a group of symptomatic atopic asthmatics resulted in marked reductions in the number of mast cells and eosinophils present in the submucosa, in parallel with a diminution in symptoms, bronchodilator usage, PEF variability and methacholine responsiveness. Although it is possible that the beneficial effect of topical corticosteroids in asthma relates to their actions on vascular permeability and in inhibiting phospholipase activity responsible for the generation of the newly formed lipid mediators, their potency in downregulating cytokine production is also likely to be of considerable importance, especially since these drugs have no direct effect on mast cell function [66].

Although not fashionable at the present time, modifications of the environment to limit the level of exposure to allergens and adjuvant factors at a critical time during the development of sensitisation are additional possibilities for future therapeutic intervention. In support of such an approach we have recently shown that the level of exposure to the major allergen (DerP1) of the house dust mite within the first year of life is a significant factor in the subsequent development of asthma when assessed at the age of 11 years [67].

Concluding comments

Much of the discussion presented in this paper has focused on allergic asthma. However, bronchial biopsy data also indicate that other clinical types of asthma such as that induced by occupationally related small molecular weight chemicals, and 'intrinsic' asthma, are also inflammatory disorders of the airways involv-

ing T-cells, mast cells, eosinophils and neutrophils. Thus, although we understand the mechanisms responsible for these variants of the disease less well than those for classical atopic asthma, it is likely that common inflammatory pathways will be found to link their pathogenesis.

A clearer understanding of how the various cellular and mediator components of asthma interact provides rational explanations for the clinical expression of asthma, its variation and how currently used anti-asthma drugs work. As mediator and cytokine networks become better defined, new opportunities arise for designing drugs to interrupt various stages of the inflammatory response. At present there are no animal models of human asthma, only models of components of the disorder. Thus, further careful investigation of various clinical forms of the disease is warranted in the hope of shedding further light on pathogenetic mechanisms.

With increasing emphasis being placed on the importance of inflammatory processes underlying the airways obstruction and increased airways lability in asthma, it would seem logical that our therapeutic strategy should move away from heavy reliance on bronchodilators, more towards the prophylactic anti-inflammatory drugs such as sodium cromoglycate, nedocromil sodium and inhaled corticosteroids. Now that patients can objectively monitor the progress of their disease using peak expiratory flow meters and learn more about the underlying nature of the disease and how to use the various forms of treatment available to them, it should be possible to move away from the current practice of crisis management and more towards prophylaxis and self management. It is reasonable to hope that implementation of these strategies will produce the much needed reduction in morbidity and mortality of this common affliction of modern society.

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