

## THE 1992 COUNAND LECTURE

# Asthma: past, present and future

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**ABSTRACT:** The association of asthma with the release of inflammatory mediators, through a mechanism that involves the immune system, has taken almost 100 years to evolve. While studies on lung tissue from patients who had died from asthma pointed to inflammation of the airways as a major lesion, clinicians have, until relatively recently, preferred to consider the disease more in terms of airways dysfunction rather than the cause(s) of this dysfunction.

Bronchial mucosal biopsy and lavage has reaffirmed the view that asthma is a special type of airway inflammation involving mast cells and eosinophils which is orchestrated by T-lymphocytes of a Th2-like phenotype. Through cytokine release, these cells upregulate the function of mast cells and eosinophils. However, other factors are now considered important in the maintenance and chronicity of the inflammatory response both through tissue remodelling (e.g. fibrosis) and through the secretion of cytokines from epithelial, endothelial and constitutive mesenchymal cells.

Evidence is accumulating that these events are genetically-linked although this is likely to involve multiple genes. Of special importance is the role of the environment both in the induction of allergen sensitised airways and the subsequent inflammatory response that follows. Included in these are intrauterine events, early life exposure to allergens, pollutants and viruses. If prevention of asthma is to be a future target for intervention, then future work should concentrate on those early genetic and environmental factors that initiate the inflammatory response rather than relying on strategies that attempt to reverse established disease.

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Bronchial asthma has been referred to in the literature for over 2,000 yrs, but it was only in the middle of the last century that real progress was made towards defining the nature of the condition and its possible causes. In his treatise (1886) entitled "On Asthma, Its Pathology and Treatment", Dr Henry Hyde Salter, a physician and later Dean of Medicine 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 own personal experience as an asthma sufferer and to an analysis of the few cases of the disease that he was able to find in London Teaching Hospitals at that time. Almost 100 yrs 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 caliber over short periods of time either spontaneously or in response to treatment" [2]. Further consideration was given to this description at a Ciba Foundation Study Group in 1971, but the conclusion of those who participated was that there was insufficient information for a clear definition to be agreed [3].

A lack of understanding of the underlying processes

responsible for asthma has resulted, in the main, from the inaccessibility of airway tissue for detailed study. This, in part, has been responsible for a strong research emphasis being placed on physiological methods to assess airway function. With the introduction of the spirometer and peak expiratory flow meter for use both in the clinic and in the community, physicians became increasingly aware of the chronicity of the disease, and often of the disparity between the presence of symptoms and objective measures of air flow observation. The additional feature of airways hyperresponsiveness as a characteristic of asthma was incorporated into the American Thoracic Society definition of asthma in 1962 [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], but its association with the disease mechanism was not fully appreciated until methodological aspects of inhalation bronchoprovocation had been developed. Careful work has subsequently enabled these tests to be standardized and, as a result, to provide an additional method for assessing asthma. However, the significance of bronchial hyperresponsiveness in terms of the underlying disease process may have been over-interpreted. Thus, while broadly indicating the severity of the disease [6], studies in which methacholine and histamine responses have been measured repeatedly over

prolonged periods, have failed to indicate a close relationship with disease severity [7, 8]. In general terms, the concept of airway hyperresponsiveness, whether specific or nonspecific, provided a plausible mechanism to explain the paroxysmal symptomatology of asthma and a 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, propranolol, bradykinin, sulphur dioxide and sodium metabisulphite, has broadened the concept of bronchial hyperresponsiveness to include indirect mechanisms involving the release of bronchoconstrictor mediators from nerves and inflammatory cells (fig. 1). While indices of hyperresponsiveness derived from using direct-acting agonists, such as methacholine and histamine, correlate quite well, their relationship to hyperresponsiveness measured with the indirect-acting agents is at best marginal. The important question that has so far eluded definition is why asthmatic airways show a propensity for becoming more easily obstructed in responding to these many different stimuli.

**Inflammation as the basis of asthma**

'OSLER [9], in his first edition of the Principles and Practice of Medicine published in 1892, refers to asthma

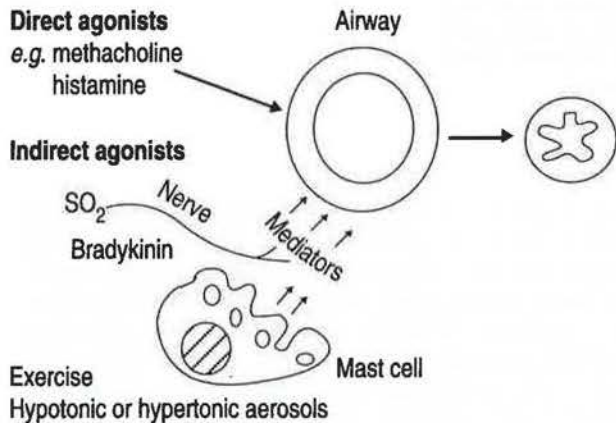


Fig. 1. - The concept of direct and indirect bronchial responsiveness.

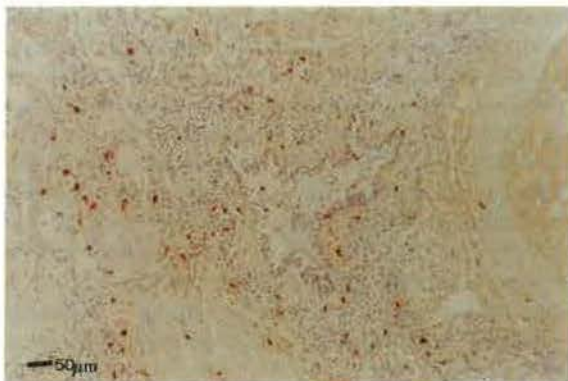


Fig. 2. - An airway from the lungs of a patient who had died from asthma. The section has been stained with a monoclonal antibody to tryptase (AA-1) to demonstrate the presence of mast cells as an important component of the inflammatory response.

as "a special form of inflammation of the smaller bronchioles - bronchiolitis exudativa (Curshmann)", which he differentiated from "spasm of the bronchial muscles". At that time, little was known about the pathological features of asthma, although frequent reference was made

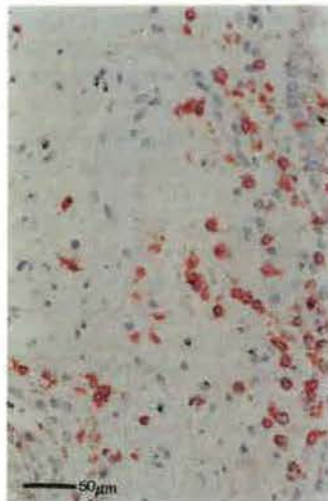
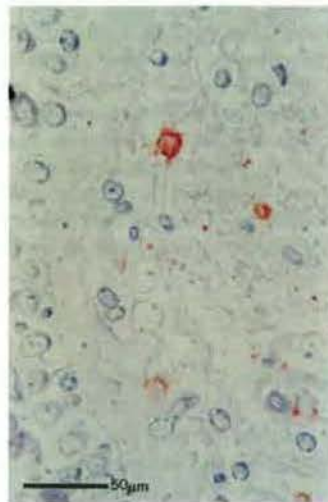
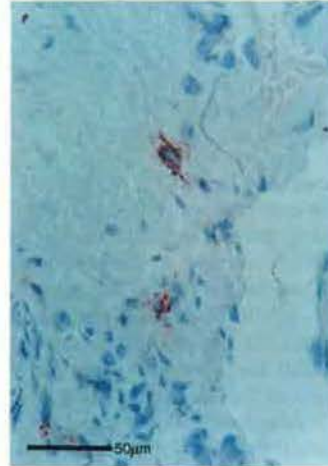


Fig. 3. - Glycolmethacrylate sections (2 μm) of bronchial mucosal biopsies from a patient with mild asthma immunostained to demonstrate the presence of mast cells (top), eosinophils (middle) and T-cells (bottom).

in the literature to the presence of "fibrinous casts" occluding the smaller airways of those who had died from the disease. Whilst, in the latter part of the last century, several authors [10–12] described the clinical and pathological features of severe asthma, it was not until the 1960s that an exhaustive study of the cellular components of the airways in asthma death and comparison with other airway diseases was reported by DUNNILL [13]. In these studies, various features of asthma were highlighted, including the presence of excess luminal secretions, epithelial damage, goblet cell and submucus gland hypertrophy and hyperplasia, 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 explaining the processes that led to an asthma death (fig 2).

In the 1960s and early 1970s, it was generally thought that these pathological changes related only to death from asthma, with the disease in life being largely a disorder of airways smooth muscle. A focus directed towards the mechanisms of acute bronchoconstriction and the presence of bronchial hyperresponsiveness served to further direct attention in asthma towards smooth muscle physiology, rather than to the underlying reasons for the abnormal functioning of the smooth muscle. The concept of viewing asthma as episodic bronchospasm also pointed the pharmaceutical industry strongly in the direction of developing bronchodilator drugs, based on the original observations that adrenaline and atropine relieved asthma attacks. However, the association of a blood and sputum eosinophilia with asthma, the presence of tenacious plugs of secretions from the airways during the recovery phase of acute severe asthma, the presence of clumps of epithelial cells in the sputum (creola bodies), and the increasing recognition that asthma frequently occurred in association with other atopic diseases provided clues that the disease in life extended beyond airway smooth muscle dysfunction, but a clearer understanding has been slow to emerge.

A major breakthrough came with the application of bronchoscopy to the study of asthmatic airways. On account of the risks of bronchoconstriction provoked by rigid bronchoscopy in asthma, only limited information was obtained by a few investigators [14]. Fiberoptic bronchoscopy directed the way forward, in first providing a means for obtaining lavage and subsequently mucosal tissue specimens from the asthmatic airway, thereby enabling a detailed study of the airway cellular events in patients with mild-to-moderate disease. Bronchoalveolar lavage studies indicated that asthmatic airways were subject to an inflammatory response involving eosinophils, mast cells and mononuclear cells, and that disordered airway function was the result of the secretion of an array of preformed and newly generated vaso- and bronchoactive mediators. Under direct vision, fiberoptic bronchoscopy proved to be an invaluable technique for obtaining small mucosal biopsies for detailed 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] (fig. 3). Thus, a picture began to

emerge of asthma, even in its milder forms, being a chronic and persistent inflammatory disorder that was responsible for much (if not all) of the symptomatology and disordered airway physiology that had been described previously by physicians and physiologists.

### Allergens as an important cause of asthma

While it has long been known that asthma could be provoked by inhaling respirable materials, the reasons for this had to await the astute attention of BLACKLEY [19], a general practitioner in the English city of Manchester, who made some fundamental observations about environmental factors that accompanied hay fever and asthma, from which he himself suffered. In his treatise entitled "Catarrhus Aestivus", published in 1873, he describes painstaking experiments linking increased pollen counts across the spring and summer period to the occurrence of his rhinitis and asthma. In 1880, BLACKLEY [20] was first to report the allergen-induced skin wheal on introducing pollen into the skin with a lancet. SALTER [1] had recognized that dust, especially in British trains, also triggered asthma. However, almost a century passed before VOORHORST *et al* [21] finally proved that the domestic dust mite (HDM) (*Dermatophagoides* sp.) was responsible for the major allergenic component of perennial asthma, and this has since been the subject of intensive research. It is now known that HDMs and, in particular their faecal particles, are responsible for releasing large amounts of allergen into the asthmatic airway. At least seven separate groups of allergens have been identified with HDMs, the first four of which are known to have proteolytic or other enzyme activities [22]. For example, Der p1, the major allergen of *D. pteronyssinus*, is a cysteine protease derived from the mite's gastrointestinal tract. The potent biological activities of other allergens might explain why these particular proteins are able to penetrate epithelial surfaces so easily, and as a consequence lead to specific sensitization.

Recent studies have emphasized 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 eluded definition [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, HOPKIN and co-workers [24, 25] 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). Recently, the same group have identified this locus as coding for the  $\beta$ -chain of the high affinity immunoglobulin E (IgE) receptor (Fc $\epsilon$ R1), and that linkage is manifest most strongly if the "atopy gene(s)" are inherited through the mother (genomic imprinting) [26]. However, at least five other groups, who have studied somewhat smaller families, have failed to confirm linkage of atopy to 11q13, and there is even controversy over whether or not dominant inheritance for the atopic

trait is correct [27]. The genetics of atopy is further compounded by important associations between IgE responses to specific allergens and the human leukocyte antigen (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 immunoglobulin G (IgG) responses to environmental allergens, including ragweed, pollen, rye grass and house dust mite [28]. 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 [29, 30].

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 sensitization in genetically at risk children. In a collaborative study with Platts Mills, 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 hyperresponsiveness by the age of 11 yrs [31]. 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 are also 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 when compared with babies whose mothers do not smoke [32]. It has been proposed that smoking adversely affects maternal nutrition, which then influences foetal growth and immune responses. In two separate studies, we have shown that large head circumference and low birth weight have a powerful predictive effect on levels of serum IgE both in children (9–11 yrs) and adults (50 yrs) [33] (table 1). It is known that nutritional and endocrine factors influence growth and maturation of the thymus in parallel with brain growth, suggesting that head circumference may be a surrogate marker for the development of the immune system.

The implications of these findings for the prevention of allergic disease are profound. ARSHAD *et al.* [34] 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. Whether this is sustained throughout childhood can only be answered with further follow-up of this cohort. Thus, while not definitely proven, there is increasing evidence to indicate that intrauterine nutrition and early life exposure to allergens are critical factors in determining the level of sensitization and the subsequent development of allergic disease. At a cellular level WARNER *et al.* [35] have recently shown that T-cells isolated from the cord blood of babies that subsequently develop atopic dermatitis and/or asthma exhibit higher proliferated responses, with lower interferon- $\gamma$  (IFN- $\gamma$ ) and higher interleukin-4 (IL-4) messenger ribonucleic acid (mRNA) expression at birth to foods (egg and milk proteins) and/or inhalant allergens. Since cytokines are critically involved in the allergic tissue response, IL-4 being responsible for isotype switching of B-cells to IgE synthesis [36], and for the maintenance of the Th2 lymphocyte subpopulation [37], and IFN- $\gamma$  serving to oppose the actions of IL-4 [38] (fig. 4), a possible mechanism is provided for early expression of the atopic phenotype in these at risk infants.

#### Adjuvant factors in sensitization 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. It is interesting to note that cigarette smoking later in life also elevates serum IgE, and that this has a synergistic interaction with the direct effects of cigarette smoking in accelerating the decline in pulmonary function with age [39] (table 2). An adjuvant effect of cigarette smoking on the development of occupational asthma related to such sensitizing agents as acid anhydrides and platinum salts further indicates an important interactive effect of cigarette smoke and its products with the development of sensitization of the respiratory tract [40]. The mechanism(s) responsible for this effect on the immune response may be the direct destructive effect of cigarette smoke on the bronchial epithelium, thereby facilitating access of allergens to the mucosal

Table 1. – The predictive effect of body size at birth on serum total IgE levels measured 47–55 yrs later, in a random population of 28 residents in Preston, England

	Mean size at birth		Difference (raised IgE - normal)			p-value**
	Male	Female	Male (41 raised IgE 105 normal)	Female (21 raised IgE 113 normal)	All† (62 raised IgE 218 normal)	
Birth weight oz	111.3	113.0	4.2	8.0	5.6 (0.3 to 10.9)	0.04
Head circumference in	13.6	13.6	0.33	0.25	0.30 (0.10 to 0.50)	0.004
Length* in	20.4	20.3	0.12	0.15	0.13 (-0.17 to 0.44)	0.4
Ponderal index* oz·in <sup>-3</sup> ×1000	13.1	13.4	0.19	0.63	0.36 (-0.15 to 0.87)	0.2
Ratio of head circumference to length×100*	66.9	66.9	1.1	0.7	1.0 (-0.1 to 2.0)	0.07
Placental weight oz	21.8	20.8	-1.1	2.7	0.3 (-1.1 to 1.8)	0.6

\*length at birth not recorded for one subject; \*\*: adjusted for sex. IgE: immunoglobulin E; †: 95% confidence interval in parentheses. oz: ounce, 1 oz = 31.1 g; in: inches, 1 in = 2.54 cm. (personal communication, Godfrey, Barker and Osmond).

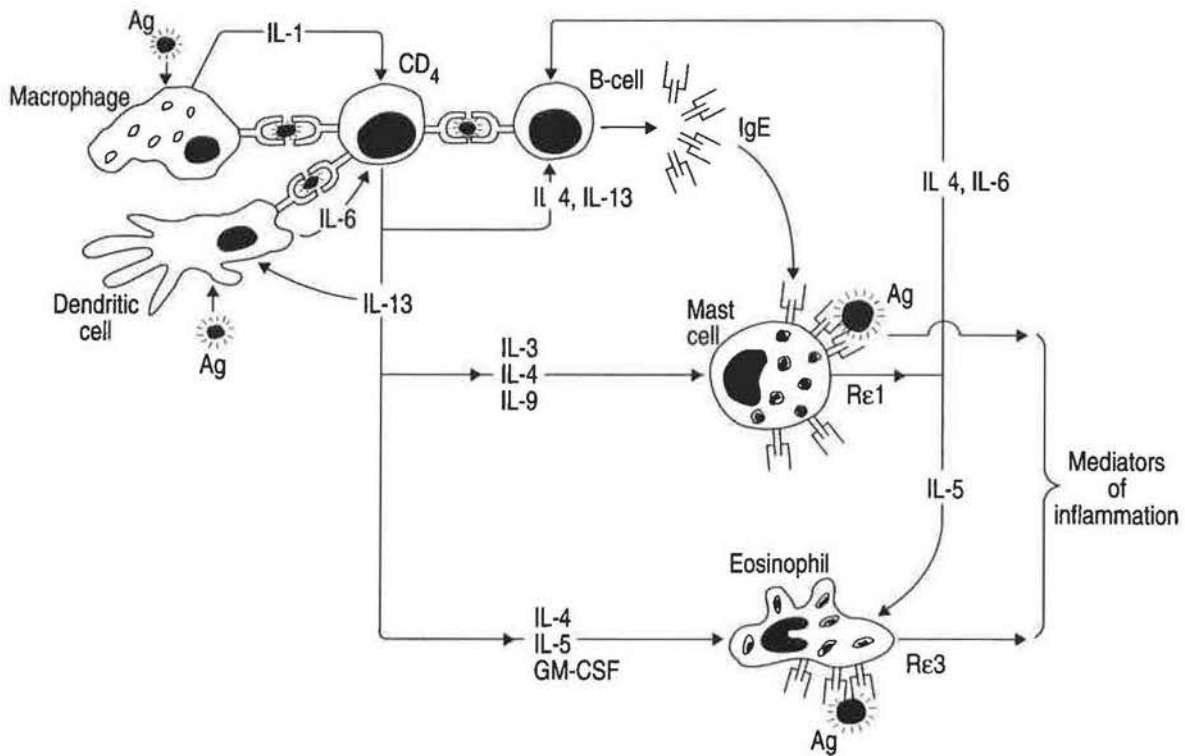


Fig. 4. - Schematic representation of the mucosal immune events leading to an allergic tissue response. Ag: antigen; IL: interleukin; GM-CSF: granulocyte macrophage colony stimulating factor.

Table 2. - Differences in FEV<sub>1</sub>/FVC % according to combinations of smoking habits and serum IgE in a population of Southampton residents over 65 yrs of age

Smoking IgE Interaction	n	All subjects (95% CI)	n	Nonasthmatics (95% CI)
NS IgE ≤80 IU·ml <sup>-1</sup>	61	0	48	0
NS IgE >80 IU·ml <sup>-1</sup>	18	-0.70 (-7.46 to 6.06)	15	-0.66 (-7.49 to 6.16)
XS IgE ≤80 IU·ml <sup>-1</sup>	79	-2.31 (-6.92 to 2.31)	72	-3.37 (-7.98 to 1.24)
XS IgE >80 IU·ml <sup>-1</sup>	23	-5.11 (-11.49 to 1.27)	17	-2.72 (-9.39 to 3.96)
CS IgE ≤80 IU·ml <sup>-1</sup>	15	-6.24 (-13.73 to 1.24)	13	-4.95 (-12.35 to 2.44)
CS IgE >80 IU·ml <sup>-1</sup>	15	-15.37* (-22.66 to -8.08)	12	-14.16* (-21.61 to -6.71)

Estimates were obtained by multiple linear regression, with and adjustment for age (treated as a continuous variable) and sex. (\*: p<0.005). NS: nonsmoker; XS: ex-smoker; CS: current smoker. For further abbreviations see legend to table 1.

immune system, or may influence the mucosal immune system itself by facilitating antigen presentation or by biasing T-cell differentiation along the Th2 phenotype pathway (*vide infra*).

Other adjuvant factors have also been implicated in the early life origins of asthma, and include respiratory tract virus infections, particularly respiratory syncytial virus (RSV). In a mouse model, ALWAN *et al.* [41] have shown that two virulence proteins, designated F and G, in the virus capsid are able to bias T-cell development along

either 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. Some evidence has accumulated that early life infection with RSV is a predisposing factor for the development of IgE hyperresponsiveness [42] and asthma [43] but, until studies on T-cells similar to the mouse studies described by ALWAN *et al.* [41] can be demonstrated in humans, this remains speculative.

Another effect of respiratory tract viruses is to damage the bronchial epithelium, thereby augmenting the penetration of the airway mucosa by inhaled allergens. The recent suggestion that certain air pollutants (e.g. passive cigarette smoke and  $\text{NO}_2$ ) impair the lower respiratory tract's capacity to resist virus infection, offers a possible link between two environmental factors that predispose the airways to becoming sensitized to specific allergens [44]. Exposure to environmental air pollutants, such as ozone, sulphur dioxide and oxides of nitrogen, has been shown in animal models to augment allergen sensitization of the lower respiratory tract [45]. Although a number of studies have directed attention towards air pollution as a predisposing factor for developing asthma, it is not known whether this is a direct effect of the pollutant on sensitive airways, or an indirect effect in augmenting the airway response to inhaled allergen [46].

### The cell biology of allergen sensitization

Atopy, the genetic predisposition for directing an IgE response to common environmental allergens, is the strongest identified risk factor for the development of asthma. In 1909, Block and Massini demonstrated that local sensitisation to *Trichophyton* sp. could be passively transferred. Twelve yrs later, PRAUSNITZ and KÜSTNER [47] demonstrated the ability of serum from an individual to mediate an immediate hypersensitivity to a specific allergen of the localized recipient site of passive transfer and fixation. It took a further 46 yrs before the Ishizakas identified this "reaginic" agent of serum as IgE.

The mechanism through which IgE determines the expression of atopy is through its high affinity to specific receptors (FcεRI) expressed on the surface of tissue mast cells and basophils and with somewhat lower affinity to macrophages, eosinophils and platelets (FcεR2, CD23). Cross-linkage of IgE with specific allergen results in the non-cytotoxic release of an array of preformed and newly generated inflammatory mediators. For the mast cell, these include histamine, tryptase, prostaglandin  $\text{D}_2$  ( $\text{PGD}_2$ ) and leukotriene  $\text{C}_4$  ( $\text{LTC}_4$ ) (a component of slow reacting substance of anaphylaxis (SRS-A)) which, through their direct effects on airway smooth muscle and microvasculature, are responsible for the allergen-induced early asthmatic response (EAR) [48]. A similar function for IgE on other accessory cells will serve to augment this response by releasing additional autacoid mediators including thromboxane  $\text{A}_2$  ( $\text{TXA}_2$ ) (macrophages), prostaglandin  $\text{I}_2$  ( $\text{PGI}_2$ ) (eosinophils) and platelet-activating factor (PAF) (macrophages and eosinophils).

The last half decade has witnessed considerable advances in the understanding of the regulation of IgE synthesis by lymphocytes (fig. 4). Interleukin-4 is a key cytokine involved in the isotype switching of B-cells from synthesis of immunoglobulins M and G (IgM and IgG) to IgE, involving a sequence of intracellular transcriptional events and the transient generation of germ-line mRNA transcripts [36]. IL-4 interacts with B-cells via specific cell surface receptors, which exist in both high and low

affinity forms. Recently, a second cytokine designated interleukin-13 (IL-13), exhibiting a 30% homology with IL-4, has also been shown to be a ligand for IL-4 receptors, but, unlike IL-4, it is also a differentiation factor for dendritic cells [49]. Switching of B-cells to IgE synthesis is potently inhibited by interferon- $\gamma$  and  $-\alpha$  [38].

It is now recognized that in human allergic disease, epitopes on allergen molecules are recognized by dendritic cells and subsequently presented as fragments to T-cells involving Class II molecules and T-cell receptors, resulting in their differentiation along a pathway leading to the Th2 phenotype [50]. When activated by antigen, these cells direct cytokine synthesis towards interleukins-3, 4, 5, 10, 13 and GM-CSF. Apart from controlling IgE synthesis, IL-4 is an obligatory cytokine for the development and maturation of the Th2 lymphocyte phenotype [37]. Interleukin-3 and a further factor (from fibroblasts) designated stem cell factor (*c-kit* ligand) are involved in the growth, differentiation and regulation of mast cells and basophils [51], whilst interleukin-5 (together with granulocyte macrophage colony-stimulating factor (GM-CSF)) serve similar functions for eosinophils [52]. Thus, 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 differentiate 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- $\gamma$ , IL-2, tumour necrosis factor- $\beta$  (TNF- $\beta$ ) together with variable amounts of GM-CSF [50]. Whilst there is agreement that in humans antigen-specific T-cells exhibiting characteristics of both the Th1 and Th2 phenotype exist in both bronchial biopsies and in bronchoalveolar lavage taken from the airways of asthmatic subjects, the dominant cytokine repertoire exhibited by identification of mRNA using *in situ* hybridization and the polymerase chain reaction strongly suggests a dominance of the Th2 phenotype [53, 54]. The crucial question that requires answering is how the various genetic and environmental factors in the airways interact to direct the immune response along the Th2 lymphocyte pathway, and what is it that selects this response for some proteins (allergens) but not others?

### The cell biology of airway inflammation in asthma

Once sensitized, the lower respiratory tract responds to inhaled allergens in a highly specific manner, resulting in widespread airway obstruction and hyperresponsiveness. Almost 30 yrs ago, PEPYS [55] showed that in sensitized subjects inhalation of specific allergen led to both early (5–15 min EAR) and late (2–6 h LAR) bronchoconstrictor responses that lasted approximately 60 min and 12–24 h, respectively [55]. Later studies by COCKROFT and MURDOCK [56] showed that the LAR was accompanied by an acquired increase in bronchial responsiveness to such stimuli as inhaled histamine and methacholine (fig. 5). Because hyperresponsiveness is considered an important

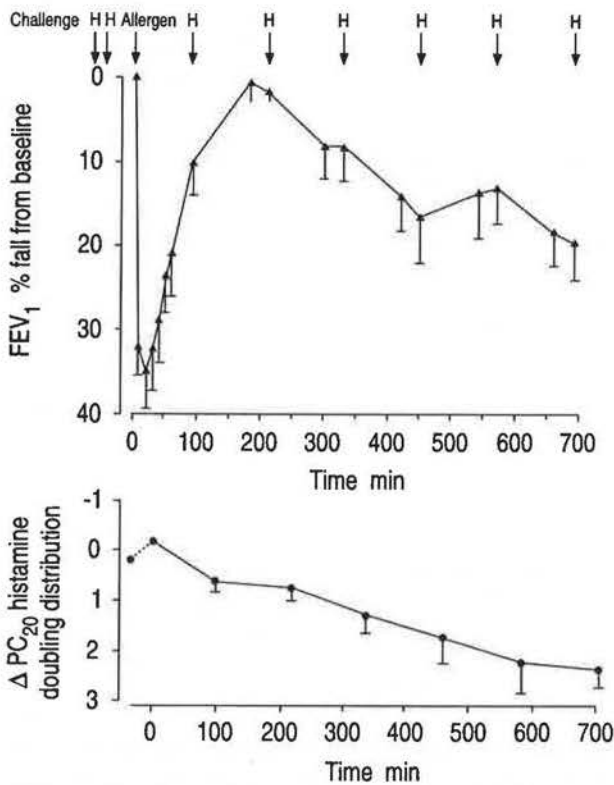


Fig. 5. -- Time related changes in FEV<sub>1</sub> and histamine (H) reactivity following allergen challenge in seven patients with asthma. Allergen-induced early and late bronchoconstrictor responses and the accompanying decrease in PC<sub>20</sub> indicative of acquired airway hyperresponsiveness. FEV<sub>1</sub>: forced expiratory volume in one second; PC<sub>20</sub>: provocative concentration producing a 20% fall in FEV<sub>1</sub>.

component of airway dysfunction in naturally occurring asthma, these findings have received considerable attention as models for studying pathogenetic mechanisms.

Measurement of mediators in the peripheral blood and bronchoalveolar lavage fluid, together with their metabolites in urine, has shown that the EAR is a mast cell-dependent response resulting from the IgE-dependent secretion of constrictor mediators. Together, these mediators contract airway smooth muscle, stimulate afferent neurones and increase microvascular leakage. The type of mast cell involved in this reaction contains predominantly tryptase as its neutral protease. Tryptase exists as a tetramer, of molecular weight 134,000 Da and constitutes 20–30% of the total protein of the human mucosal mast cell [51, 57]. Amongst its biological actions, tryptase is able to increase microvascular permeability and enhance airway smooth muscle responsiveness (at least in the dog) [58]. Histamine produces most of its airway effects by stimulating H<sub>1</sub> receptors, which are present both on airway smooth muscle and on the microvasculature, while PGD<sub>2</sub> and its immediate metabolite 9 $\alpha$ 11 $\beta$ -PGF<sub>2</sub> contract airways smooth muscle by interacting with thromboxane (TP1) receptors. Mast cell-derived LTC<sub>4</sub> is rapidly metabolized to LTD<sub>4</sub> and subsequently to LTE<sub>4</sub>, these three sulphidopeptide leukotrienes comprising the smooth muscle contractile and vasoactive properties of the biological activity, previously described as slow-reacting substance of anaphylaxis (SRS-A).

While the allergen-induced late reaction in the skin has been known to have an inflammatory basis (originally described by Pepys as an "Arthus" response), until recently it has been difficult to provide a cellular basis for the LAR. It has been known for some time that during the LAR there appeared in the circulation an increase in neutrophil and eosinophil chemoattractants that were defined physicochemically but not structurally. During the LAR, circulating eosinophils exhibited characteristics of cell activation, including an increased expression of specific cell surface markers. Just prior to the onset of the late reaction (approximately 2 h), COOKSON *et al.* [59] demonstrated a transient decrease in the circulating eosinophil count which, when taken with the observations of DE MONCHY *et al.* [60] of an increased lavage eosinophilia 24 h after challenge, suggested the selective recruitment of these cells into the airways. A number of additional studies have confirmed that both inhalation and local allergen provocation of the airways result in an eosinophil influx into the bronchial lumen of sensitized subjects at intervals up to 24 h post-challenge [14].

To address the cellular mechanisms of the LAR directly, we have recently completed a study in which the bronchial mucosa was biopsied 5–6 h after either segmental allergen or saline challenge, and the immunopathological changes in small mucosal biopsies examined [61]. Surprisingly, 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 these cells appeared to be in a highly degranulated state. Other findings included an increase in eosinophils, T-cells and, somewhat surprisingly, mast cells. Further similar studies carried out with allergen challenge of the skin, conjunctiva and nasal mucosa in sensitized individuals have confirmed that at 4–6 h the dominant leucocyte infiltrating the inflamed lesion is the neutrophil, and not the eosinophil as previously thought. This does not, of course, exclude the eosinophil as an important cell contributing to events later in the inflammatory reaction but, in the author's opinion, it is difficult to provide a strong case for this leucocyte at the peak of the late response; rather, it contributes to later events, for example, in the lower airways the persistence of hyperresponsiveness, which may extend for up to three weeks following a single allergen exposure.

Of considerable interest is the mechanisms by which leucocytes move into the airway and become activated. Using a panel of monoclonal antibodies to endothelial and leucocyte adhesion molecules, we have been able to show that 6 h following allergen challenge there is marked upregulation of E-selectin (endothelial leucocyte adhesion molecule-1, ELAM-1) the ligand of which on neutrophils and other leucocytes, is sialyl Lew<sup>x</sup>, and intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily [62] (fig. 6). One ligand for ICAM-1 is designated lymphocyte functional antigen-1 (LFA-1, CD11a-CD18), an integrin expressed on a large number of leucocytes, but especially on lymphocytes, neutrophils and eosinophils. Expression of ICAM-1 was also observed in the bronchial epithelium, although no difference could be observed in the intensity or distribution

of immunostaining at this site, when comparing the allergen and saline challenges [61]. Another member of the immunoglobulin superfamily, vascular cell adhesion molecule-1 (VCAM-1) [63] was expressed in the airway microvasculature at a low level, but this was not increased within the time-frame of 6 h following allergen provocation. A positive correlation was observed between the extent of ICAM-1 expression and LFA-1<sup>+</sup> leucocyte 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.

Considerable knowledge has accumulated 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 and leucocytes (e.g. sialyl Lew<sup>x</sup>) results in the rolling of leucocytes along the endothelial cell, whereas upregulation of ICAM-1 and VCAM-1 arrests the leucocytes, thereby facilitating trans-endothelial migration [62]. Elegant studies by GUNDELL and co-workers [64, 65] in non-human primates naturally sensitized to *Ascaris* sp. antigen have shown that blocking antibodies directed to E-selectin and ICAM-1 abrogate the late airway response and acquired bronchial hyper-responsiveness with allergen challenge in parallel with a reduction in neutrophils and eosinophils, respectively.

In order to understand how allergen provocation can lead to an upregulation of endothelial leucocyte adhesion molecule expression, it is important to understand more about how these molecules are regulated. The P- and L-selectin are rapidly expressed on endothelial cells after exposure to a range of short-acting mediators, including histamine and leukotrienes. Within one hour of autacoid exposure, the expression of these molecules diminishes (probably by shedding) and they are replaced by E-selectin, the expression of which is upregulated by cytokines, especially interleukin-1, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  [62] (fig. 6). The same cytokines are also responsible for the upregulation of the ICAM-1; whereas, optimal expression of VCAM-1 requires a combination of IL-1 and/or TNF- $\alpha$  together with IL-4. Recently, DURHAM and co-workers [66] have shown that 24 h following allergen challenge, VCAM-1 is upregulated on the vascular endothelium of the nasal mucosa and associated with an increased influx of leucocytes (T-cells and eosinophils) bearing the integrin ligand for VCAM-1, very late activation antigen (VLA-4) ( $\alpha_4\beta_6$ ). The source of cytokines responsible for the upregulation of vascular adhesion molecules in the short period required to initiate leucocyte recruitment following allergen provocation has been the subject of some speculation. Initially, it was thought that T-cells and monocyte/macrophages were the prime source of these cytokines, but since these cells require 4–6 h to generate cytokines *de novo* prior to their

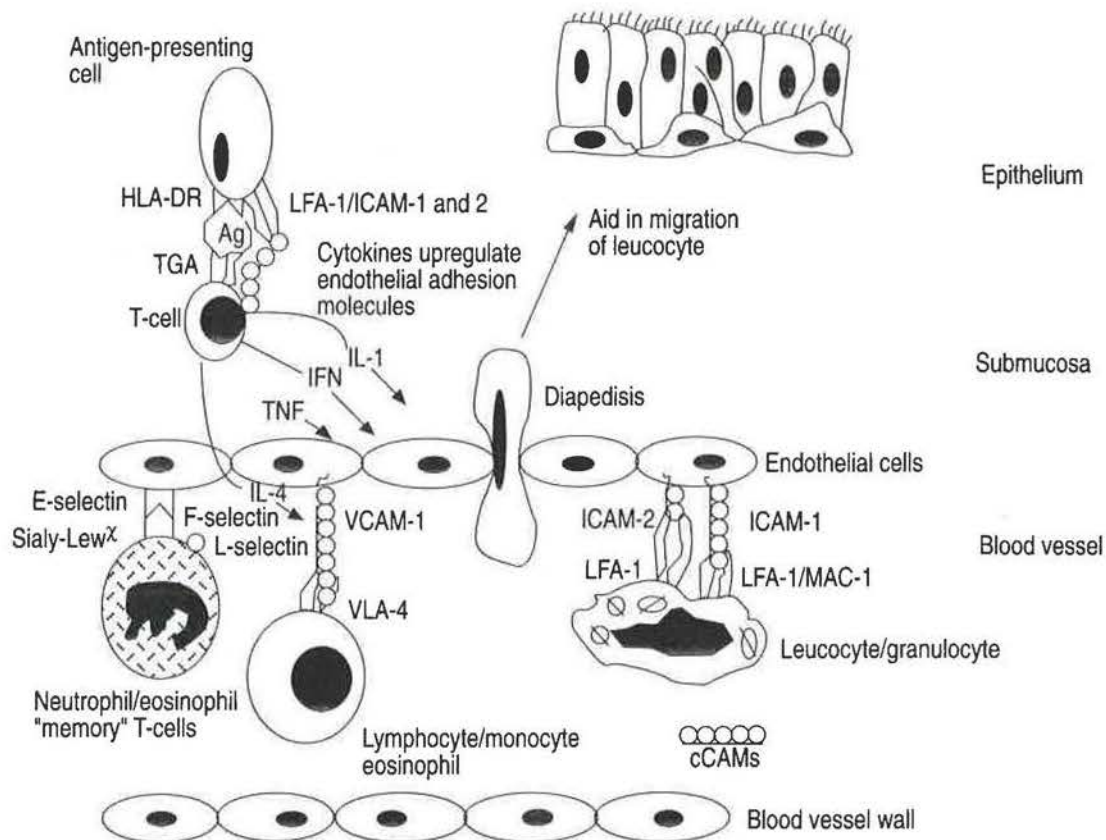


Fig. 6. — Schematic representation of the role of leucocyte endothelial adhesion molecules in T-cell mediated inflammation. Ag: antigen; cCAMS: soluble cell adhesion molecules; HLA-DR: human leucocyte antigen-DR; ICAM-1: intercellular adhesion molecule-1; IFN: interferon; IL: interleukin; LFA-1: lymphocyte functional antigen-1; TCR: T-cell receptor; TNF: tumour necrosis factor; VCAM-1: vascular cell adhesion molecule; VLA-4: very late activation antigen-4.



secretion, it is difficult to explain the expression of E-selectin and ICAM-1 and the associated leucocyte influx that is already well-established 6 h after local allergen challenge. Another possibility is that there exists a source of preformed cytokines.

Using immunohistochemistry applied to 2  $\mu\text{m}$  thick sections embedded in the water soluble resin glycol-methacrylate, we have been able to show that mucosal mast cells store IL-4, IL-5, IL-6 and TNF- $\alpha$  [67–69]. Following cross-linkage of IgE receptors on the surface of mast cells, the cytokines are rapidly released [66] and could, therefore, provide a mechanism for the early upregulation of vascular adhesion molecules. Thus, allergen-induced release of preformed TNF- $\alpha$  from mast cells could explain the observed upregulation of E-selectin and ICAM-1 during the LAR, with IL-5 serving to promote eosinophil chemotaxis and priming, and IL-4 augmenting the recruitment of eosinophils and T-cells through its capacity to upregulate the expression of VCAM-1. Recently, we have been able to confirm the presence of mast cell cytokines within the secretory granules by immunoelectron-microscopy, and their release following IgE-independent activation (fig. 7).

Once recruited into the airway, both neutrophils and eosinophils become activated and secrete a wide array of preformed and newly generated inflammatory products. These include the toxic granule components of the eosinophil (major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN)) and a range of lipid products including prostanooids, leukotrienes and PAF. The availability of potent and selective sulphopeptide leukotriene antagonists has provided a useful tool for determining the contribution of these potent vaso- and bronchoactive mediators in the EAR and LAR. The administration of LTD<sub>4</sub> antagonists prior to allergen provocation of sensitized airways have shown marked inhibitory effects both on the EAR and LAR and attenuation of the acquired increase in bronchial hyperresponsiveness [70]. Although PAF was at one time regarded as a prime mediator of late phase inflammatory responses and bronchial hyperresponsiveness [71], 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 [72].

A greater understanding of the mechanisms of early and late phase allergen responses has helped to explain how various anti-asthma drugs might operate in asthma. Thus, sodium cromoglycate (SCG) and nedocromil sodium (NS) not only inhibit the release of preformed and newly generated autacoids from activated mast cells, but might also inhibit cytokine release. Oral and topical corticosteroids reduce the late phase response, probably by inhibiting the cytokine-mediated upregulation of vascular adhesion molecules, in addition to their effect in reducing cytokine secretion from mast cell and T-cells.

### Mucosal inflammation in clinical asthma

Bronchoalveolar lavage and, more recently, bronchial biopsy studies have provided overwhelming evidence that a specific form of airway inflammation underlies asthma, irrespective of its cause. The dominant mediator secreting cells leading to airway dysfunction appear to be mast cells and eosinophils, although with more severe disease monocytes, macrophages and platelets play an important role. There is also convincing evidence of a key role for T-cells in 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 expression of which appears to correlate with clinical indices of disease activity [73, 74]. Recently, CORRIGAN and co-workers [53, 54] have shown that these activated T-cells exhibit mRNA transcripts for IL-3, 4 and 5, thereby suggesting that they are of the Th2 phenotype. More recently, we have shown that the level of CD25 (IL-2 receptor) expression on bronchoalveolar lavage T-cells in active asthma correlates with their proliferative response to the major allergen of house dust mite Der p1. Clearly, further work now needs to be focused on the mechanisms by which these antigen specific T-cells are recruited and maintained in the airway, and how they relate to the other components of the

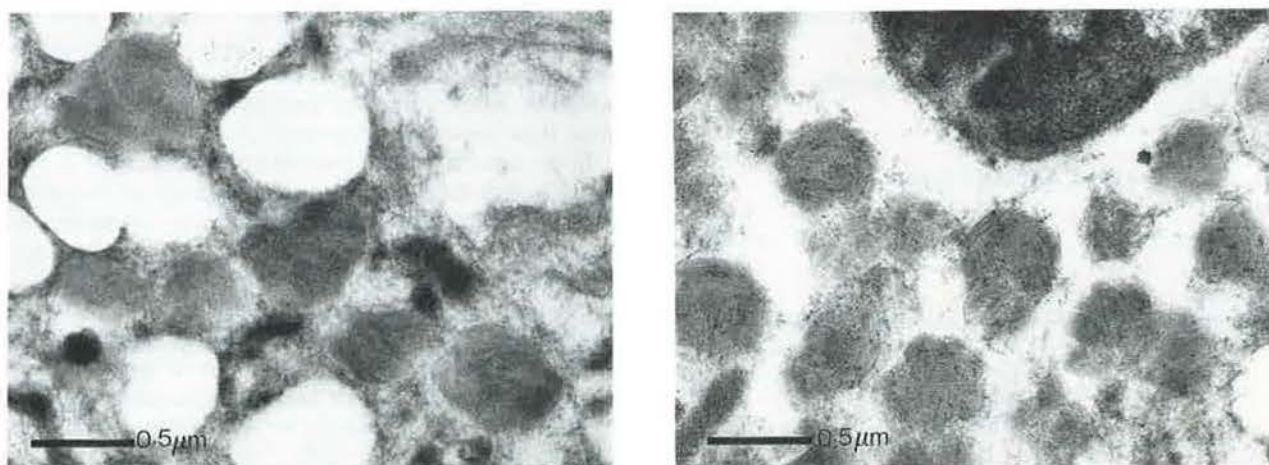


Fig. 7. — Freeze substitution immunogold electron microscopy of a human mast cell to show localization of immunoreactive IL-4 to the secretory granules (right), compared to a control antibody (left) ( $\times 46,000$ ). IL-4: interleukin-4.

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, in an attempt to identify those factors that draw the T-cell towards differentiating along the Th2 phenotype. The recent demonstration 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 discussion "*asthma is 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*" [75] (fig. 8). Positioning airway inflammation at the beginning of this new definition of asthma, with the physiological and clinical consequences following it, has profound implications in terms of disease diagnosis and management. It is on the basis of this, and the increasing concern over the regular use of inhaled  $\beta_2$ -agonists, that future treatment strategies are being aimed towards preventing or inhibiting underlying airway inflammation, rather than simply treating symptoms [75]. Although at one time viewed with some suspicion, there have been several randomized 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 hyperresponsiveness [76]. Similarly, in parallel with the excellent clinical response observed with the regular use of inhaled topical corticosteroids, mast cell, eosinophil and T-cell responses in the airway are all reduced in parallel with a reduction in bronchial hyperresponsiveness [77]. Regular use of inhaled SCG also reduces the levels of eosinophils recovered from the airways by lavage. More recently [78] nedocromil sodium (a novel pyranoquinolone with wide-ranging activities on inflammatory cells, including mast cells) has been shown to decrease eosinophil numbers in mucosal biopsies from asthmatics after 16 weeks of treatment [79].

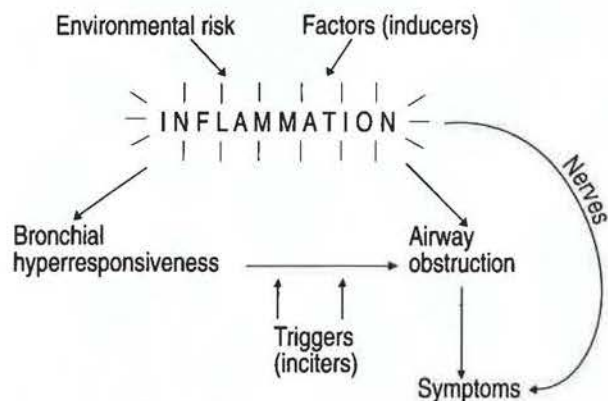


Fig. 8. — Schematic representation of the factors which underlie a definition of asthma based on inflammation.

By contrast, 12 weeks of regular treatment with the long acting  $\beta_2$ -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, or on indices of their activation [80]. However, while  $\beta_2$ -agonist may not reduce the background inflammation of asthma, there is some evidence in animals (and more recently in humans) that this drug may reduce the cellular events associated with the allergen-provoked LAR [81].

These clinical observations raise some important points about the factors that may maintain the inflammatory response in asthma. Whilst it is clearly recognized that mucosal immune responses to inhaled antigens leading to inflammation is a key feature of extrinsic asthma, there is almost nothing known of those factors which lead to an almost identical pathological picture in asthma where there is no obvious inducing agent (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. Even in extrinsic asthma, withdrawal from environmental sensitizing agents often produces only partial remission. It remains possible, therefore, that the chronicity of asthma is dependent upon processes that escape the control of the mucosal immune system.

#### The epithelium as a target for the inflammatory attack in asthma

The observation by NAYLOR [82] 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. This has been amply confirmed in postmortem studies of asthma when, in addition to an airway lumen filled with secretions, there are large areas where the pseudostratified ciliated epithelium is stripped to a single layer of basal cells. Elegant studies by FRIGAS and GLEICH [83] have presented a convincing case for the arginine-rich proteins of eosinophils playing a key role in epithelial damage. Our own work leads us to believe that eosinophils require a cognate interaction with the epithelium, whereupon there is release of metalloendoproteases, e.g. gelatinase, in parallel with an increase in epithelial permeability and detachment of columnar cells from their basal cell attachments [84].

In patients with mild-to-moderate asthma, there is evidence to indicate that a major site of damage to the epithelium is localized to a plane between the columnar and basal cells, and that this involves weakening of the major adhesion structures responsible for maintaining the integrity of the epithelium, desmosomes [85, 86]. These are complex structures that are 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  $\alpha_6\beta_4$  [87]. The precise mechanisms whereby the adhesive function of desmosomes is disturbed in asthma is not known, although parallels might

be drawn to the skin blistering that occurs in the related allergic disorder, eczema.

The consequences of epithelial disruption in asthma may be important in serving to augment the inflammatory response. Integrity of the epithelium is required for the adequate formation of the airway lining fluid, which is rich in components such as antioxidants and immunoglobulins, that help protect the airway from noxious environmental insults. Loss of ciliary function will impair mucus clearance, while areas of increased permeability will allow allergens and other inflammatory stimuli to penetrate the airway wall. In response to this, growth factors are released that may lead to proliferation of myofibroblasts situated just beneath the epithelial basement membrane. Myofibroblasts are thought to 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 a "thickened basement membrane", characteristic of the disease [88]. The number of myofibroblasts correlates well with the thickness of this collagen layer [89]. Factors that may be important in initiating this response include TGF- $\beta$  present in eosinophil granules, platelet-derived growth factor (PDGF) localized to the bronchial epithelium, and endothelin, a peptide found both in endothelial and epithelial cells and the expression of which is increased in the epithelium in asthma [90]. In addition to stimulating myofibroblasts to contract, endothelin is a potent chemoattractant for these cells.

The capacity of the epithelium to serve as a source of cytokines has aroused considerable interest. In both normal and asthmatic airways preformed IL-1 $\beta$ , IL-8, GM-CSF can be immunolocalized to the epithelium (fig. 9). 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. There is also some evidence that in atopic asthmatics, but not in normals, IL-8 is a chemoattractant for eosinophils, in addition to its well-known effects on neutrophils [91].

Other constitutive cells of the airway may contribute cytokines to the inflammatory response in asthma. The microvascular endothelium is important, with its capacity to secrete IL-5, GM-CSF and IL-8. Myofibroblasts may also be an important source of cytokines. In the presence of TNF- $\alpha$ , 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 mature and prolong the survival of mast cells, in part due to their capacity to secrete *c kit*-ligand and fibronectin.

Another factor that may be important in augmenting the airway inflammatory response of asthma is the establishment of autocrine feedback pathways. In addition to mast cells serving as a source of cytokines, there is considerable evidence to incriminate eosinophils as an important source of these molecules including GM-CSF, IL-3,

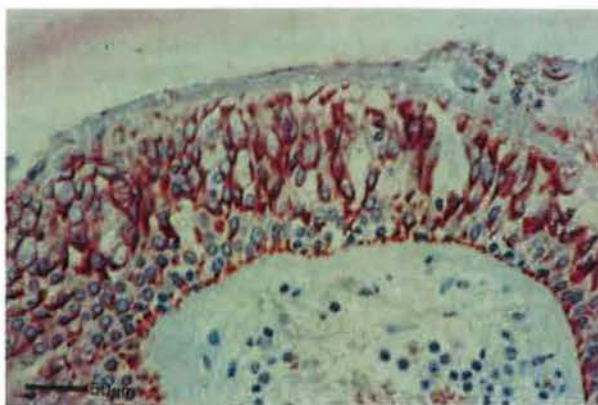


Fig. 9. - Immunolocalization of IL-8 to the bronchial epithelium in a thin section of a glycolmethacrylate embedded bronchial mucosal biopsy. IL-8: interleukin-8.

IL-5, TNF- $\alpha$  and TGF- $\beta$  [92-95]. Thus, with extensive eosinophil infiltration, these cells may be a major source of proinflammatory cytokines, and might account for the cascade of inflammatory events that lead to acute severe asthma and occasionally death from the disease.

### Concluding comments

There can now be few who doubt that inflammation underpins much of the disordered airway function which occurs in asthma. However, there is still much to learn about how this is initiated and maintained. André Courmand brought to the study of the lung and its circulation an enquiring mind and a scientific discipline in the field of physiology that has greatly influenced clinical practice in respiratory and cardiovascular medicine. The advent of novel molecular and cellular biological techniques has now revealed further exciting challenges for the clinical scientist. In the field of asthma and allied disorders there exists a realistic expectation that substantial discoveries will be made to prevent and cure the disease, rather than relying on drugs that relieve symptoms or suppress the inflammatory process.

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