The role of allergy in the development of asthma

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Recent studies have shown that initial sensitization to airborne environmental allergens occurs typically in early childhood, but subsequent progression to persistent atopic asthma, which may not manifest for several years, is restricted to only a subset of atopics. The key to establishing the link between atopy and asthma lies in the development of persistent inflammation in the airway wall, resulting in structural and functional changes in local tissues which are responsible for the symptoms of the disease. This review summarizes recent findings on the nature of the cellular and molecular mechanisms underlying this process, and addresses the issue of why the intensity and duration of these tissue-damaging responses in the airway wall apparently exceeds the critical threshold required for development of persistent asthma in only a minority of allergy sufferers.

*Allergy** is acknowledged as a major risk factor for *asthma*, and inhalation challenge of *atopic* (allergic) asthmatics with specific *allergen* evokes a biphasic response comprising discrete acute- and late-phase reactions separated in time by several hours[1–3]. The defining clinical feature of these two reactions is airflow obstruction, which is reversible over short periods of time either spontaneously or as a result of treatment, and which is believed to be the direct result of inflammation of the airway wall.

At the cellular epicentre of this process are $CD4^+$ T-helper memory cells (see review by Corry & Kheradmand, this supplement). These produce an array of *cytokines* that directly or indirectly 'program' the leukocytes that are responsible ultimately for acute and chronic allergic inflammation in the airways. As illustrated in Fig. 1, the principal type 2 helper (*Th2*) cytokines implicated in this process include interleukin (IL)-4, which is required to drive production of allergen-specific *immunoglobulin E* (IgE)[4], IL-3, which controls mast-cell and *basophil* development[5], and IL-5 in conjunction with IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which regulate the *eosinophil* component of allergy[5,6]. In addition, there is growing interest in the role of Th2-derived IL-9 in regulation of IgE production[7] and mast-cell growth[8], and in IL-13 in relation to airways hyperreactivity (see below).

At the effector end of this process, the acute-phase component of allergy represents classical immediate-type *hypersensitivity*, triggered through allergen-induced crosslinking of specific IgE antibody bound to *mast cells* through high-affinity receptors. These cells release a range of granule-associated preformed *mediators* which are responsible for the immediate symptoms of the acute allergic response (in this case, bronchoconstriction), and which contribute directly to some aspects (for example, oedema) of the late-phase reaction. In addition, mast cells release a variety of *chemokines* and cytokines which contribute to recruitment and activation of secondary effectors, in particular the eosinophils[1,2] that are one of the hallmarks of the late phase of allergic reactions. This late-phase response is characterized by airway perivascular oedema, mucous plugging and the presence of activated Th2 cells which represent the principal source of the cytokines responsible for the sustained recruitment and activation of eosinophils. As the late response develops at challenge sites the overall inflammatory infiltrate may include, in addition to eosinophils, significant numbers of *monocytes*, *neutrophils* and platelets together with representatives from a variety of subpopulations of T cells other than Th2 cells[1].

Opinion on the relative importance of the contribution of mast cells to the late phase of the allergic response has been divided. But evidence from studies in mast-call 'knock-in' mice, which has demonstrated that these cells can contribute to aerosol allergen-induced granulocyte recruitment in a murine asthma model[9], has reawakened interest in this issue, in particular in the potential role of mast-cell-derived tumour necrosis factor (TFN)-α[10]. Indirect evidence also suggests a role for mast-cell-derived TFN-α in human asthma[3].

It is also now recognized that structural cells such as airway epithelial cells are important sources of mediators in asthma, in particular in chronic disease[1]. As discussed below, the recruitment of these cell populations into the overall inflammatory response may be an important component of the chronic phase of *atopic asthma*.

Allergy to airborne *antigens* has become increasingly common in many countries during recent years. However, despite the fact that most of the offending 'allergens' are present continuously in the natural environment, particularly the indoor environment[10], only a small proportion of allergic individuals manifest symptoms of asthma, indicative of persistent allergic inflammation in their airways. Why is progression from allergic sensitization to disease expression in the airways not automatic, when all the necessary elements would seem to be present in the allergic individual?

In seeking to identify the missing link(s) in this process, this review traces the development of atopic asthma from the initial sensitization of naive Th cells against environmental allergens, through to the ultimate expression of chronic allergen-driven Th2-mediated inflammation in the airway mucosa.

The sensitization phase of respiratory allergy

There is increasing interest in the concept that asthma may be preventable if the early events in the process, in particular those involved in initial sensitization of the immune system in atopics against airborne environmental allergens, can be circumvented or forestalled. This has led to an increase in studies focusing on the initiation of immune responses to these agents in immunologically naive humans.

Our perception of the mechanism(s) underlying the primary allergic sensitization process has changed radically over the past few years. Following rapidly on the heels of pioneering work on Th-cell heterogeneity in the mouse[4], T-cell cloning studies revealed major variations in cytokine production by inhalant allergen-specific Th-memory cells within the human population, with atopics expressing a cytokine pattern similar to murine Th2 cells[11,12] or Th0 cells manifesting a mixed cytokine profile[13,14], in contrast to the Th1-like profile typical of non-atopics. The key cytokines in the atopic

* Terms in italic font are defined in the glossary on p. B39.

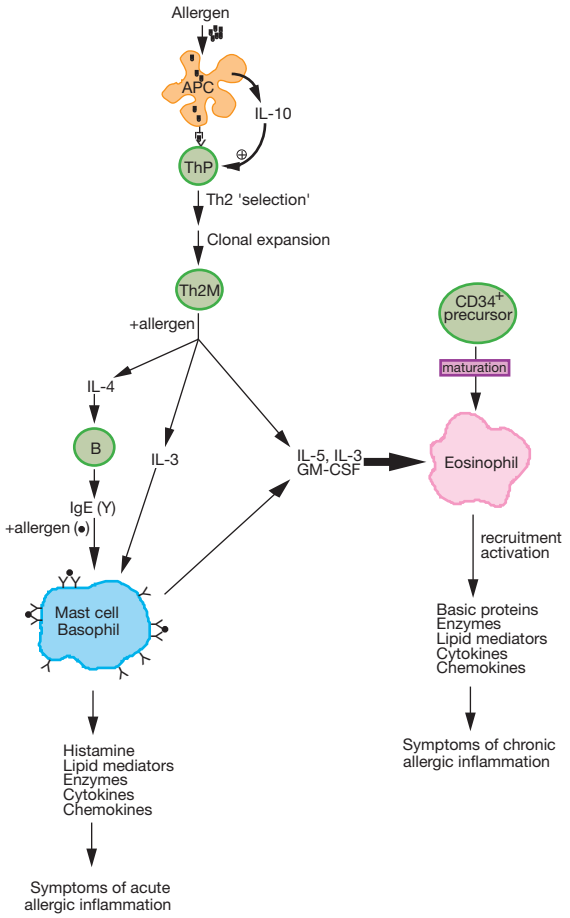

Figure 1 Major cell types implicated in the pathogenesis of acute and chronic allergic reactions. The initiation of this process involves presentation of processed allergen to naive Th-precursor (ThP) cells by antigen-presenting cells (APCs) within a cytokine milieu that favours the selective expansion of Th2-polarized memory cells (Th2M), resulting eventually in production of specific IgE by B cells. Re-exposure to allergen elicits an acute-phase response that is triggered through crosslinking of antibody-loaded high-affinity IgE receptors on mast cells/basophils. This is followed after several hours by a late-phase response involving inflammatory mediator production by Th2 cells and eosinophils with additional contributions from mast cells/basophils, and may also include contributions from macrophages, $CD8^+$ T cells, neutrophils and platelets.

response seem to be IL-4 and IL-5, which drive IgE production and eosinophilia, respectively, after allergen exposure. The central issue in relation to the aetiology of allergy is thus how these disparate cytokine production patterns become programmed into long-term immunological memory.

The key events that determine the cytokine phenotype of allergen-specific Th-memory cells seems to occur very early in life, often many years in advance of the expression of persistent atopic disease. Surprisingly, initial priming of Th cells against environmental allergens commonly occurs *in utero*, presumably by means of transplacental transport of allergens to which the mother is exposed during pregnancy[15]. Paradoxically, these early allergen-specific Th-cell responses are dominated by production of the same Th2 cytokines that are associated with expression of atopy and asthma in later life[15]. This appears to be a direct result of selective downregulation at the fetomaternal interface of local production of Th1 cytokines, such as interferon (IFN)-γ, which are highly toxic towards the placenta[16]. The principal control mechanism in this process (reviewed in ref. 17) involves constitutive production by trophoblasts and other cells within the placenta of prostaglandin E2, progesterone, IL-4 and IL-10, which are Th2-trophic and/or Th1-inhibitory.

However, beyond birth these Th2-trophic control mechanisms wane, and the weakly primed fetal Th-cell system is suddenly exposed to much higher concentrations of environmental antigens, and as a result fetal allergen-specific Th-cell responses are subjected to allergen-driven regulation. The emerging evidence indicates that high-level allergen exposure during infancy and early childhood, notably to dietary allergens such as egg albumen, leads to negative regulation of Th-cell responses[18,19] via T-cell *anergy* and/or deletion[17]. In contrast, postnatal exposure to inhalant allergens, which occurs at much lower concentrations, results either in redirection of these responses towards a Th1-like cytokine pattern (in non-atopics)—a process termed immune deviation—or in further boosting of fetally primed Th2-polarized immunity in potential atopics[17,19,20].

The increasing tendency for failure of this allergen-driven immune deviation process during early life seems to lie at the core of the progressively rising prevalence of atopic disease among successive birth cohorts in the developed countries[21]. The cause of this failure is unclear, but some indications have emerged. First, it is apparent from our original studies[22] and subsequent work of many laboratories (reviewed in ref. 17) that the rate of postnatal transition of Th-cell function from the Th2-skewed state characteristic of fetal life towards the relatively Th1-polarized adult state occurs significantly slower in children with genetic predisposition to atopy. We have hypothesized that this may compromise capacity to develop 'protective' Th1-like immunity against inhalant allergens during infancy[17,21,22]. Second, much experimental literature indicates that the principal stimulus for normal postnatal maturation of Th1-associated functions is microbial exposure[17,23], which may partly explain some of the inverse relationships reported between childhood 'infections' and atopy development[24,25]. It is of interest to note in this context the recently described association between a *polymorphism* in the gene encoding the high-affinity receptor for bacterial lipopolysaccharide (CD14) and intensity of allergic sensitization[26]. This gene is located on the long arm of chromosome 5 adjacent to the IL-4/IL-9 gene cluster, and controls (*inter alia*) levels of soluble CD14, which in turn is central to the regulation of production of Th1-trophic IL-12 (ref. 26).

Clinical asthma and airways hyperresponsiveness

The term asthma describes a heterogeneous collection of clinical phenotypes as opposed to a single condition. A notable example is the distinction between the manifestation of the disease in children compared with adults. The point prevalence of asthma is actually greatest in young children, but up to half of these cease wheezing by adolescence[27]. The most common trigger of asthma exacerbations (~85%) in children is virus infections, whereas infections account for only a minority of adult exacerbations[28]. It is noteworthy, however, that children with the most severe asthma have symptoms which begin in early life, are likely to be clearly atopic, and do not tend to lose their symptoms in adolescence.

The key feature of persistent asthma is the development of the state of *airways hyperresponsiveness* (AHR). In the context of atopic asthma this equates to an exaggerated bronchoconstrictor response not only to allergens to which the subjects are sensitized, but also to a range of non-specific stimuli, including agents as diverse as cold air and methacholine.

How this hyperresponsive state is acquired is poorly understood. It is likely that AHR can result from several mechanisms, some or all of which may be operative in individual asthmatics. The result of chronic allergic inflammation in atopic asthma is airway remodelling (Fig. 2), involving thickening of the airway wall secondary to increased subepithelial collagen deposition (types III and V) and increased vascularity as well as hypertrophy and hyperplasia of airway smooth muscle (ASM)[1]. In addition, increased numbers of

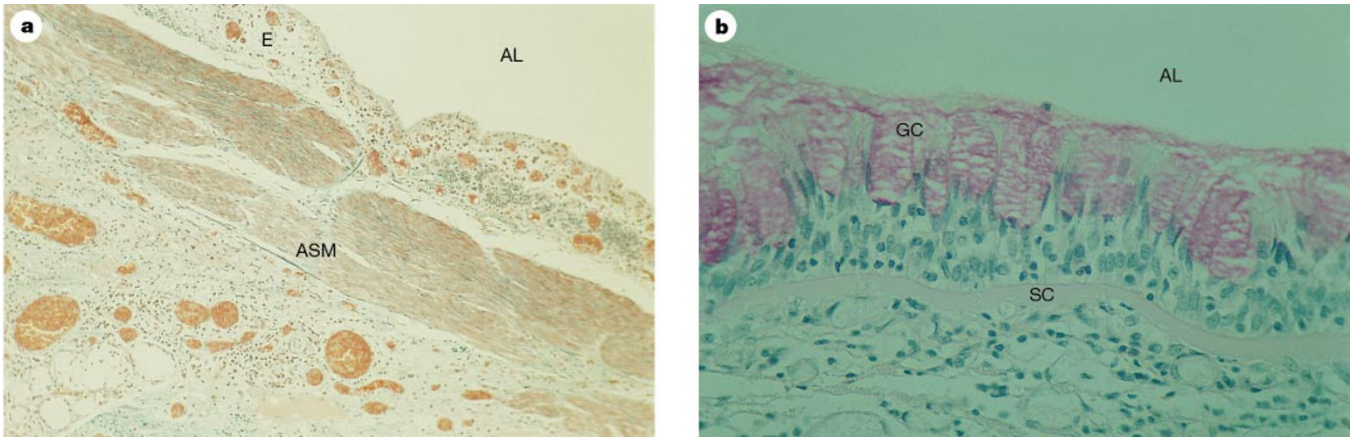

Figure 2 Transverse sections of the central airway from a subject with chronic asthma. **a**, Section stained with modified elastic trichrome, showing denudation of epithelium, enlarged muscle mass and prominent vessel dialation. AL, airway lumen; E, epithelium; ASM, airway smooth muscle. Magnification ×500. **b**, Section stained with Periodic Acid Schiffs reagent, showing goblet cell hyperplasia and collagen deposition below epithelial basement membrane. AL, airway lumen; GC, goblet cells; SC, Subepithelial collagen. Magnification ×1,000. Images provided by N. Carroll, Sir Charles Gairdiner Hospital, Perth.

goblet cells in the epithelium and alteration of autonomic neural input to the airways, especially the non-adrenergic non-cholinergic inhibitory and excitory nervous systems, have been described.

Inflammation-induced airway remodelling may mediate AHR development through several discrete mechanisms, including those that alter neural regulation of ASM, increase ASM contractility, or that alter the sensitivity of ASM muscarinic receptors to cholinergic stimuli. In addition, airway remodelling may contribute to hyperresponsiveness through purely mechanical means. For example, an increase in the volume of tissue within the muscle layer—which may occur a result of mucosal oedema, influx of inflammatory cells into the submucosa or increased vascularity of the epithelium—will result in greater airway narrowing and airway obstruction for a given degree of ASM shortening. Alternatively, an increase in the amount of ASM as a result of hypertrophy and hyperplasia will produce more shortening of ASM for a given stimulus and a greater increase in airway narrowing and airway obstruction. The combination of increased ASM and thickening of the airway wall would in theory produce an exaggerated airway narrowing response to environmental stimuli similar to that observed in asthma.

There are, however, additional mechanisms involving the lung parenchyma. In particular, the lung parenchyma is coupled to the airways so that when the lungs inflate, this mechanical interdependence results in the airways being pulled open. It has also been suggested[29] that the airways and parenchyma could be uncoupled by parenchymal inflammation and oedema, leading to changes in overall lung mechanics. Studies using transbronchial biopsies have indeed shown eosinophilic inflammation in alveolar walls in adult asthmatics[30]. In addition, invasive studies that have used catheter-tipped manometers inserted into small airways of humans[31], or that have directly measured alveolar pressure in animals[32], have shown parenchymal responses to inhaled methacholine. Non-invasive techniques are being developed[33,34] for studying lung parenchyma mechanics in humans in more detail and these promise to produce new information on the contribution of parenchymal lung inflammation to the overall changes in airway mechanics in asthma.

Although it is generally believed that airway remodelling in asthma is due to a disturbance in the balance of various growth and inhibitory factors within the airway mucosa, and thus secondary to chronic inflammation, our knowledge of the underlying cellular and molecular mechanisms is incomplete. For example, passive sensitization of ASM *in vitro* has been shown to alter muscle contractility. Although the process is dependent on the presence of high levels of IgE antibodies, the mechanism is not understood with certainty[35]. With respect to the deposition of interstitial collagens beneath the airway epithelial basement membrane during the remodelling process, there is compelling evidence implicating myofibroblasts as a major source[36,37], and recent *in vitro* studies indicate a potential stimulatory role for a variety of growth factors released from injured airway epithelial cells, including platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)-1, basic fibroblast growth factor (bFGF) and transforming growth factor (TGF)-β[38]. But the relative contribution of individual growth factors to myofibroblast proliferation in this disease is still to be determined.

In vivo studies that seek to identify the precise aspect(s) of the atopic response that are responsible for AHR development and the attendant structural changes in the airway wall have only begun relatively recently, but some insight has been gained from animal models. In particular, experimental models have been developed in both mice[39,40] and rats[41]. These demonstrate that $CD4^+$ T cells that are transferred adoptively from sensitized to naive animals can induce AHR that is associated with IL-5 production and eosinophilia. Transgenic mouse models involving hyperexpression of individual cytokines in the airway epithelium are also providing insight into this process[42], and into potential mechanism(s) underlying inflammation-induced deposition of extracellular matrix proteins in the airway wall[43–45]. Receptor-blockade techniques are also proving useful in this regard, the prime example being studies demonstrating the importance of IL-13 in AHR development in mice[46,47].

Allergy and the development of clinical asthma

One of the least understood aspects of the pathogenesis of allergic disease is target-organ selectivity. So far, we have no satisfactory explanation for why apparently equivalent levels of hypersensitivity to the same allergens in different subjects can manifest in some exclusively as skin disease (atopic dermatitis), in others as nasal inflammation (*rhinitis*), while in others as multi-organ disease.

The situation with respect to asthma is particularly complex: although the vast majority of asthmatics are allergic to one or more inhalant allergens, only a subset of allergic subjects go on to ultimately develop persistent airway disease. A study in Australia has indicated that by early school age up to 40% of children display atopic sensitization to $\geq$1 inhalant allergen[48], and this figure remains stable into adulthood. However, despite the fact that >90% of adult asthmatics are atopic, it is evident from asthma

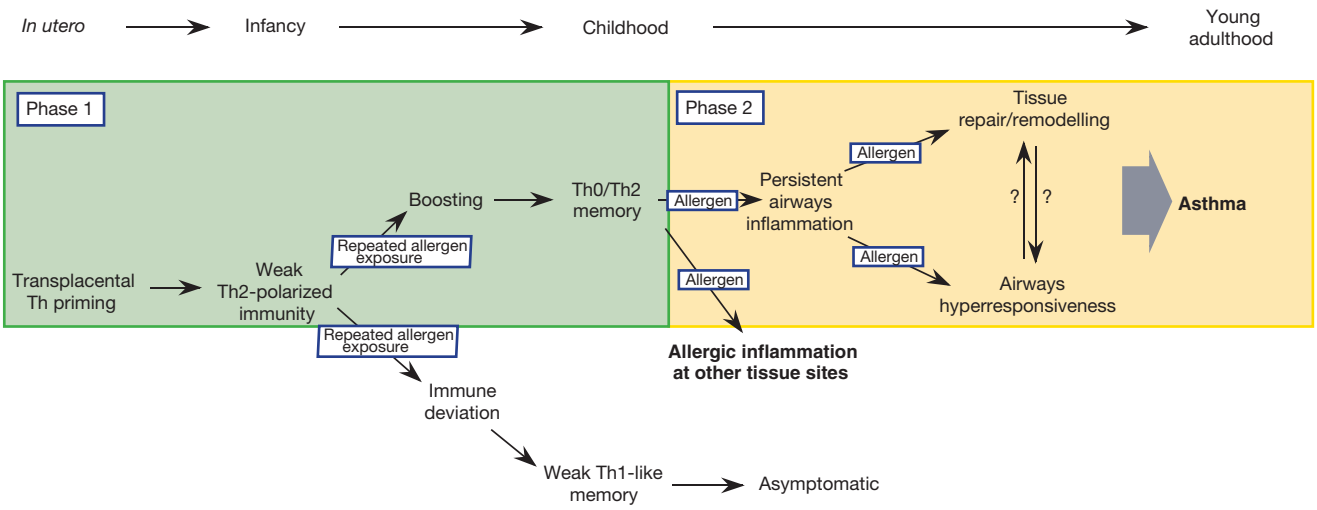

Figure 3 Progression from primary allergic sensitization in early childhood to atopic asthma in later life. Recent findings indicate that the development of asthma can be considered to occur in two phases. Phase 1 involves T-cell 'sensitization' in early life, whereas chronic airways inflammation and its sequelae occur in stage 2.

prevalence figures in older children and adults that only 25–30% of sensitized atopics progress to asthma. On this basis it is reasonable to hypothesize that, in this subset of atopics, additional co-factor(s) must be operating which focus the disease process upon the airway mucosa.

As depicted in Fig. 3, the development of atopic asthma can be considered as a two-stage process. In the scheme shown, phase 1 involves the development of allergen-specific immunological memory against inhalant allergens. This process normally occurs during childhood, and in a subset of individuals it results in development of Th0/Th2-polarized immunological memory (that is, allergic sensitization), which increases 'risk' for allergic respiratory disease but is insufficient in itself for disease expression. The latter occurs only in those subjects whose allergic sensitization to airborne antigens results in persistent inflammation within the airway mucosa.

As noted above, the characteristic histopathological feature of atopic asthma is the presence in the airway mucosa of activated Th cells that produce Th2 cytokines. Repeated cycles of allergen-driven activation of these cells, resulting ultimately in a chronic 'wound healing/repair' response in these tissues, is believed to be central to the structural and functional changes in the airway wall that are characteristic of the asthmatic state.

Although yet to be proven, we can hypothesize that the level of Th2-mediated inflammatory damage sustained must exceed a certain critical threshold in order to precipitate long-term pathological changes in the airway wall, and thus progression to phase 2. Given this scenario, the most likely co-factor(s) that would operate in tandem with atopy in this second phase of the asthma disease process are those that can directly or indirectly add to the level of local airway inflammation. Possibilities for the latter include the following.

Exogenous airborne inflammatory stimuli. Current evidence indicates that the most important of these stimuli in quantitative terms are respiratory virus infections[49] followed by indoor levels of inhalant allergens to which the atopics are sensitized[50], both of which have been linked repeatedly to asthma exacerbations in children and adults. Additional possibilities include indoor irritants such as environmental tobacco smoke, and non-allergenic inflammatory particulates in house dust[51], as well as outdoor air pollutants such as diesel exhaust components. Maternal smoking, both during pregnancy and postnatally, is associated with reduced infant lung function at birth and is a major independent risk factor for developing asthma[52].

Failure of immunoregulatory mechanisms in airway mucosa. A series of overlapping control mechanisms serve collectively to limit the duration and intensity of T-cell mediated immunoinflammatory responses within the airway mucosa. Included among these is the local production of nitric oxide (NO), which can inhibit Th-cell activation through effects on T-cell signalling kinases[53]. However, the T-cell inhibitory effects of NO are considerably more effective against Th1 than Th2 cells, and hence this mediator can potentially skew ongoing T-cell responses towards the Th2 cytokine phenotype via differential 'sparing' of the latter[54]. NO is produced at high levels within the asthmatic airway[55].

Dendritic cells are the principal antigen-presenting cell (APC) population in the airway wall, but the functional phenotype of these cells is normally tightly controlled and is restricted to antigen uptake and processing[56,57]. The cells require overnight exposure to GM-CSF to induce maturation of antigen-presentation functions[56]. Although this cytokine is normally produced in only trace amounts in resting airway tissues, it is present locally at very high levels in atopic asthmatics[58]. Recent evidence also suggests that these changes in cytokine production within the airway epithelium of atopic asthmatics are accompanied by increases in the local population density of intraepithelial dendritic cells[59,60], with concomitant upregulation of surface expression of activation markers[61]. Dendritic cells have been shown to have a key role in $CD4^+$ Th-cell-induced experimental airway eosinophilia in an animal model of asthma[62].

An additional control mechanism within the airway wall that may be disturbed in asthma involves the functions of local *macrophages*, which in the steady state downregulate T-cell responses via direct effects on T-cell cycling[63] and via inhibitory effects on APC[56]. There is increasing evidence that monocytes recruited into inflammatory sites in the airways, including in asthma, express the alternative T-cell-trophic phenotype[64], and may thus contribute to persistence of the underlying immune responses which were responsible for their initial recruitment into the tissue.

Variation within Th2 responses. Considerable quantitative and qualitative variation is evident when allergen-specific Th2-like responses are compared between individual atopics[65], and some of these variations may contribute to the absolute level and tissue distribution of ongoing allergen-induced immunoinflammatory damage. Such variations may include allergen-specific T-cell clone size(s), and also the balance between crossregulating cytokines within individual responses, in particular the relative contribution of anti-inflammatory IL-10 (ref. 66). In this context, we have

recently shown that the magnitude of *in vivo* skin-test reactions in children to inhalant allergen is inversely related to the capacity of their T cells to produce IL-10 in response to *in vitro* challenge[67]. An additional source of variation may be levels of surface expression on allergen-specific Th2 cells of mucosal 'homing' molecules (such as $\alpha 4\beta 7$; see ref. 68) and others affecting T-cell trafficking to specific tissues such as the cutaneous lymphocyte-associated antigen.

Recruitment of structural cells. The level of inflammatory cytokine production by structural cells in the airway wall in the absence of inflammatory stimulation is relatively low. However, in atopic asthmatics, airway epithelial cells, fibroblasts, endothelial cells and smooth muscle cells produce a wide range of cytokines and mediators[1], presumably as part of the tissue's response to chronic inflammation. These phenotypic changes in the airway mucosa may in part account for the persistence of airway inflammation in chronic disease sufferers, even in the apparent absence of ongoing exposure to inhalant allergens to which the subjects are sensitized.

Conclusions and speculation

Considerable progress has been achieved in recent years in identifying the many cellular and molecular effector mechanisms that contribute to acute- and late-phase allergic inflammation at challenge sites. The greatest impediment to the rapid translation of this growing information into development of more precisely targeted anti-inflammatory drugs is the redundancy inherent in most of these processes. However, a hierarchy of effector mechanisms is slowly becoming evident, particularly in relation to chronic allergic inflammation in which $CD4^+$ Th-cell-mediated eosinophil participation has emerged as a key rate-limiting component of the disease process, in virtually all manifestations of allergy.

It is becoming increasingly clear that the development of severe allergic disease within individual tissues is in a sense an iterative process in which exogenously stimulated immunoinflammatory reactions provoke responses from the injured tissues (for example, 'repair'), which feed back to modulate the original immunoinflammatory response, and this altered response can in turn elicit further changes within the tissue. The chronically inflamed airway wall may represent the prime example of this process, with acute-phase allergy-induced inflammation providing the initial stimulus, which steadily intensifies as the contribution of the late-phase reaction increases with progressive expansion of the T-cell-driven component of the overall response. The reaction of the airway tissue to these insults includes a range of adaptive changes familiar to cell biologists with interests in 'wound healing', culminating eventually in structural changes that can have permanent effects on lung function. However, it is still not clear as to precisely which of these long-term changes within tissues of the airway wall are responsible for the individual symptoms of asthma.

It is also important to acknowledge that alternative immunoinflammatory pathways exist for asthma development. The best characterized examples are intrinsic asthma[69] and occupational asthma induced by toluene di-isocyanate[70]. No role for IgE has been found in these diseases, despite the presence of cellular infiltrates and associated cytokine production in airway-wall biopsies which closely resemble the Th2-biased pattern that is typical of atopic asthma[69,70]. It has been proposed that the underlying mechanisms driving these (and possibly other) forms of asthma may include airway epithelial-mediated deviation of all local immune responses towards the Th2 profile[71], and this suggestion merits more detailed investigation.

Our current understanding of the relative contributions of these different types of mechanisms to disease expression in the airways is derived from extensive research that has concentrated on established (chronic) atopic asthma. An alternative approach to these complex issues, which is just starting to bear fruit, is to focus instead on the inductive phase of the disease process. As illustrated in Fig. 3, recent findings in the paediatric literature indicate that issues associated with the development of allergy *per se* can be logically segregated from those associated with the expression of allergic inflammation in the airway wall, and in turn from the response of the target tissue to this repeated inflammatory stimulus. Placed within the appropriate developmental context, namely that these events occur typically over the period of life during which lung growth is maximal, this approach may provide a new perspective for finer dissection of the many interacting components of this complex disease process, and hopefully may shed new light on the factors that underlie the progression from acute to chronic disease. □

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