

Innate and adaptive immune responses in asthma

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The recognition that asthma is primarily an inflammatory disorder of the airways associated with T helper type 2 (T_H2) cell-dependent promotion of IgE production and recruitment of mast cells and eosinophils has provided the rationale for disease control using inhaled corticosteroids and other anti-inflammatory drugs. As more has been discovered about the cytokine, chemokine and inflammatory pathways that are associated with T_H2 -driven adaptive immunity, attempts have been made to selectively inhibit these in the hope of discovering new therapeutics as predicted from animal models of allergic inflammation. The limited success of this approach, together with the recognition that asthma is more than allergic inflammation, has drawn attention to the innate immune response in this disease. Recent advances in our understanding of the sentinel role played by innate immunity provides new targets for disease prevention and treatment. These include pathways of innate stimulation by environmental or endogenous pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) to influence the activation and trafficking of DCs, innate sources of cytokines, and the identification of new T cell subsets and lymphoid cells.

What is asthma?

Asthma is a disorder of the conducting airways leading to variable airflow obstruction in association with airway hyperresponsiveness (AHR). Airway inflammation is central to disease pathophysiology and relates to airway dysfunction partly through the release of potent inflammatory mediators and partly through remodeling of the airway wall. Thus, as the disease becomes more severe, the airway, similar to a chronic wound, is more susceptible to a wide range of environmental insults (for example, biologically active allergens, viruses, air pollutants, certain drugs and chemicals), and has an altered repair response with secretion of growth factors that induce mucus cell metaplasia, smooth muscle proliferation, angiogenesis, fibrosis and nerve proliferation. Varying combinations of these processes help explain the different asthma subphenotypes, their response to treatment and their natural history over a person's lifetime.

Most asthma begins in childhood in association with sensitization of the airways to common aeroallergens, especially those derived from house dust mites, cockroaches, animal dander, fungi and pollens. In most cases, this occurs through the selective expansion of T lymphocytes (particularly of the T_H2 type) that secrete a cluster of cytokines encoded on chromosome 5q31–33, including interleukins IL-3, IL-4, IL-5, IL-9, IL-13 and granulocyte macrophage colony-stimulating factor (GM-CSF), which are coordinately regulated¹.

Activation of T_H2 CD4⁺ cells occurs through phosphorylation of the *trans*-acting T cell-specific transcription factor GATA-3 (ref. 2). In other affected individuals, alternative immunological pathways drive the inflammatory response. These will be discussed later in this Review.

T_H2 -type cytokines orchestrate the allergic inflammatory cascade that occurs in asthma, including T_H2 cell survival (regulated by IL-4), B cell isotype switching to IgE synthesis (IL-4 and IL-13), mast-cell differentiation and maturation (IL-3, IL-9 and IL-13), eosinophil maturation and survival (IL-3, IL-5 and GM-CSF) and basophil recruitment (IL-3 and GM-CSF). A similar inflammatory response is triggered in response to parasitic infection, suggesting that, in some way, allergy is a manifestation of 'frustrated' parasite elimination.

Changing worldwide trends in asthma

Asthma and related allergic diseases have increased markedly in Western countries over the past 50 years, and this has been attributed to urbanization and associated changes to diet and lifestyle³. The urban-rural gradient in prevalence has been demonstrated most strongly in children who grow up in environments with a wide range of microbial exposures (such as traditional livestock farms or in families who have adopted a more 'naturalistic' diet and lifestyle), who are protected from childhood asthma and atopy (the predisposition to develop IgE against common environmental allergens) in proportion to their level of exposure to bacterial and fungal microbes⁴. This protective effect against the onset of asthma in children is even more apparent if the microbial exposure (for example, working with animals or drinking unpasteurized milk)⁵ occurred throughout the mother's pregnancy⁶. To gain further insight into the geographical

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differences in asthma and allergy prevalence, an understanding of the mechanisms underlying the process of allergen sensitization itself is fundamental.

Allergen sensitization

In association with the airway epithelium and underlying mucosa is a specialized population of antigen-presenting (and allergen-presenting) cells (APCs) called dendritic cells (DCs). These cells express receptors of the innate immune system and also have the potential to take up allergens, process them into small peptides and then present them via the major histocompatibility complexes MHC class I and MHC class II for recognition by T cell receptors (Fig. 1). Uptake of allergens by airway DCs is an active process; in allergic individuals, it is facilitated by interaction of the allergen with IgE attached to FcεRI, the high-affinity receptor for IgE⁷. At birth, the airways contain no DCs⁸. Damage to and/or activation of the respiratory epithelium by microbes and irritants are probably the main stimuli that initiate the ingress of immature DCs from the bone marrow⁹. These stimuli cause the release of a range of chemoattractants, such as CCL20, CCL19 and CCL27, the ligands for CCR6, CCR7 and CCR10, respectively, which direct DC migration toward the epithelium and underlying mucosa¹⁰ (Fig. 1). The presence of GM-CSF, which is released from epithelial cells and a range of other structural and immune cells in the presence of IL-4 and tumor necrosis factor-α (TNF-α), leads to DC maturation to a fully competent, antigen-processing cell. However, during initial allergen sensitization of the airways, the mechanisms responsible for T_H2 lymphocyte differentiation from naive T cells remain poorly understood. What is clear is that T_H2 polarization requires an obligatory source of IL-4 to activate the transcription factors signal transducer and activator of transcription 6 (STAT6) and GATA-binding protein 3 (GATA3).

Two competing ideas have been advanced to explain the cellular source of the IL-4. First, a T_H2 response occurs by default in the absence of strong T_H1- or T_H17-promoting cytokines or when only a weak MHC class II T cell receptor (TCR) interaction occurs in the immunological synapse, resulting in IL-4 production from naive CD4⁺ T cells¹⁰. Alternatively, the obligatory IL-4 may originate from accessory cells, especially mast cells and basophils¹¹. Support for this second mechanism comes from the

demonstration that, in parasite-infected mice, basophils migrate into the lymph nodes, draining the site of antigen exposure. Here, the basophils serve both as APCs and as the source of IL-4 (ref. 12). Basophil depletion and reconstitution experiments in mice have added credence to this second proposed mechanism. However, the basophil-depleting antibody (antibody to FcεRI) also depletes a subpopulation of 'inflammatory' DCs that express this receptor¹⁰. Inflammatory DCs, but not basophils, have been suggested to be necessary and sufficient for the development of T_H2 immunity to house dust mite allergen when the first exposure occurs by inhalation. For inhaled allergens, such as those from house dust mites, it is proposed that basophils amplify the T_H2 immunity that is initiated by DCs and, in part, influenced by innate signaling through Toll-like receptor 4 (TLR4) and C-type lectin signaling on epithelial cells and DCs¹³.

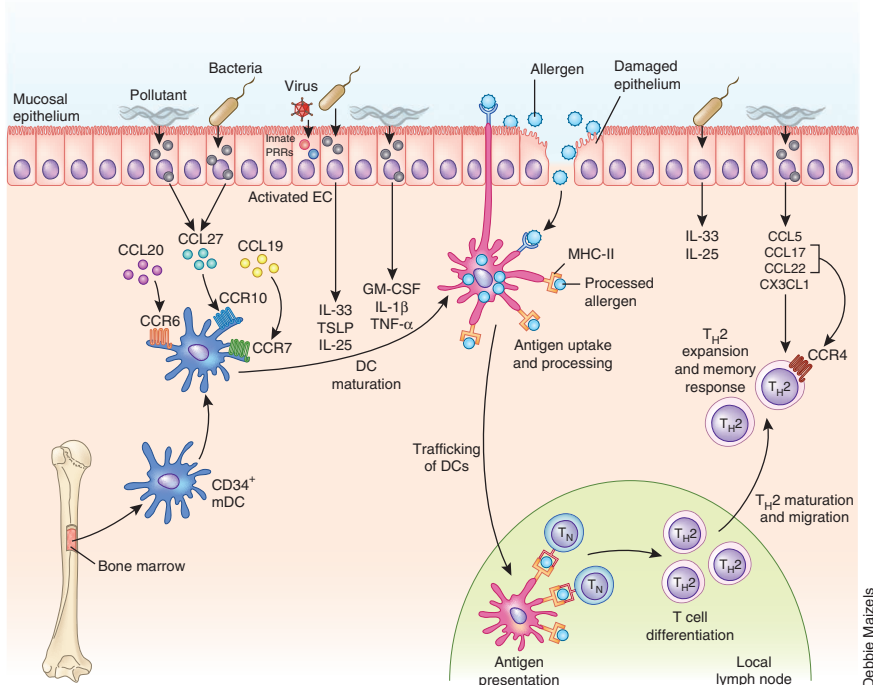
Polarization toward a T_H2 subtype is also under epigenetic regulation. In mice, microRNA miR-21 has been shown to exert a pivotal role in setting a balance between T_H1 and T_H2 responses. It does this by binding the promoter of the gene encoding IL-12 p35 and inhibiting its activation in favor of a T_H2 profile. Conversely, reduced microRNA levels lead DCs to produce more IL-12, and allergen-challenged T cells to produce more interferon-γ (IFN-γ) and less IL-4, enhancing T_H1 delayed-type hypersensitivity¹⁴.

Propagation of the allergic response

Enhancement of airways sensitization is augmented by two additional factors:

Breakdown of barrier function. In developed countries, atopy afflicts up to 40% of children and young adults, but only one-third of cases develop into asthma. Furthermore, systemic atopy itself does not seem to be as important as local sensitization of the lower airways to aeroallergens associated with the development of asthma¹⁵. Indeed, genome-wide association studies (GWASs) are revealing new asthma susceptibility genes that are mostly expressed in the epithelium and innate immune pathways, including *ORMDL3*, *IL33* and *SMAD3*

Figure 1 Primary sensitization of the airways in the induction of allergic-type asthma. Perturbation of the epithelium with infection and pollutants provides the initial danger signal and activates innate signaling receptors, leading to chemokine secretion from airway epithelial cells and trafficking of immature DCs to the mucosal epithelium. DCs respond to danger signals via PRRs, resulting in their maturation to competent antigen-presenting myeloid-type DCs. Once activated, mature DCs process allergens detected through processes extended into the airways, or by capturing allergens that have breached the epithelium. The allergen-loaded DCs then migrate to local lymph nodes where they interact with naive T cells via TCR, MHC class II and co-stimulatory molecules to drive differentiation of T cells. Additional epithelial-derived cytokines and chemokines such as IL-33, IL-25, CCL17 and CCL22 influence DC activation and T_H2 maturation and migration into the mucosa.



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(ref. 16), rather than genes associated with atopy and serum IgE such as the HLA-DQ locus¹⁶, *FCER1A*, *STAT6* and *IL13* (ref. 17).

The asthmatic epithelium is intrinsically defective in its physical barrier function with incomplete formation of tight junctions, thereby facilitating penetration of inhaled allergens into the airway tissue¹⁸ (Fig. 1). Related to this defect, a proportion of the asthma-related allergens have intrinsic biological properties that increase their capacity to penetrate the epithelial barrier and trigger a danger signal to DCs. Components of house dust mite, cockroach, animal and fungal allergens are proteolytic and can disrupt epithelial tight junctions and activate protease-activated receptors¹⁹. Other allergens have more subtle biological effects (Table 1). Beyond proteolytic allergens, additional environmental stimuli such as respiratory viruses and air pollutants (for example, ozone, particulates and environmental tobacco smoke)^{20,21} also disrupt tight junctions to impair barrier function.

Defective epithelial barrier function has also been described in the pathophysiology of food allergy²², allergic rhinosinusitis²³ and conjunctivitis²⁴. However, it is in the pathogenesis of atopic dermatitis (or eczema) that genetic defects in barrier function are most clearly understood, with defective filaggrin (FLG) production being the most frequent²⁵. In atopic children, it is not infrequent that atopic dermatitis precedes asthma in the 'atopic march', suggesting that epicutaneous allergen transfer may contribute toward the development of asthma²⁶.

Enhancement of DC antigen-presenting capacity. Once sensitized, memory T cells drive the ongoing allergic response through interactions with DCs. DCs extend their processes between epithelial cells into the airway lumen²⁷, and, by forming tight junctions with adjacent epithelial cells, they can detect inhaled allergenic molecules without disturbing the epithelial barrier²⁸. In the mouse lung, two distinct DC subsets have been described in accordance with their expression of the integrin CD11c as myeloid (conventional DCs (cDCs), CD11c⁺) or plasmacytoid DCs (pDCs, CD11c⁻)²⁹. Similarly, human DCs are subdivided into CD11c⁻ (CD123⁺CD303⁺CD304⁺) plasmacytoid DCs and CD11c⁺ myeloid DCs. The myeloid DC can be further subdivided into three subsets on the basis of differential expression of CD1c, CD141 and CD16 (ref. 30). Induced sputum from asthmatic airways and peripheral blood contain increased numbers of both pDCs and cDCs, which further increase in number upon allergen challenge³¹.

Proteolytic and other biological activities of allergens (Table 1) provide one set of danger signals (PAMPs) to initiate maturation of DCs. Within a few hours of contact with allergen, activation of pattern-recognition receptors (including TLRs on DCs) augments their homing capacity by upregulating chemokine receptors to direct them toward the T cell area of regional lymph nodes³² (Fig. 1). It is the myeloid-type cDC subtypes that are predominantly responsible for antigen presentation, whereas plasmacytoid pDCs are more strongly linked to acquisition of tolerance and antiviral defense. During this time, DCs undergo a substantial phenotypic change, from quiescent cells to cells expressing an array of cell-adhesion and co-stimulatory molecules that are recognized by naive T cells and by the processing of the allergen. Mature DCs occupying the T cell area form an 'immunological synapse' with the few allergen-specific T lymphocytes to initiate a T_H response. Whereas some of the T_H cells make their way to the B-cell follicle to facilitate immunoglobulin class switching from IgM to IgE, others move back to the airway mucosa to elicit the classical T_H2 response through the coordinated secretion of the proallergic cytokines.

Epithelial consolidation of DC activation. Pattern-recognition receptors (PRRs) have a crucial adjuvant role in directing allergen sensitization.

Table 1 Examples of biological activities of aeroallergens

Source of allergens	Identified biological activities
Grass pollen	Pectate lyase, RNase, polygalacturonase, lipid transfer protein, profilin, expansin
Tree pollen	Profilin, isoflavone reductase, pathogenesis-related protein, pectin methylesterase, peptidyl-prolyl isomerase, 1,3-β-glucanase, calcium-binding protein, pectate lyase, superoxide dismutase
Fungi	Protein disulfide isomerase, aldehyde dehydrogenase, RNase, vacuolar serine protease, alkaline serine protease, enolase, aspartate protease, dipeptidyl peptidase, subtilisin-like protease
Pet dander	Uteroglobin-like protein, cystatin, lipocalin, albumin
Mites	Cysteine protease, β-glucan moiety, trypsin, amylase, chymotrypsin, chitinase, collagenase, glutathione transferase

TLRs are key components of the innate immune system that mediate recognition and response to PAMPs in the form of microbial, fungal and viral products and their ligands, including endotoxin (recognized by TLR4), lipoproteins (TLR2 and TLR6), viral double- and single-stranded RNA (TLR3 and TLR7/8) and bacterial CpG-containing DNA (TLR9)³³. Other PRRs respond to endogenously generated danger signals (DAMPs) produced during tissue damage, such as free ATP and uric acid³⁴. Small amounts of TLR ligands from microbes in house dust, when inhaled along with allergens, provide the necessary adjuvant effect to drive the allergic response. Activation of selective TLRs on epithelial cells enhances DC motility and antigen sampling through the production of T_H2-promoting chemokines (CCL17 and CCL22 via CCR4)³⁵ and cytokines (IL-25, IL-33, GM-CSF and thymic stromal lymphopoietin (TSLP)) (Fig. 1). In the absence of TLR ligation, however, DCs remain relatively inactive³⁶.

Viral infection and predisposition to asthma. The discovery that early-life sensitization to multiple aeroallergens carries the greatest identifiable risk for developing asthma¹⁵ raises the question of what factors result in a predisposition to this phenotype. Although infection with rhinoviruses is the main cause of asthma exacerbation, in those genetically at risk of asthma, rhinovirus-induced wheezing in the first three years of life is also the greatest risk factor for developing asthma at 6 years of age³⁷. The range and magnitude of allergen sensitization is also affected by rhinovirus infection³⁸, with the latter now known to precede the former³⁹. As an intrinsic abnormality, impaired TLR3-mediated interferon-β (IFN-β)⁴⁰ and IFN-λ⁴¹ production by asthmatic epithelial cells would predispose to both viral lung infection and allergic sensitization^{42,43}. Reduced primary IFN production by lower-airway epithelial cells enables rhinovirus and other viruses to replicate, leading to cytotoxic cell death (rather than apoptosis), release of inflammatory products and enhanced viral shedding. Such events provide a strong stimulus for recruitment of immature DCs and their priming for allergen sensitization^{9,44}. Asthmatic epithelial cells also respond to rhinovirus infection by generating increased amounts of the pro-T_H2 cytokine TSLP⁴⁵ (Fig. 1), whereas exogenous IFN-β applied to asthmatic epithelium exerts anti-T_H2 as well as antiviral properties⁴⁶.

Environmental protection against T_H2-mediated sensitization

The ability of microbial and other environmental stimuli to shape the immune response provides a mechanism for the rising trends of asthma in those countries adopting a Western lifestyle and also helps explain protection afforded by traditional livestock-farming and rural

lifestyles⁴⁷. The amount and range of microbial exposure, rather than exposure to specific organisms or their products, is what drives protective immunity^{48,49}. Previous studies have found that children living on farms have a wider range of microbial exposures than occurs in the reference groups, and this is suggested to explain the protective effect of a farming environment on asthma development^{4–6}. As a function of urbanization, loss of microbial biodiversity in air, soil, water and food, along with the adoption of intensive farming methods and use of biocides, widespread use of antimicrobials and disinfectants, changes in the microbiota and dietary changes, go some way in explaining the rise in asthma and allergy in countries adopting components of the western lifestyle⁵⁰. Experimental support for this concept comes from the increase in T_H2 and IgE responses in animals bred in the absence of microbial exposures (gnotobiosis)⁵¹.

The idea that exposure to a wide variety of microorganisms and their products protects against allergy and asthma has been reinforced further by the discovery that the microbiome of the airways in asthma is completely different from that of normal subjects. Contrary to popular opinion, the bronchial tree is not sterile; it possesses at least 2,000 bacterial genomes per square centimeter⁵². Pathogenic Proteobacteria (for example, *Haemophilus* spp.) occur most frequently in asthmatic airways of adults and children as compared to normal controls, whereas Bacteroidetes (for example, *Prevotella* spp.) are more frequent in controls. *Haemophilus*, *Moraxella* and *Neisseria* spp. found in neonatal throat swabs have been reported to correlate with an increased risk for asthma in early life, when the disease most frequently begins⁵³.

Mechanisms of microbial protection against asthma and allergy

A new paradigm for allergy states that, in genetically susceptible subjects, low-dose exposure to PAMPs and DAMPs in the presence of allergens leads to priming of DCs and enhanced allergic responsiveness⁵⁴. In contrast, high-dose exposure (for example, as occurs on livestock farms, in rural environments in developing countries and in traditional lifestyles) leads to allergenic tolerance⁵⁵. Scalable triggering of innate immunity, as a principal determinant of allergy, is attractive: in different environments, ambient air concentration of the TLR4 agonist, lipopolysaccharide, can vary by as many as 5 logs, with the highest levels being encountered in association with livestock farming⁴ and pet ownership⁵⁶.

Immunological tolerance in protecting against asthma. Dampening of immunological responsiveness and induction of allergen tolerance have been attributed to the regulatory T (T_{reg}) cell. T_{reg} cells are identifiable through their coexpression of the transcription factor forkhead box protein 3 (Foxp3), CD4 and CD25, and they exist in either naturally occurring (thymus-derived or nT_{reg}) or inducible (T_R1 or iT_{reg}) forms⁵⁷. The redirection of allergen-specific effector T cells to a regulatory phenotype is a key event in the development of a normal immune response to allergens, and it accounts for at least some of the clinical efficacy of allergen-specific immunotherapy. Naturally occurring T_{reg} cells influence allergic pathways by secreting IL-10 and TGF- β to mediate (i) suppression of DCs involved in programming effector T cells, (ii) direct inhibition of T_H1 , T_H2 and T_H17 cells, (iii) suppression of allergen-specific IgE and induction of IgG4, (iv) inhibition of mast cells, basophils and eosinophils and (v) prevention of effector T cell migration into the target tissue⁵⁸ (Fig. 2). Although controversy remains over how IgG4 is able to suppress allergic responses, this process probably involves activation of immunoreceptor tyrosine-based inhibitory motif (ITIM) signaling

through aggregation of the low-affinity IgG receptors Fc γ RIIB and Fc γ RIIA on mast cells and basophils, rather than inhibiting allergen binding to IgE⁵⁹.

Early-life protection against allergy and asthma. Prenatal exposure to high levels of microbial products (for example, in milking parlors and cow sheds) is especially protective, and although multiple mechanisms have been suggested, the inhibitory response seems most closely linked to high circulating numbers of T_{reg} cells⁶⁰. In mice, selective depletion of T_{reg} cells during the allergen-sensitization phase markedly enhances experimental allergic lung inflammation⁶¹. A study in humans showed that maternal cells have the capacity to traverse the placenta and enter fetal lymph nodes to induce iT_{reg} cells, preventing antimaternal immunity that persists until early adulthood. This study also challenges the idea that fetal lymphocytes are incapable of mounting an immune response⁶². A similar mechanism of maternal-fetal transfer may operate in the protection against allergic sensitization to induce allergen-specific T_{reg} cells in the presence of TGF- β . However, although membrane-bound TGF- β has been implicated in mucosal tolerance to microorganisms⁶³, answering the question of how these pathways are activated in the presence of high maternal and offspring microbial exposure involving PRRs and epigenetic pathways requires further research.

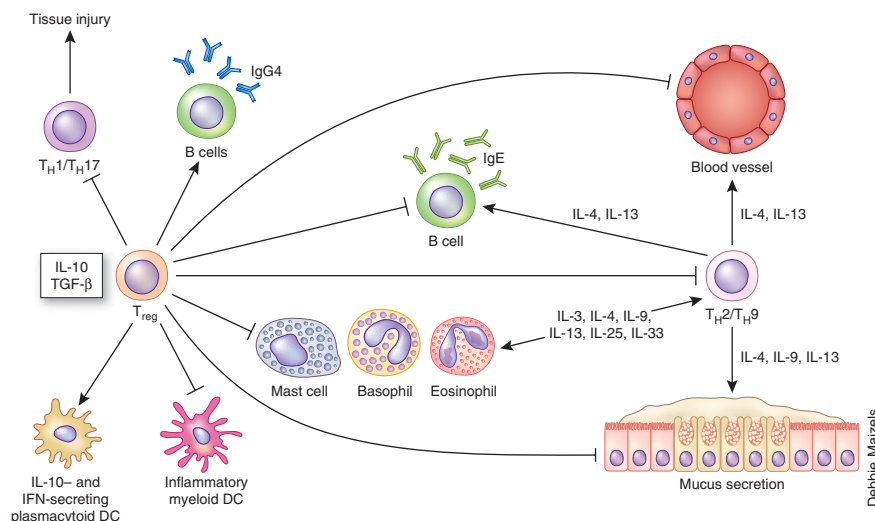
Further strong support for maternal programming of protection against infant sensitization comes from exposing pregnant mice to the Gram-negative, nonpathogenic bacterium *Acinetobacter lwoffii* F78, which is found in cow sheds and activates TLR2, TLR3, TLR4, TLR7 and TLR9. Prenatal exposure of pregnant mice to *A. lwoffii* F78 protects the offspring from developing T_H2 -mediated allergic airway inflammation⁶⁴. The mechanisms resulting in this protection involve the upregulation of maternal lung TLR expression, initiation of a transient, acute, local T_H1 -like inflammatory response, systemic release of inflammatory cytokines and downregulation of TLR and cytokine expression in the placenta. Offspring from *A. lwoffii* F78-treated *TLR2/3/4/7/9*^{-/-} mothers were no longer protected from T_H2 -type airway responses to inhaled antigen; thus, maternal TLR signaling seems to be crucial in mediating the transfer of this transgenerational protective effect. The inhibitory effects of *A. lwoffii* F78 on allergic responsiveness in the offspring is dependent on IFN- γ and epigenetically controlled through chromatin histone 4 (H4) acetylation of the *IFNG* promoter of CD4⁺ T cells; conversely, inhibition of H4 acetylation abolishes the T_H2 -protective phenotype⁶⁵.

Engagement of adaptive cellular immunity in asthma

Once DCs have become primed by the environment to promote allergen sensitization, a common sequence of events occurs. Presentation of allergen peptides in the cleft of MHC class II to the TCR on naive T cells in parallel with engagement of co-stimulatory molecules on each cell type (for example, CD80 or CD86 on DCs with CD28 on T cells, or OX40L on DCs with OX40 on T cells) in the presence of low levels of IL-12 and high levels of IL-4 results in T-cell differentiation toward the T_H2 phenotype (Fig. 1). If the co-stimulatory second signal in the immunological synapse is missing (for example, engagement of CD80/86 with cytotoxic T-lymphocyte antigen-4 (CTLA-4) on T cells), then anergy or T cell apoptosis results⁶⁶.

Among the family of co-stimulatory molecules, OX40L is selectively upregulated by the IL-7-like cytokine TSLP, which derives largely from the epithelium following activation of TLRs⁶⁷. Several other newly identified epithelial-derived cytokines have also been shown to exert T_H2 -polarizing activity. These include the IL-1 and

Figure 2 The pleiotropic effects of T_{reg} cells in suppressing allergic inflammatory pathways. T_{reg} cell development and suppression is fundamental in protecting normal airways against allergen sensitization. T_{reg} cells are also induced in response to allergen-specific immunotherapy and promote the production of the 'blocking' antibody class IgG4. Solid arrows indicate stimulatory signals; blunt arrows indicate inhibitory signals.



IL-18 family member IL-33 acting through its receptor, ST2 (ref. 68), and IL-25, one of six members of the IL-17 cytokine family⁶⁹. TSLP, IL-33 and IL-25 are generated by the airway epithelium in response to activation of PRRs such as TLR3 (and its ligand, viral double-stranded RNA) and TLR5 (and its ligand, bacterial flagellin) or following cytotoxic epithelial injury. IL-33 functions as an alarmin to alert the immune system to physical stress or infection (Fig. 1). The IL-33 receptor, comprising ST2 and IL-1 receptor accessory protein, is expressed at a high level on T cells and mast cells, where its activation expands the T_H2 cell population (possibly via induction of nuocytes) and greatly enhances release of their cytokines⁶⁸. In contrast, IL-25 functions to enhance the T_H2 memory response initiated by TSLP-primed DCs⁶⁹, with marked upregulation of IL-25 and its receptor occurring in asthmatic airways following allergen challenge⁷⁰. All three cytokines also interact with effector cells of the allergic response—mast cells, basophils, eosinophils and endothelial cells—to enhance their inflammatory functions⁷¹. Thus, by operating together, these three epithelial cytokines have the potential to bridge innate and adaptive immunity to sustain the T_H2 response toward a more chronic state that is characteristic of asthma.

The recent recognition of a new, innate type-2 immune effector leukocyte, the nuocyte, is an exciting new breakthrough in allergic disease in providing a missing link between the innate and adaptive T_H2 response (Fig. 3). Nuocytes expand in number in response to epithelial-derived IL-25, IL-33 (similar to IL-12 in driving the T_H1 response)⁷², Notch signaling and activation of the transcription factor retinoic acid-related orphan receptor γ (ROR γ) and are an early source of IL-13, as shown in gastrointestinal elimination of parasites. Thus, in the absence of both IL-25 and IL-33, nuocytes fail to expand, resulting in defective worm expulsion, which is reversible by the adoptive transfer of *in vitro*-cultured wild-type, but not IL-13-deficient, nuocytes⁷³. Thus, nuocytes represent a new, key innate effector cell in type-2 immunity, and recent observations suggest that this cell population is also incriminated in allergic airway sensitization and parasite-induced lung eosinophilia in mouse models^{74,75}. It is now crucial to show whether nuocytes have a role in the pathophysiology of asthma in humans.

New T cell subsets in asthma subphenotypes

T_H9 cells. A second T_H2 cell subtype, designated T_H9 , has been linked to asthma as a subpopulation that requires both IL-4 and TGF- β ⁷⁶ (Fig. 3). During the allergic response *in vivo*, it remains to be established whether IL-9-secreting T cells are distinct from T_H2 cells or whether T_H2 cells become reprogrammed into T_H9 cells. T_H9 cells do not express well-defined transcription factors such as T-bet, GATA3, ROR γ and Foxp3, indicating that they are different from T_H1 , T_H17 and iT_{reg} cells. The sequential cytokine interactions that

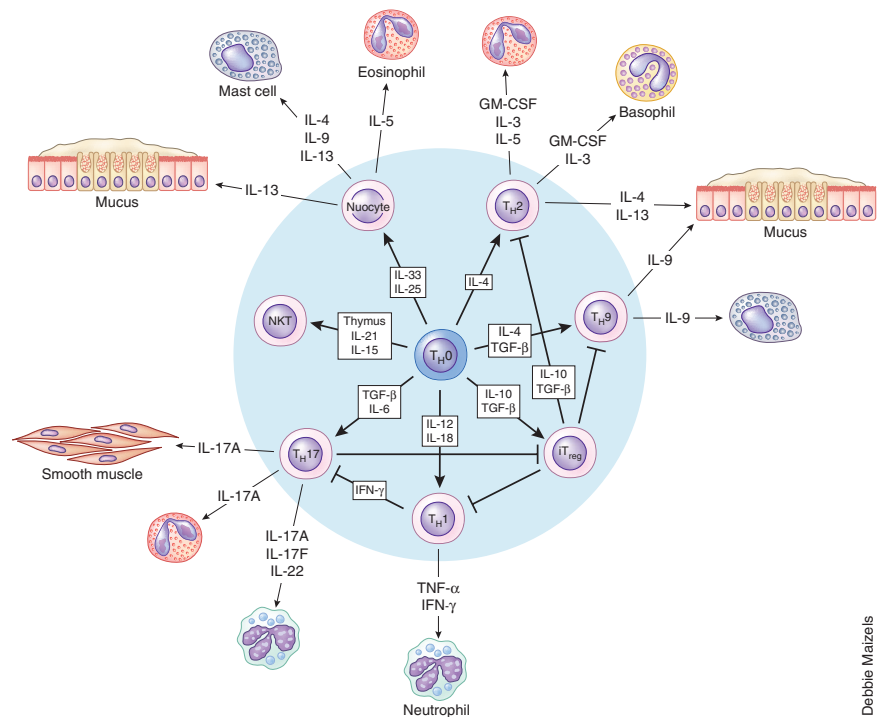
lead to T_H9 differentiation are becoming clearer⁷⁷. TGF- β induces the PU.1-encoding *Sfp1* locus, which is independent of IL-4-induced STAT6 activation, whereas IL-4 represses T-bet and Foxp3 (inhibitors of IL-9 production) but is required for the induction of T_H9 -promoting IRF4 (ref. 78).

Signaling through IL-2R and CD28 can increase IL-9 secretion by allergen-specific T cells and requires the simultaneous presence of both IL-4 and IL-10, although IL-4-independent production of IL-9 has also been demonstrated from human memory (CD45RO⁺ CCR8⁺) T cells, nT_{reg} cells in peripheral tissues and iT_{reg} cells in the presence of TGF- β . Other sources of IL-9 relevant to diseases such as asthma include eosinophils, basophils and mast cells. IL-9 is highly expressed and localized to tissue lymphocytes during intestinal parasite infection⁷⁹ and to CD3⁺ cells in bronchial submucosa and bronchoalveolar lavage⁸⁰. IL-9 expression also increases markedly in response to allergen challenge⁸¹. In studies using IL-9 transgenic and knockout mice, direct IL-9 instillation into the lungs and blocking monoclonal antibodies (mAbs), it has been shown that IL-9 drives mucus production, both by a direct effect on airway epithelia⁸² and also by interacting with IL-13 (ref. 83). Other features of airway remodeling such as sub-epithelial fibrosis have also been described in IL-9 transgenic mice, possibly owing to immune-cell recruitment (for example, by promoting eotaxin release from smooth muscle) and inflammatory-mediator release⁸⁴. Along with IL-4 and stem cell factor, IL-9 is also a potent stimulus for mast-cell development⁸⁴. As IL-9 has been implicated in both inflammatory and remodeling components in mouse models of allergic airway disease, it seems an attractive therapeutic target. Two first-in-human, open-label dose-escalation trials of a monoclonal antibody against IL-9, MEDI-528, in normal subjects and subjects with mild asthma have been successfully completed, showing some evidence of efficacy⁸⁵.

T_H17 cells. T_H17 cells are a distinct T cell lineage that does not share developmental pathways with either T_H1 or T_H2 cells. Indeed, both IFN- γ and IL-4 inhibit IL-17-producing T cells, whereas blocking antibodies to IFN- γ and IL-4 are facilitatory^{86,87}. T_H17 cells have a role in regulating both neutrophilic and macrophage inflammation in autoimmune disease and psoriasis, and more recently they have been suggested to be involved in asthma and corticosteroid insensitivity⁸⁸. T_H17 lymphocytes differentiate in response to a combination of TGF- β and IL-6, leading to the expression of the

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Figure 3 Different T cell subtypes involved in the pathogenesis of asthma and its differing endotypes. Combinations of cytokines and contact signals cause DCs and the thymic epithelium to conspire to drive the differentiation of an array of different leukocyte subsets (inner, blue circle) that augment or protect the airways from inflammatory responses linked to asthma. These different leukocyte subsets generate cytokines that influence different cell types and the attendant inflammatory response, driving allergic airway inflammation and airway hyperreactivity.



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transcription factor ROR γ t. Conversely, their differentiation is restricted by both T_H1 and T_H2 cytokines including IFN- γ , IL-4 and IL-13 (ref. 87). T_H17 cells are characterized by the production of IL-17A, IL-17F and IL-22. IL-17A and IL-17F both induce IL-6, GM-CSF, CXCL10 and CXCL8 in epithelial and endothelial cells, fibroblasts, neutrophils and eosinophils⁸⁹. Specifically, the induction of CXCL8, a potent neutrophil chemokine whose expression is elevated in airway secretions in severe asthma, has directly implicated T_H17 cells in neutrophilic airway inflammation⁹⁰.

In mice, allergic sensitization followed by challenge of the airways induces a strong T_H17 response in association with airway neutrophilia and hyperresponsiveness, and this response is abrogated in *Il17f*^{-/-} mice⁹¹. Thus, although IL-17 is considered to be essential during antigen sensitization to establish allergic airway responses, in mice already sensitized to allergen, IL-17 attenuates the allergic response by inhibiting DCs and chemokine (CCL11 and CCL17) synthesis⁹². Also in mice, there seems to be an inverse relationship between IL-25 and IL-17A in regulating allergic lung responses. In ovalbumin-driven allergic-type lung inflammation, neutralization of IL-25 correlates with a decrease in IL-13 concentrations, but an increase in IL-17A production, and prevention of AHR. In contrast, blockade of IL-17A reverses any protective effect of antibodies to IL-25 including re-expression of IL-13 and eotaxin production, eosinophilia and AHR⁹³. Moreover, IL-17A itself, but not IL-17F or IL-22, enhances the contractile force of airway smooth muscle. Sensitized mice lacking the integrin $\alpha_v\beta_8$ on DCs show reduced activation of this IL-17A-linked pathway with antigen challenge. This reduction in smooth muscle contraction in the airways is reversible by IL-17A, indicating involvement of this cytokine on allergen-induced AHR by acting directly on airway smooth muscle⁹⁴.

Experimental models of allergic rhinitis and asthma have also suggested that the complement cleavage products C3a and C5a, and activation of their corresponding receptors in antigen-presenting cells, regulate the development of altered T_H2 and T_H17 immunity⁹⁵. Thus, C3aR-deficient mice have impaired T_H2 responses, along with protection from AHR, following allergic antigenic stimulation^{96,97}. Similarly, when C5a is inhibited, the severity of AHR is reduced during the effector phase of the allergic airway response in rodents⁹⁸. Complement probably has a multifunctional role in the allergic tissue response: on the one hand, it exacerbates AHR and the T_H2 response during the effector phase; on the other, it induces tolerance during allergen presentation to T cells⁹⁹.

In humans, a newly recognized subset of T_H2 memory and effector cells has been found expressing both GATA3 and ROR γ t and, as

a consequence, producing T_H17 and T_H2 cytokines¹⁰⁰. However, although a good case can be made for IL-17A and IL-17F in mouse models of neutrophilic and corticosteroid-refractory lung responses to allergens, evidence for IL-17 involvement in human asthma is less robust, despite some emerging genetic evidence and a potential role for IL-17A and IL-17F in moderate-to-severe disease^{101,102}.

Several groups have reported that the number of circulating T_H17 cells as well as plasma concentrations of IL-17 and IL-22 increase in proportion to disease severity. However, in a recent bronchial biopsy in asthma of varying severity compared to normal controls, the main cellular source of IL-17 was neutrophils, not T cells, with no correlation between IL-17A or IL-17F expression and the extent of neutrophilia, nor any link to asthma severity¹⁰². Rather, the number of bronchial submucosal IL-17F⁺ cells correlated most closely with the extent of eosinophilia. It is clear that functional evidence is needed to associate T_H17 cells causally to a specific subphenotype of human asthma. It should be noted that prosurvival factors for neutrophils are also produced by the epithelium in severe asthma and asthma associated with current tobacco smoking¹⁰³, possibly involving transactivation of the epidermal growth factor receptor¹⁰⁴.

Invariant natural killer T cells. Invariant natural killer T (iNKT) cells are characterized by an invariant variable region TCR α -chain 14–joining region 18 (V(α)14-J(α)18), which selectively recognizes the glycolipid α -galactosylceramide (α -GalCer) presented to the TCR by the antigen-presenting molecule CD1d¹⁰⁵. Recently, the endogenous lipid β -D-glucopyranosylceramide (β -GlcCer) has been shown to be a potent cell self antigen in mice and humans, and its activity is dependent upon the composition of the N-acyl chain. Because β -GlcCer is generated in response to TLR agonists, it is suggested that recognition of β -GlcCer by CD1d translates innate danger signals into iNKT cell activation¹⁰⁶.

A role for subsets of iNKT cells in asthma has also been suggested by extensive studies in animal (mostly mouse) models of lung inflammation driven by allergen, viral infection, ozone exposure or bacterial components. This idea requires that, in causing airway hyperreactivity,

iNKT cells function together with T_H2 cells or independently of adaptive immunity¹⁰⁷. However, whereas the mouse studies are highly suggestive, the role of CD1d-restricted iNKT cells in human asthma remains controversial. An initial study reporting that iNKT cells were the predominant T cells in the lung and bronchoalveolar lavage fluid of all asthmatic subjects studied¹⁰⁸ has not subsequently been confirmed. Indeed, the methods used to detect human iNKT cells in this first report were suboptimal. Instead, iNKT cells seem to be present only as a small fraction of the total T cells populating asthmatic airways¹⁰⁹. An emerging view is that iNKT cells are likely to have a role in modulating the asthmatic phenotype, but they are probably not the critical drivers of the asthmatic response, as originally suggested. However, in line with a modifying influence, a recent study reported that most sterile house dust extracts contain antigens capable of activating both mouse and human iNKT cells¹¹⁰.

The humoral immune response

Isotype switching of B cells to IgE synthesis is a prerequisite of atopy and provides the critical trigger mechanism for the immediate allergic response upon exposure to allergen. This process requires the obligatory presence of IL-4 or IL-13, which, by interacting with its receptor on B cells in the presence of MHC class II-associated allergen presentation by T_H2 cells and co-stimulation involving CD40 and CD40L, results in immunoglobulin class switching from IgM to IgE. The intracellular molecular sequence involves germline gene transcription, DNA recombination and B cell differentiation, resulting in the synthesis and secretion of allergen-specific IgE antibody, followed by the maturation of clonal B cell subsets into plasma cells secreting large amounts of IgE¹¹¹. IgE antibodies with high affinity for allergens undergo cross-linking at low concentrations by trace amounts of allergen, whereas IgE antibodies with low affinity bind allergens weakly. High-affinity IgE is formed by sequential class switching ($\mu \rightarrow \gamma \rightarrow \epsilon$), with an intermediary IgG phase required for the affinity maturation of the IgE response¹¹². As a result, the IgE inherits somatic hypermutations and high affinity from the IgG1 phase. Low-affinity IgE is formed through direct class switching ($\mu \rightarrow \epsilon$) and is therefore much less mutated. It follows that mice deficient in IgG1 production cannot produce high-affinity IgE, even with repeated immunizations. Low-affinity IgE competes with high-affinity IgE for binding to Fcε receptors, thereby reducing IgE-dependent mast-cell and basophil activation-secretion coupling¹¹².

IgE binds the α -chain of high-affinity FcεR1 on mast cells and basophils. Cross-linking of receptor-bound IgE by allergen initiates cell activation and the release of preformed and newly generated mediators, cytokines, chemokines and growth factors. FcεR1 receptors facilitate allergen uptake and processing, with the extent of IgE occupancy of FcεR1 positively regulating the level of receptor expression. IgE can also bind low-affinity FcεRII (CD23) on B cells, where it serves to enhance both allergen-specific B cell and T cell recall functions¹¹³. In the bronchial mucosa of asthmatics, class switching to IgE has been shown to occur irrespective of atopic status¹¹⁴, providing a possible mechanism for non-atopic as well as atopic asthma. More than 50% of individuals with non-atopic asthma also have circulating IgE antibody against *Staphylococcus aureus* endotoxins, which serve as superantigens by interacting with selective TCR Vβ families¹¹⁵.

Modulation of innate and adaptive immunity in asthma

The sentinel role of T cells, especially of the T_H2 type, in orchestrating the inflammatory response in asthma makes them a particularly attractive therapeutic target.

Immunosuppressants in difficult asthma. Severe corticosteroid 'refractory' asthma presents a particularly difficult problem, as it is associated with the greatest morbidity and mortality and the accompanying health service and economic costs. Many attempts to treat this condition have used immunosuppressants such as gold salts, methotrexate, azathioprine, cyclosporine and tacrolimus, but, although some clinical benefits have been found in individual patients, the beneficial effects are sporadic and often associated with unacceptable side effects. Intravenous immunoglobulin also has limited use in severe asthma, by reducing T cell activation and increasing corticosteroid responsiveness¹¹⁶. A more recent approach has been the use of biologics to selectively block T cell responses. Keliximab, a chimeric mAb targeted to the CD4 receptor, when administered to individuals with moderate-to-severe corticosteroid-dependent asthma produced some beneficial effect on the diurnal variation in lung function. However, it had limited effect on other asthma end points¹¹⁷, despite markedly reducing T cell numbers, CD4 receptor density and allergen-driven T cell proliferation¹¹⁸.

In asthmatic airways and circulating cells, the extent of cell-surface CD25 expression increases in proportion to disease severity. Daclizumab is a blocking humanized mAb to CD25 and a powerful immunosuppressant used in the management of organ transplant rejection¹¹⁹. In moderate-to-severe asthma, treatment with daclizumab for 150 d resulted in significant but small improvements in asthma outcome measures but was accompanied by the expected side effects of immunosuppression¹²⁰.

Following allergen challenge in asthma, T_H2 cells are recruited into the airways by the release of CCL17 and CCL22 from epithelial cells interacting with CCR4 on T_H2 cells¹²¹. The supernatant from allergen-challenged asthmatic bronchial explants is highly chemoattractant to T_H2 cells, and its chemoattractant activity is completely blocked by a mAb to CCR4 (ref. 122). A single injection of a defucosylated human IgG mAb to CCR4 with enhanced antibody-dependent cell-mediated cytotoxicity (mogamulizumab, AMG 761) in nonhuman primates and in atopic normal subjects caused profound and sustained reductions in numbers of circulating T_H2 cells lasting several months¹²³. Amgen is planning a phase 2 trial of mogamulizumab in asthma, but this awaits the selection of doses that are sufficient to attenuate T_H2 cytokine production without depleting T_H2 cells. Therapeutic efficacy of mogamulizumab has already been shown in mycosis fungoides, Sézary's syndrome and related T cell lymphomas involving CCR4-bearing T cells¹²⁴.

Another T_H2 chemoattractant is prostaglandin D_2 (PGD₂), an eicosanoid produced in large amounts from activated mast cells¹²⁵. The G protein-coupled receptors TP1 and DP1 mediate PGD₂'s bronchospastic and vasodilator actions, respectively; however, stimulation of the third PGD₂ receptor, CRTH2 (DP2), which is expressed on T_H2 cells, eosinophils and basophils, leads to their recruitment into the airways, as well as promoting cytokine release¹²⁶. Potent small-molecule CRTH2 antagonists such as MK-7246 are highly active at inhibiting eosinophilic responses in allergen-challenged animal models¹²⁷. However, whereas in asthma CRTH2 expression is restricted to only 2.3% of airway T cells compared to 0.3% in normal airways, a recent 4-week clinical trial of the orally active antagonist OC000459 (Oxigen) has shown some efficacy in moderate disease not requiring corticosteroids, and caused a 60% reduction in the frequency of sputum eosinophils¹²⁸.

An attractive approach to attenuating T cell responses in asthma is to block T cell and DC co-stimulation through the 'second signal' (Fig. 1). Maturation and activation of T cells involving

MHC-restricted antigen presentation requires parallel engagement of CD28 on T cells by CD80 or CD86 (second signal)⁶⁶. This co-stimulation is interrupted if CD28 interacts with CTLA-4, another adhesion molecule that delivers a suppressive signal to T cells by inhibiting Akt phosphorylation to induce anergy or apoptosis, and directs early T cell differentiation into Foxp3⁺ T_{reg} cells¹²⁹. A human immunoglobulin CTLA-4 fusion protein (abatacept) has been developed that is efficacious in rheumatoid arthritis¹³⁰ and in slowing the loss of pancreatic islet β cells in type 1 diabetes and that is associated with increasing the proportion of T_{reg} cells in insulinitic lesions¹³¹. In human asthmatic bronchial explants, CTLA-4 fusion protein inhibits allergen-specific T_H2 cytokine¹³² and chemokine¹³³ release, but only in explants from the airways of mild allergic asthmatics¹³⁴. A trial in mild atopic asthma is investigating whether abatacept is effective in decreasing allergic airway inflammation following segmental allergen challenge (<http://clinicaltrials.gov/ct2/show/NCT00784459>). A phase 2 trial to evaluate the efficacy, safety and tolerability of an intravenous human mAb to OX40L (RO4989991) in the prevention of allergen-induced asthma has also just been completed, and the outcome is awaited (<http://clinicaltrials.gov/ct2/show/NCT00983658>).

Targeting T_H2 cytokines in asthma. The key cytokines incriminated in T_H2-type inflammation are those encoded in the IL-4 gene cluster on chromosome 5q31, containing the genes encoding IL-3, IL-4, IL-5, IL-9, IL-13 and GM-CSF¹. Confidence in the T_H2 pathway as being crucial to asthma pathophysiology has been the driving force for a range of biologics targeting their specific cytokines. These have mostly been directed at the IL-4 and IL-13, IL-5 and, for T_H1, the TNF pathways. What is clear from all these studies is that overall clinical efficacy is relatively low but, in each of the tested populations, substratification has identified a small, well-defined responder group¹³⁵.

A good example is the IL-4 and IL-13 pathway. Although there is overwhelming evidence from *in vitro* and animal (mostly mouse) studies indicating a sentinel role for this cytokine pathway in allergic processes¹³⁶, attempts to validate this hypothesis in human asthma revealed that only 50% of individuals with asthma had elevated IL-13 levels in sputum, irrespective of the severity of the disease¹³⁷.

To emphasize the importance of disease substratification, Woodruff *et al.*¹³⁸ have shown that only 50% of individuals with asthma attending a clinic express IL-13-responsive genes in their airway epithelial cells, and this is linked to a strong T_H2 ('T_H2-high') response in bronchial biopsies, as opposed to in 'T_H2-low' asthmatics, whose IL-13-responsive gene expression signature was indistinguishable from that of normal subjects. T_H2-high asthmatics had significantly greater expression of IL-5 and IL-13 in bronchial biopsies along with greater AHR and higher serum IgE, blood and airway eosinophilia, subepithelial fibrosis and airway mucin gene expression. The clinical response with a trial of inhaled corticosteroids was also restricted to T_H2-high asthmatics only¹³⁸. If only half of the subjects with asthma who had been enrolled into clinical trials to examine the efficacy of IL-4 and IL-13 blockade engaged the IL-13 pathway as a key component of pathobiology, then perhaps the negative outcomes of trials involving unselected patients is to be expected. One IL-13 biomarker in the Woodruff study was periostin¹³⁸. In a recently published trial, the mAb to IL-13, lebrikizumab (Genentech/Roche), when administered to patients with chronic moderate-to-severe asthma for 12 weeks, significantly increased baseline spirometry (5.5%). This result was enhanced in those with elevated serum periostin (high periostin

8.2% versus low periostin 1.2%)¹³⁹. However, as with the other IL-13-specific antibody trials (AMG-307 and CAT-354), there was little or no effect on patient-related outcome measures.

IL-5 is a key cytokine involved in eosinophil maturation and survival whose blockade in various animal models has a strong effect on acute and more sustained pulmonary eosinophilia and attendant changes in lung function. However, despite markedly reducing both circulating and sputum eosinophilia, two humanized mAbs, mepolizumab and reslizumab, when administered to patients with moderate-to-severe asthma, had no overall effect on any asthma outcome measures. Nevertheless, two studies of mepolizumab in patients with severe asthma requiring oral corticosteroids and persistent sputum eosinophilia showed a good clinical response^{140,141}, as also found in Churg-Strauss and other hypereosinophilic syndromes¹⁴². Similar results have also been obtained with reslizumab^{143,144}. Efficacy of mepolizumab has also been described in severe eosinophilic nasal polyposis in proportion to nasal lavage IL-5 levels¹⁴⁵. A further development of this approach has been the introduction of a highly active mAb targeting IL-5R α (MEDI 563, benralizumab), which has been defucosylated to enhance its antibody-dependent cell-mediated cytotoxicity potential¹⁴⁶. A phase 1 study in mild asthma has shown a strong dose-related reduction of circulating eosinophils lasting 8–12 weeks after a single injection¹⁴⁷.

Targeting TNF- α in severe asthma with golimumab also yields responders and nonresponders¹⁴⁸. In the case of anti-TNF α therapies, which include the soluble receptor fusion protein etanercept and mAbs, the identification of more neutrophilic asthma that is less dependent upon T_H2 mechanisms and, as a consequence, less responsive to corticosteroids might help identify a responsive target subpopulation. Such patients have been shown to have high circulating TNF- α and CXCL-8 as biomarkers¹⁴⁹. A transcriptomic analysis applied to induced sputum from such patients has identified a unique signature with prominence of IL-1 and the TNF- α and nuclear factor- κ B pathways¹⁵⁰. This stratification of asthma into pathway-selective phenotypes is likely to be a key driver for future drug development, as is proving so successful for cancer treatments.

BOX 1 Unanswered questions relating to adaptive and innate immunity in allergy and asthma

How does the epithelium program DCs during initial allergen sensitization and upon re-exposure?

What is the relationship between the increase in and altered function of smooth muscle cells and the immune and inflammatory responses in asthma?

What factors localize the immune and inflammatory responses to the conducting airways while sparing the alveoli?

How do the different immune and inflammatory phenotypes relate to different asthma endotypes, their responses to treatment and natural history?

What are the prenatal immune and structural changes in the lungs that predispose someone to acquiring an asthma phenotype?

What roles do biological properties of some allergens have in enhancing sensitization of the airways?

What are the causative mechanisms for the protective effects of microbes and their products in allergy and asthma?

Concluding comments

Knowledge of the underlying mechanisms of allergen sensitization and the airway events that follow allergen exposure has provided an important underpinning of the cellular and mediator mechanisms of asthma. However, important questions remain to be answered, such as how genetic factors predispose individuals to asthma (beyond atopy), the precise mechanisms for the rising trends in disease associated with the Western lifestyle, how exposure to microorganisms at crucial stages across the life course help to shape the asthmatic response or protect against it, and the cellular mechanisms that connect multiple different environmental exposures to asthma persistence and chronicity (see **Box 1**).

Knowledge gained from detailed immunology *in vitro* and in animal models of allergic-type lung inflammation has transformed our understanding of the crucial roles of both the innate and adaptive immune responses pertinent to asthma. However, what is disappointing is the lack of translation of this mechanistic understanding into patient benefit, unlike what has occurred for some other chronic immunological and inflammatory diseases (for instance, rheumatoid arthritis, systemic lupus erythematosus, psoriasis and inflammatory bowel disease). Part of this lack of success may rest on the reliance on relatively simple antigen sensitization and challenge models as surrogates for human asthma¹⁵¹. What are needed are models that more closely reflect asthma pathophysiology as it occurs in humans, and we should harness some of the new 'omics' technologies to explore in detail how different pathways translate into disease subtypes in deeply phenotyped patients. The identification of these pathways and their biomarkers, coupled with targeted biologics, will undoubtedly transform disease management, especially if these are upstream factors in the pathogenetic sequence and closer to the disease origins where stratified medicine is most effective¹³⁵. Moreover, a greater understanding of the early-life origins and factors leading to disease consolidation and persistence will open new doors for both asthma prevention and treatment.

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