

Pathophysiology of allergic inflammation

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Summary: Allergic inflammation is due to a complex interplay between several inflammatory cells, including mast cells, basophils, lymphocytes, dendritic cells, eosinophils, and sometimes neutrophils. These cells produce multiple inflammatory mediators, including lipids, purines, cytokines, chemokines, and reactive oxygen species. Allergic inflammation affects target cells, such as epithelial cells, fibroblasts, vascular cells, and airway smooth muscle cells, which become an important source of inflammatory mediators. Sensory nerves are sensitized and activated during allergic inflammation and produce symptoms. Allergic inflammatory responses are orchestrated by several transcription factors, particularly NF- κ B and GATA3. Inflammatory genes are also regulated by epigenetic mechanisms, including DNA methylation and histone modifications. There are several endogenous anti-inflammatory mechanisms, including anti-inflammatory lipids and cytokines, which may be defective in allergic disease, thus amplifying and perpetuating the inflammation. Better understanding of the pathophysiology of allergic inflammation has identified new therapeutic targets but developing effective novel therapies has been challenging. Corticosteroids are highly effective with a broad spectrum of anti-inflammatory effects, including epigenetic modulation of the inflammatory response and suppression of GATA3.

Keywords: Th2 cells, cytokines, chemokines, lipid mediators, T lymphocyte

Introduction

All allergic diseases are characterized by a distinct pattern of inflammation that is largely driven via immunoglobulin E (IgE)-dependent mechanisms. Genetic factors play an important influence on whether atopy develops, and several genes have now been identified in asthma and other allergic diseases using genome-wide association studies (GWAS), although few have been reproduced between studies or between different ethnic groups (1). So far the novel genes identified by over 20 GWAS have provided few new insights into the pathophysiology of allergic diseases, although T-helper 2 (Th2) cytokine genes have been consistently identified. In a GWAS of total serum IgE the most striking hits were functional variants in the genes encoding the α -chain of the high affinity receptor for IgE (Fc ϵ R α 1), RAD50, located adjacent to the gene for interleukin-13 (IL-13), and signal transducer and activator of transcription 6 (STAT6), which is regulated by

IL-4 and IL-13 (2). Environmental factors appear to be more important in determining whether an atopic individual develops a particular allergic disease, although genetic factors may exert an influence on how severely the disease is expressed and the extent of the allergic inflammatory response.

The inflammatory process has several common characteristics shared between different allergic diseases, including asthma, allergic rhinitis/rhinosinusitis, and atopic dermatitis (eczema) (3–5). Allergic inflammation is characterized by IgE-dependent activation of mucosal mast cells and an infiltration of eosinophils that is orchestrated by increased numbers of activated CD4⁺ Th2 lymphocytes. However, in patients with more severe disease, other cells such as neutrophils, Th1, and CD8⁺ (cytotoxic) lymphocytes may also be involved. The clinical differences between these diseases are largely determined by anatomical differences and the interaction between allergic inflammation and structural cells, such as airway smooth muscle cells in the lower airways, resulting in bronchoconstriction, vasodilatation in the upper airways leading to nasal blockage and rhinorrhea and activation of keratinocytes in the skin. The fact that there are common characteristics of allergic diseases suggests that it may be possible to treat these common diseases with single agents, particularly, as they often coexist.

This review focuses on how allergic inflammation is orchestrated, whereas detailed information about the different cellular components (mast cells, eosinophils, basophils, lymphocytes, dendritic cells), inflammatory mediators (cytokines, chemokines, lipid mediators), and structural cells (epithelial cells, airway smooth muscle cells, keratinocytes, endothelial cells, mucus-secreting cells) is provided in the detailed specific reviews included in this issue. No single cell or mediator can account for all the features of allergic disease and different cells and mediators may be more important in one manifestation of allergic disease than another. For example, histamine clearly plays a key role in rhinitis yet has a relatively minor role in asthma, as judged by the differences in efficacy of antihistamines between these conditions.

The allergic inflammatory response has evolved from the inflammatory process mounted in response to parasite and worm infections (6). This inflammatory response not only provides an acute defense against these organisms but is also involved in healing and restoration of normal function after tissue damage as a result of infection or toxins. Helminth infections appear to provide protection against the development of allergic diseases, but the nature of this protective immunity is not yet clear (7). In allergic diseases, the inflammatory response is activated inappropriately so that it becomes

harmful rather than protective. Allergens, such as house dust mite and pollen proteins, activate mast cells and eosinophil inflammation, which has evolved to kill the invading parasites and would therefore be self-limiting, but in allergic disease, the non-infective inciting stimulus persists and the normally acute inflammatory response becomes converted into a persistent inflammation that may have structural consequences in the airways and skin.

The relationship between inflammation and clinical symptoms of allergy is not yet completely clear. There is evidence that the degree of inflammation is related to airway hyperresponsiveness (AHR), as measured by histamine or methacholine challenge. AHR is an exaggerated airway narrowing in response to many stimuli, which is characteristic of asthma, and the degree of AHR relates to asthma symptoms. Inflammation of the airways may increase airway responsiveness, which thereby allows triggers that would not narrow the airways to do so (8). But inflammation may also directly lead to an increase in asthma symptoms, such as cough and chest tightness, by activation of airway sensory nerve endings. In rhinitis, nasal blockage is due to vasodilatation, and nasal discharge is due to plasma exudation and mucus secretion. The characteristic sneezing is a manifestation of hypersensitivity of sensory nerves and is the equivalent of cough in asthma. In skin, disruption of the epithelial barrier leads to infection and hypersensitivity of sensory nerves that result in itching.

Inflammatory cell interactions

Many inflammatory and structural cells are involved in allergic inflammation (Fig. 1), but it is not clear how these cells interact or how the inflammatory process is maintained.

Mast cells and symptoms

Mast cells appear to play a critical role in initiating allergic inflammation in the airways, since they are directly activated by allergens through an IgE-dependent mechanism. This leads to the rapid release of preformed and synthesized mediators that result in bronchoconstriction, vasodilatation, and plasma exudation thus leading to symptoms of asthma (wheezing and dyspnea) or allergic rhinitis (nasal blockage and secretions). Mast-cell activation therefore plays a key role in generating the symptoms of asthma and rhinitis, as mast cells may be activated by many of the triggers causing symptoms, including allergens, cold air, and osmolality changes (which mediate exercise-induced asthma). Mucosal mast cells are maintained at mucosal surfaces in allergic diseases by cytokines such as IL-9 and stem-cell factor (SCF) (9). SCF interacts with mast cells

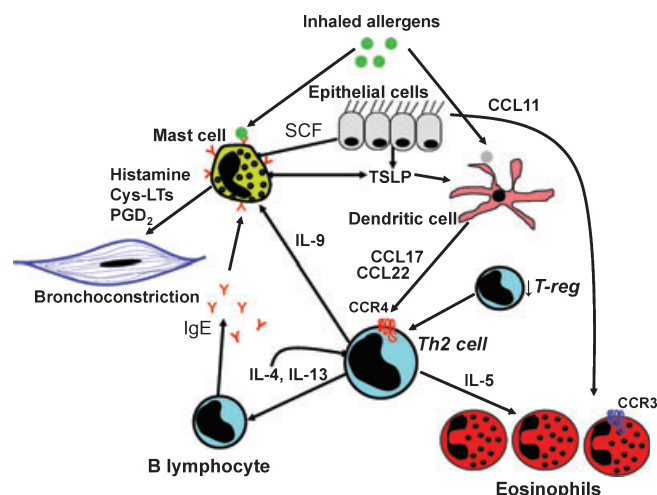


Fig. 1. Inflammation in allergy. Inhaled allergens activate sensitized mast cells by cross-linking surface-bound IgE molecules to release several bronchoconstrictor mediators, including cysteinyl-leukotrienes (cys-LT) and prostaglandin D₂ (PGD₂). Epithelial cells release stem-cell factor (SCF), which is important for maintaining mucosal mast cells at the airway or skin surface. Allergens are processed by myeloid dendritic cells, which are conditioned by thymic stromal lymphopoietin (TSLP) secreted by epithelial cells and mast cells to release the chemokines CC-chemokine ligand 17 (CCL17) and CCL22, which act on CC-chemokine receptor 4 (CCR4) to attract T-helper 2 (Th2) cells. Th2 cells have a central role in orchestrating the inflammatory response in allergy through the release of interleukin-4 (IL-4) and IL-13 (which stimulate B cells to synthesize IgE), IL-5 (which is necessary for eosinophilic inflammation) and IL-9 (which stimulates mast-cell proliferation). Epithelial cells release CCL11, which recruits eosinophils via CCR3. Patients with allergic disease may have a defect in regulatory T cells (Treg), which may favor further Th2-cell activation.

through its tyrosine kinase receptor c-Kit, and several c-Kit inhibitors, based on imatinib, are in development for asthma and other mast cell-mediated diseases (10). While mast cells account for the early response to allergen in the airways and skin, it is not clear how important they are for the development of late responses and for the chronic inflammatory changes that occur. Allergen-derived T-cell peptides derived from Fel d1 have been shown to induce isolated late responses and AHR without any mast-cell activation or eosinophil infiltration in the lower airways through direct activation of CD4⁺ T cells (11).

Th2 cells as orchestrators of allergic inflammation

Th2 cells play an important role in orchestrating allergic inflammation through the release of cytokines that include IL-4, IL-5, IL-9, and IL-13 (12) (Fig. 2). Th2 cells are recruited and activated at the sites of allergic inflammation, and a major focus of research has been to understand how topical allergens regulate Th2 cells via antigen-presenting cells (APCs). Dendritic cells (DCs) are the main APCs that process

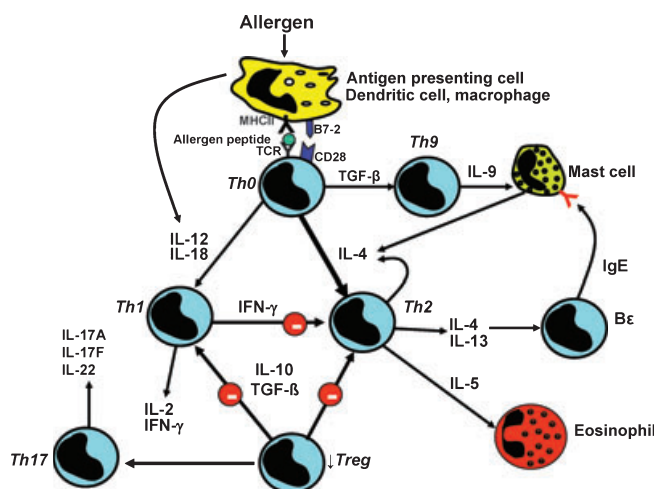


Fig. 2. T lymphocytes in allergy. Asthmatic inflammation is characterized by a preponderance of T-helper 2 (Th2) lymphocytes over T-helper 1 (Th1) cells. Regulatory T cells (Treg) have an inhibitory effect, whereas T-helper 17 (Th17) cells have a proinflammatory effects. Th9 cells secrete IL-9 important for mast-cell differentiation. MHCII, Class II major histocompatibility complex; IL, interleukin; IFN- γ , interferon γ ; TGF- β , transforming growth factor β ; IgE, immunoglobulin E; Th0, uncommitted T cell; TGF, transforming growth factor; B ϵ , B cell producing ϵ transcripts and IgE.

allergens and present T-cell peptides to naive T cells, and DCs play a critical role in the recruitment and activation of Th2 cells through the secretion of chemokines CCL17 and CCL22 (13). IL-4 is necessary for differentiation of Th2 cells but is not secreted by DCs, so accessory cells may be needed as a source of IL-4. There is increasing evidence that basophils, which are c-Kit negative, express Fc ϵ R1, major histocompatibility complex (MHC) class II, and costimulatory molecules CD80/CD86, may play a role in antigen recognition and processing and when recruited to lymph nodes are able to induce Th2 cell differentiation through the release of IL-4 (14–16). Other cells also have the capacity to process and present allergens, including mast cells, macrophages, eosinophils, and natural helper cells. Natural helper cells are innate cell populations that include innate Th2 cells and are activated by IL-25 and IL-33 to secrete Th2 cytokines (17).

Other T cells involved in allergy

Although Th2 cells predominate in allergic disease and Th2 cytokines play a key role in the pathophysiology of these diseases, other types of T cells may also play a role, particularly in more severe disease (18). Th1 cells and CD8⁺ cells that secrete Th2 cytokines (Tc2 cells) have also been implicated in allergic disease, as have natural killer cells and invariant natural killer T cells. Th9 cells are CD4⁺ T cells that produce IL-9, which are dependent on transforming growth factor- β

(TGF- β) for their development through activation of the transcription factor PU.1, and are regulated by IL-25 (19). Th17 cells may be associated with more severe asthma and may mediate a more neutrophilic pattern of inflammation (see below). Although Th17 cells produce IL-22, a distinct set of CD4⁺ IL-22 producing cells (Th22) has been described in allergic inflammatory diseases (20).

Regulatory T cells (Tregs) may play an important role in suppressing allergic inflammation. Several types of Tregs are now recognized, including CD4⁺CD25⁺ innate Tregs that express the transcription factor forkhead box protein 3 (FoxP3) and inducible Tregs. They may suppress inflammation through secretion of IL-10 or by inducing IL-10 secretion for bystander cells, as well as direct inhibition of cells, such as DCs, by cell–cell contact. There is some evidence that Treg function is impaired in patients with allergic diseases and that Treg function is enhanced by specific immunotherapy (21).

Role of eosinophils

Eosinophils are also common to allergic inflammation at different sites and are orchestrated by Th2 cells through the release of IL-5. Indeed, blocking IL-5 with a monoclonal antibody (mepolizumab) virtually eliminates eosinophils from the circulation and markedly reduces eosinophils in sputum (22, 23). Eosinophils contribute to the pathophysiology of asthma through the release of cysteinyl-leukotrienes (cys-LTs) but are less important as a source of these mediators than mast cells. They may also function as APCs and also produce several Th1 and Th2 cytokines (24). Eosinophils express high levels of TGF- β , and this has been linked to the characteristic subepithelial fibrosis in asthma (25), which has also been observed in patients with eosinophilic bronchitis who present with chronic cough and no AHR (26). Cationic proteins released from eosinophils are important in helminth killing but may also be profibrotic and lead to shedding of epithelial cells in asthma, although this does not occur in nasal epithelial cells. Depletion of eosinophils with anti-IL-5 reduces exacerbations of asthma and the requirement for oral corticosteroids in selected patients with severe asthma but does not reduce either symptoms or AHR, suggesting that eosinophils play no role in these features of asthma (23, 27). The clinical effects of anti-IL-5 therapies in other allergic diseases are not yet known.

B lymphocytes

B lymphocytes play a key role in allergic inflammation through the synthesis of IgE, and these cells are mainly local-

ized to draining lymph nodes. Isolated B cells are also found in the respiratory mucosa, but they are rarely organized into lymphoid tissue as in the gut (28). IgE synthesis is regulated by IL-4 and IL-13 and may be augmented by IL-9. Local IgE synthesis may occur on the upper and lower airways in allergic diseases. In the mucosa of patients with allergic rhinitis and asthma, C ϵ germline transcripts as well as IL-4 and IL-13 are produced after exposure to allergen *in vitro* (29, 30). B cells also function as APCs, and in murine models of chronic allergen exposure, MHC class II-expressing B cells drive Th2 cells (31). B cells may also play a regulatory role through secretion of IL-10, but the role of different subsets of B cells in allergic diseases remains to be determined. A member of the tumor necrosis factor (TNF) called B-cell activating factor (BAFF) plays an important role in B-cell survival, and immunoglobulin class switching is increased after segmental allergen challenge in asthmatic patients (32).

Neutrophils

The role of neutrophils in allergic diseases is still uncertain and remains poorly explored. Patients with severe asthma are more likely to have infiltration of neutrophils in bronchial biopsies and sputum (33, 34), but increased neutrophils in sputum may be seen even in mild asthma. Increased sputum neutrophils are also found in smoking asthmatics and during virally induced exacerbations. Patients with neutrophilic asthma appear to be less responsive to corticosteroids, and high doses of corticosteroids may increase neutrophils by reducing apoptosis (35). The mechanism for increased neutrophils may be due to secretion of several neutrophil chemotactic factors, including LTB₄, CXCL1, and CXCL8, and may be driven by Th17 cells through the secretion of IL-17A and IL-17F (36). However, the increased IL-17A and IL-17F expression in asthmatic airways does not appear to be correlated with neutrophil numbers (37). Neutrophils may be increased in asthma as a result of high doses of corticosteroids or they may be recruited by chemotactic factors and Th17 cells. The functional role of neutrophils in asthma will only become clear when specific therapies that selectively reduce neutrophils, such as CXCR2 antagonists, are tested in asthmatic patients.

Non-atopic allergic disease

Some patients with asthma, rhinitis, rhinosinusitis, and eczema have negative skin prick responses to common inhalant allergens, and no allergen-specific circulating IgE yet have clinical features very similar to patients with allergic disease.

These non-atopic forms of 'allergic' disease might give insights into the nature of allergic inflammation. Furthermore, the allergic pattern of inflammation with activated mast cells, eosinophils, and Th2 cells is found in the tissues (38). Total circulating IgE is elevated in around 30% of patients with non-atopic (intrinsic) asthma (39). There is evidence for local IgE production in the airways of patients with non-atopic asthma, with local expression of ϵ germ-line transcripts (30). This observation suggests that the disease is driven by an unidentified allergen, an endogenous allergen or by an infective agent.

Role of infective agents

Staphylococcus aureus is thought to be an important amplifying mechanism in allergic and non-allergic atopic dermatitis and chronic rhinosinusitis with nasal polyps, but it might also be important in asthma (40, 41). *S. aureus* is able to invade epithelial cells and release its enterotoxins (exotoxins), which function as superantigens that have the capacity to stimulate T and B-cell proliferation directly. They are also able to induce class switching to IgE and the production of allergen-specific IgE in mucosal B cells. Specific IgE antibodies against *Staphylococcal* enterotoxins (termed 'superallergens') have been found in patients with severe asthma, aspirin-sensitive asthma, and nasal polyposis and are able to sensitize mast cells and DCs (42–44). *S. aureus* is also capable of invading mast cells and causing the release of cytokines (45). It is therefore possible that patients with non-atopic allergic diseases may in some way be susceptible to colonization of the lower airways with *S. aureus*, which through the local release of superantigens drives an allergic inflammatory response and local IgE synthesis. Superantigens are associated with more severe disease, as the anti-inflammatory response to corticosteroids is reduced (46). It is also possible that *S. aureus* superantigens lead to chronic rhinosinusitis, which then affects the lower respiratory tract without the need for direct infection of the lower airways.

Superantigens

Superantigens are also derived from other bacteria (including *Mycoplasma*), viruses, parasites, and fungi, and all of them are immunostimulatory, acting as V β -restricted extremely potent polyclonal T-cell mitogens (47). They bind MHC class II molecules without any prior antigen processing by APCs and stimulate large number of T cells on the basis of epitopes specified by this receptor though their unique ability to cross-link MHC class II and the β -subunit of the T-cell receptor (TCR), form-

ing a tight trimolecular complex. They also appear to cause class switching in B cells by binding to immunoglobulins, resulting in clonal expansion and expression of polyclonal IgE and specific IgE directed against the superantigen. Some superantigens may even become incorporated into the genome and thus may provide continuous endogenous stimulation (47).

Nasal and inhaled *Staphylococcal* enterotoxin B (SEB) induces lymphocytic inflammation and eosinophilia in the lungs of mice with increased production of IL-4, together with AHR in both IgE and non-IgE producing strains, demonstrating the capacity of superantigens to induce allergic inflammation independently of any allergen (48). Inhaled SEB also enhances the eosinophilic inflammatory response to inhaled allergen in sensitized mice (49). In nasal polyp tissue, there are IgE antibodies to SEB, and these are able to activate mast cells (50). *Staphylococcal* enterotoxin A also results in lung inflammation characterized by increased CD8⁺ cells and increased secretion of interferon- γ (IFN- γ) (51). These animal studies demonstrate the ability of small concentrations of locally applied superantigens to induce complex inflammatory responses in the lungs and suggest a mechanism for intrinsic or non-allergic asthma. Inhaled bacteria may invade airway epithelial cells and result in the release of various superantigens, which may directly activate Th2 cells to release IL-4 and other Th2 cytokines, as well as causing class switching of B cells in the airways to release IgE locally, which then sensitizes mast cells so that they are more easily activated. SEB stimulates the release of several cytokines with a Th2 cell bias, as well as mast-cell mediators from nasal polyp tissue in vitro and to a greater extent than from normal nasal tissue, indicating some sort of sensitization to the effects of superantigens (52). There is evidence for local clonal expansion of airway T cells in patients with intrinsic asthma compared to circulating T cells, suggesting that this could be induced by local superantigens (53).

Staphylococcal superantigens also inhibit the immunosuppressive activity of regulatory T cells (Tregs) and may therefore amplify the activity of Th2 cells and CD8⁺ cells (54). SEB inhibits CD4⁺CD25⁺ Tregs by inducing the protein glucocorticoid-induced TNF receptor-related protein ligand in monocytes (55). Superantigens induce corticosteroid resistance by activating mitogen-activated protein kinase (MAPK) cascades and through the increased expression of a form of the glucocorticoid receptor (GR β), which may act as a decoy to block the action of corticosteroids (56). Finally, SEB has recently been shown to stimulate Th17 cells directly (57, 58).

Role of autoantibodies

Another possible mechanism of activating IgE signaling without an exogenous allergen may be through the production of autoantibodies that activate this pathway. This would also be consistent with the fact that intrinsic asthma often has a late onset and is more frequently seen in women. Anti-nuclear antibodies are more common in patients with asthma than non-asthmatics, but there is no difference between allergic and non-allergic asthmatics, although patients with aspirin-sensitive asthma have a higher frequency of these antibodies (59). However, there is no increase in autoantibodies against double-stranded DNA or anti-neutrophil cytoplasmic antibodies in asthma. Autoantibodies to FcεRI have been detected in the serum patients with asthma (approximately 35% compared to 10% in non-asthmatics) but do not appear to be more common in intrinsic compared to extrinsic asthma (60). Circulating antibodies to cytokeratin-18 and α-enolase, which occur in epithelial cells, have been described in patients with intrinsic asthma and more severe asthma (61, 62). IgG autoantibodies from patients with intrinsic asthma have cytotoxic effects on epithelial cells, whereas these antibodies are not found in extrinsic asthma (63). It is possible that these autoantibodies damage airway epithelial cells so that superantigen-producing organisms are able to more easily infect the airway surface.

Inflammatory mediators

Many different mediators have been implicated in allergic disease, and they may have a variety of effects on the airways or skin, which account for all of the pathological features of allergic diseases (64–66) (Fig. 3). Mediators such as histamine, prostaglandins, leukotrienes, and kinins contract airway smooth muscle, increase microvascular leakage, increase air-

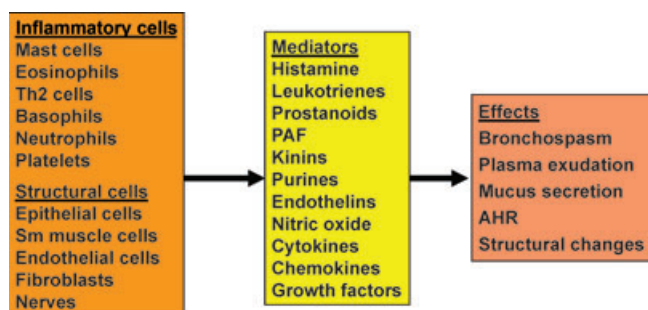


Fig. 3. Multiple cells, mediators, and effects. Many cells and mediators are involved in asthma and lead to several effects on the airways. Th2, T-helper 2 cells; Sm, smooth; PAF, platelet activating factor; AHR, airway hyperresponsiveness.

way mucus secretion, and attract other inflammatory cells. Because each mediator has many effects, the role of individual mediators in the pathophysiology of allergic diseases is often unclear. The multiplicity and redundancy of effects of mediators makes it unlikely that preventing the synthesis or action of a single mediator will have a major clinical impact on allergic diseases. However, some mediators may play a more important role if they are upstream in the inflammatory cascade and trigger a sequence of downstream events. The effects of single mediators can only be evaluated through the use of potent specific receptor antagonists or mediator synthesis inhibitors. The role of mediators may differ between allergic diseases. Thus, anti-histamines have a useful clinical effect in allergic rhinitis, whereas they are not useful in the treatment of asthma (67). In contrast, anti-leukotrienes have clinical effects in asthma but appear to be less useful in rhinitis and atopic dermatitis (68).

Lipid mediators

Several lipid mediators derived from arachidonic acid (eicosanoids) are involved in the pathophysiology of allergic disease, and this has led to therapeutic strategies to block their production or their receptors on target cells (69). Cys-LTs are potent bronchoconstrictors and antagonism of cys-LT₁-receptors provides clinical benefit in asthma (68). Cys-LTs are also involved in eosinophilic inflammation, although antagonists have only weak anti-inflammatory effects. LTB₄ has also been implicated in asthma, not only as a chemoattractant of neutrophils, which express BLT₁-receptors, but also as an important activator of T cells (70). In mice, LTB₄ plays a role in recruiting IL-13 secreting CD8⁺ cells in allergic inflammation (71). Most of the effects of LTB₄ appear to be mediated via the high affinity BLT₁, but a low affinity receptor BLT₂ is also expressed on inflammatory cells and reducing expression of this receptor by antisense oligonucleotides reduces the inflammatory and AHR response to inhaled allergen in sensitized mice, implicating this receptor in the allergic response to LTB₄ (72).

Prostaglandins have potent and diverse effects on airway function. PGD₂ has attracted particular attention as it is released in large amounts from mast cells. It is a potent bronchoconstrictor through the activation of thromboxane receptors (TP) on airway smooth muscle cells but also activates DP₁-receptors, which mediate vasodilatation and DP₂-receptors first identified as chemoattractant homologous receptor expressed on Th2 cells (CRTH2), which is expressed on Th2 cells, eosinophils, and basophils and provides a link between mast-cell activation and allergic inflammation (73). Many

CRTH2/DP₂ antagonists are now in development as oral anti-allergic therapies (74, 75).

Other lipid mediators that are less well-investigated in allergic diseases include 5-oxo-eicosatetraenoic acid (5-oxo-EETE), a product of 5-lipoxygenase that is markedly increased by oxidative stress and which is a potent chemoattractant of eosinophils, which express distinct OXE receptors (76). Oxidation of arachidonic acid by oxidative stress results in the formation of F₂-isoprostanes which are bronchoconstrictors and mediators of plasma exudation (77). Eoxins are generated from arachidonic acid by the 15-lipoxygenase pathways and are produced by eosinophils and mast cells (78). Eoxin C₄ has proinflammatory effects and causes microvascular leakage.

The cytokine network

Cytokines play a key role in the orchestration and perpetuation of allergic inflammation and are now targeted in therapy (79). Allergic inflammation is characterized by the secretion of Th2 cytokines, including IL-4, IL-5, IL-9, and IL-13, which are secreted by Th2 cells but now recognized to be secreted by other cells involved in allergic inflammation including mast cells, basophils, eosinophils, and structural cells. Details of the particular roles of these cytokines is provided in another review in this volume. The use of blocking antibodies is now beginning to shed new light on the role of individual Th2 cytokines. IL-4 and IL-13 play a key role in IgE synthesis through isotype switching of B cells and appear to play a critical role in animal models of asthma. So far blocking IL-4 and IL-13 or their common receptor IL-4R α has not yet been shown to be of clinical benefit in asthma, but many clinical trials are currently underway (80). IL-5 is of critical importance in the differentiation, survival, and priming of eosinophils. A humanized monoclonal blocking antibody (mepolizumab) induces a profound decrease in eosinophils in the blood and induced sputum in patients with mild asthma but has no effect on the response to inhaled allergen (22). Clinical trials of anti-IL-5 in unselected symptomatic asthmatic patients showed no overall clinical improvements (81). However, in highly selected patients with severe asthma and sputum eosinophils, despite high doses of inhaled or oral corticosteroids, mepolizumab reduced exacerbations and requirements for oral corticosteroids yet did not improve symptoms or AHR (23, 27). This observation suggests that blockade of individual cytokines may only provide clinical benefit in carefully selected patients.

Several proinflammatory cytokines have been implicated in allergic diseases, including IL-1 β , IL-6, TNF- α , and GM-CSF,

which are released from a variety of cells, including macrophages and epithelial cells, and may be important in amplifying the allergic inflammatory response. Although there is persuasive evidence that TNF- α may be important in patients with severe asthma and small clinical studies with anti-TNF- α therapies had been promising, a large placebo-controlled trial of an anti-TNF antibody (golimumab) in severe asthma showed no overall benefit (82). However, it is possible that there are some responders, and there was a suggestion that patients with greater bronchodilator reversibility showed a reduction in exacerbations. IL-17 is also increased in severe asthma (36), but anti-IL-17 antibodies have not yet been tested in asthma patients.

Interest has now focused on upstream regulatory cytokines in the pathogenesis of asthma, as it is considered that they may have greater therapeutic potential. Thymic stromal lymphopoietin (TSLP) is an upstream IL-7-like cytokine that may initiate and propagate allergic immune responses and plays an important role in immune responses to helminths (83). TSLP is produced predominantly by airways and nasal epithelial cells and by skin keratinocytes, and TSLP stimulates immature myeloid dendritic cells, which express the heterodimeric TSLP receptor to differentiate into mature dendritic cells (Fig. 4). TSLP-activated DCs promote naive CD4⁺ T cells to differentiate into a Th2 phenotype and promote the expansion of Th2 memory cells through the release of Th2 chemotactic cytokines CCL17 and CCL22 and expression of the costimulatory

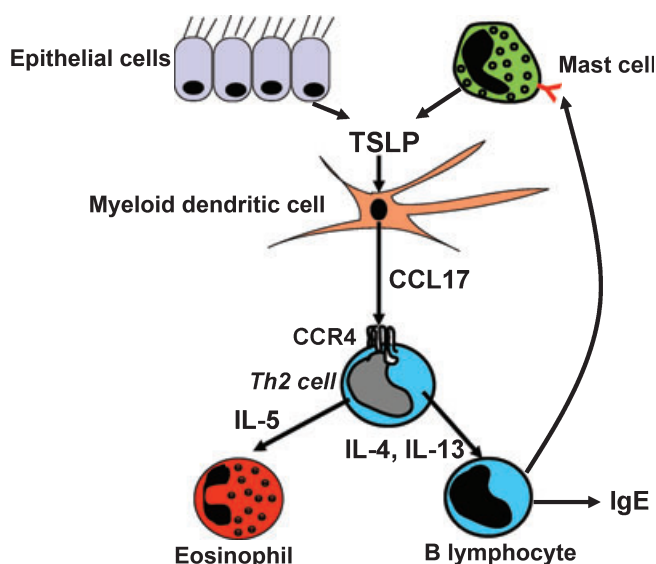


Fig. 4. Thymic stromal lymphopoietin (TSLP). TSLP is an upstream cytokine produced by epithelial cells/keratinocytes and mast cells that acts on immature dendritic cells to mature and release CCL17, which attracts Th2 cells via CCR4. Th2, T-helper 2; CCL, chemokine; CCR, chemokine receptor.

molecule OX40 ligand. In addition, TSLP suppresses the IL-12 p40 receptor in DCs and, by suppressing Th1 responses, further enhances Th2 responses. TSLP also promotes allergic inflammation by promoting the differentiation and IL-4 transcription in Th2 cells, the production of IL-13 from mast cells, by recruiting eosinophils and by amplifying responses of basophils (84). TSLP may therefore play a pivotal role in the initiation of allergic asthma, rhinitis, and atopic dermatitis. It is highly expressed in the airways of asthmatic patients, and its expression is correlated with disease severity and the expression of CCL17 (85). TSLP is also expressed in epithelial cells of patients with allergic rhinitis (86) and atopic dermatitis. Overexpression of TSLP in skin keratinocytes of mice amplifies the inflammatory response of inhaled allergen in sensitized animals, thus providing a mechanism for the 'allergic march', whereby atopic dermatitis commonly precedes the development of asthma in children (87).

IL-25 (IL-17E) is a member of the IL-17 family of cytokines and induces allergic inflammation through increased production of Th2 cytokines. Although originally shown to be produced by Th2 cells it is now known to be released from many different cells, including mast cells, basophils, eosinophils, macrophages, and epithelial cells (88). Blocking IL-25 is effective in animal models of allergic disease and blocking antibodies are now in clinical development.

IL-33 is another upstream cytokine and a member of the IL-1 family of cytokines, which is unusual in its localization within the nucleus where it may regulate chromatin structure and gene expression (89). It appears to be released only on damage to epithelial or endothelial cells and may act as an alarmin and is constitutively expressed at mucosal surfaces, such as the airways. It signals through a receptor ST2 that activates NF- κ B and MAPK pathways. Its relevance to allergic inflammation is that it enhances Th2 cell function, leading to eosinophilia, mast-cell activation, and mucus hypersecretion, and thus may act as a bridge between innate and adaptive immunity in allergic inflammation. It also directly activates eosinophils, mast cells, epithelial cells, and DCs (90). It appears to switch alveolar macrophages to the alternatively activated form (M2) that has been found in animal models of asthma with increased secretion of CCL17 (91), although whether this is relevant to human allergic disease is uncertain. IL-33 shows increased expression in airway epithelium of asthmatic patients, and this is related to disease severity (92). IL-33 is increased in the skin of patients with atopic dermatitis and is released into the circulation during as well as mediating anaphylactic shock (93). IL-33 is expressed in mast cells after activation through IgE receptors and also activates mast cells,

providing a means of maintaining mast-cell activation (94). Antibodies that block IL-33 or ST2 are now in clinical development.

Chemokines

Many chemokines are involved in the recruitment of inflammatory cells in allergic diseases and work through G-protein coupled receptors from which small molecule inhibitors have now been developed (79, 95). Chemokines appear to act in sequence in determining the final inflammatory response, and so inhibitors may be more or less effective depending on the kinetics of the response (96). Chemokines, such as CCL11, CCL24, CCL28, CCL5, and CCL13, that are chemotactic for eosinophils activate a single receptor CCR3, so blocking this receptor with small molecule antagonist was an attractive approach to treating allergic diseases (97). Several selective CCR3 antagonists have failed in toxicology testing. Other chemokine receptors are now being targeted, including CCR4, which is expressed predominantly on Th2 cells (98).

Oxidative stress

As in all inflammatory diseases, there is increased oxidative stress in allergic inflammation, as activated inflammatory cells, such as macrophages, eosinophils, and neutrophils, produce reactive oxygen species. Evidence for increased oxidative stress in asthma is provided by the increased concentrations of 8-isoprostane (a product of oxidized arachidonic acid) in exhaled breath condensates (99) and increased ethane (a product of oxidative lipid peroxidation) in exhaled breath of asthmatic patients (100). There is also persuasive epidemiological evidence that a low dietary intake of antioxidants is linked to an increased prevalence of asthma (101). Increased oxidative stress is related to disease severity and may amplify the inflammatory response and reduce responsiveness to corticosteroids, particularly in severe disease and during exacerbations (101).

Nitric oxide

Nitric oxide (NO) is produced by several cells in the airway by NO synthases (102, 103). NO is a potent vasodilator and may be a key mediator of vasodilatation in allergic inflammation. The level of NO in the exhaled air of patients with asthma is higher than the level of NO in the exhaled air of normal subjects and is increased further during the late response to inhaled allergen (104). NO production is also increased in allergic rhinitis (105). Measurement of exhaled

NO in asthma is increasingly used as a non-invasive way of monitoring the inflammatory process (106). The increased NO in asthma is derived mainly from increased expression of inducible NO synthase (NOS2) in airway epithelial cells and is normalized by selective inhibitors of NOS2 (107, 108). Exhaled NO measurements are less useful in rhinitis, as the local production of NO by the nasal mucosa is diluted by the high NO production from paranasal sinuses (109).

Purines

Purines are now recognized to be important mediators of allergic inflammation. Adenosine causes bronchoconstriction by activating A_{2B} receptors on mast cells and adenosine receptors are a target for new drugs (110). Recently there has been greater interest in adenosine triphosphate (ATP) as a mediator of allergic disease through signaling of P2X receptors (ion channels) and P2Y receptors (metabolic). ATP acts as a danger signal that alerts the immune system to tissue damage. ATP is released by danger signals such as oxidative stress. ATP, mainly acting via P2X₇ receptors, enhances the chemotaxis and activation of mast cells, eosinophils and T cells, but induces apoptosis of Tregs, thus enhancing allergic inflammation (111). ATP also activates DCs and is increased in bronchoalveolar lavage of asthmatic patients after allergen challenge (112). ATP is also a potent stimulus of mucus secretion and activation of sensory nerves. Inhaled ATP causes bronchoconstriction, cough, and dyspnea in asthmatic patients (113). ATP activates P2X₇ receptors, which associate with pannexin-1, leading to activation of the NALP3 inflammasome, resulting in cleavage of the precursors of IL-1 β , IL-18, and IL-33 by caspase-1 to release these cytokines (114).

Structural cells

Although infiltrating and resident inflammatory and immune cells are important source of inflammatory mediators, it is now apparent that structural cells in the airways and skin play a critical role in the secretion of inflammatory mediators and in maintaining chronic allergic inflammation. Perhaps the most important structural cells are epithelial cells of the airways and skin, which express a wide variety of inflammatory mediators in allergic disease. There are important interactions between epithelial cells and DCs, with the release of key mediators such as granulocyte-macrophage colony stimulating factor (GM-CSF), TSLP, IL-25, and IL-33, which promote a Th2 bias in DC precursors (13). In addition, contact between epithelial cells and DCs may involve OX40 ligand and OX40, which also drives a Th2 bias. Surface epithelial cells interact

directly with the environment and may be activated by pathogens and endotoxin through various pattern recognition receptors, such as Toll-like receptors (TLRs), thus enhancing or triggering an allergic response. Mechanical factors may also be important in releasing inflammatory mediators from epithelial cells (115). For example, compressive forces acting on airway epithelial cells release growth factors, fibrogenic mediators, and inflammatory mediators. Epithelial cells appear to be fragile in asthma and are easily shed as the intercellular connections are weak. This may be an aspect of failure to repair the structural changes that are a consequence of allergic inflammation in the airway. However, these epithelial changes that are prominent in asthma are not described in rhinitis or atopic dermatitis, indicating that epithelial cells at different anatomical sites may show different responses.

Airway smooth muscle

Airway smooth muscle cells play a key role in airway narrowing in asthma, and bronchoconstriction results mainly from the release of bronchoconstrictor mediators from mast cells, including histamine, cys-LTs, and PGD₂. But airway smooth muscle mass is increased in asthma through hyperplasia and hypertrophy as a result of various growth factors released from airway epithelial and inflammatory cells. This is particularly pronounced in severe asthma, and there is evidence for a marked increase in proliferation in patients with severe asthma (116). Airway smooth muscle cells also release multiple inflammatory mediators and growth factors and are likely to be an important source of these mediators in the airways. However, it is not certain whether these abnormal smooth muscle cells have altered contractility or response to bronchodilators. The thickened smooth muscle is space occupying and may show a poor relaxation response, thus contributing to the irreversible airway narrowing in severe asthma. Nevertheless, selective removal of airway smooth muscle cells by bronchial thermoplasty has rather small effects on airway physiology, perhaps because peripheral airways beyond the reach of the bronchoscope are more important in severe disease (117).

Fibroblasts

The role of fibroblasts in allergic inflammation appears to depend on the site and therefore the nature of the fibroblast. This has been studied most carefully in asthma where there is evidence for subepithelial fibrosis in most patients, which may be due to the activity of myofibroblasts, fibroblasts, or infiltrating fibrocytes (118, 119). TGF- β may play an

important role in mediating airway fibrosis (25). Myofibroblasts may migrate from airway smooth muscle toward the airway surface, but the origin of these cells is not certain. Eosinophils may be important in activating fibroblasts/myofibroblasts, and subepithelial fibrosis is seen in eosinophil bronchitis as well as in asthma (26). This is underlined by the demonstration that eosinophilic esophagitis is associated with marked fibrosis (120).

Blood vessels

All allergic diseases are characterized by vasodilatation, which is a key component of the inflammatory response. Vasodilatation is often accompanied by microvascular leakage from postcapillary venules, resulting in plasma exudation and local edema, which is prominent in allergic rhinitis but also is seen in asthma and atopic dermatitis. It is most directly observed in the skin, where allergen injection in atopic individuals causes a wheal and flare response. Many mediators directly relax vascular smooth muscle or release NO, a potent vasodilator. Other mediators may also cause plasma leakage and some mediators, such as histamine, PGD₂ and cys-LTs cause both (121). Airway blood flow can be measured non-invasively by the inhalation of soluble gases, such as acetylene, and is increased in asthma and correlated with increase breath temperature and exhaled NO concentrations (122). In asthma there is evidence for increased angiogenesis and this is correlated with increased production of vascular endothelial growth factor (VEGF) (123, 124). The role of angiogenesis in other allergic diseases is less certain.

Mucus hypersecretion

Mucus hypersecretion is a prominent feature of allergic inflammation at mucosal surfaces. Mucus secretion is part of the innate response of the respiratory tract and increased mucus secretion from goblet cells in the epithelium and submucosal glands is a prominent feature of the allergic inflammatory response. Several inflammatory mediators stimulate mucus secretion and cause goblet cell hyperplasia (125). Mucous plugging in asthma is due to an interaction of mucus glycoproteins (MUC5AC, MUC5B) with exuded plasma proteins and is commonly seen in the airways of patients with fatal asthma. Th2 cytokines stimulate goblet cell hyperplasia and secretion, linking this response to allergic inflammation. IL-13 is the most potent cytokine that stimulates MUC5AC and MUCB transcription and acts via IL-4R α , which activates Jak1 and STAT6. STAT6 then switches on MUC genes and causes mucus hyperplasia via a transcription factor SAM-

pointed domain-containing Ets-like factor (SPDEF) (126). Other cytokines, including IL-4, IL-9, IL-17, and IL25, also act at least in part via IL-13. The most potent stimulant of mucus secretion is ATP, which acts on P2Y₂-receptors on goblet cells (127). Epithelial growth factor receptors (EGFRs) also regulate mucus hypersecretion and may be activated via its ligand TGF- α and indirectly by oxidative stress and by various proteinases, including neutrophil elastase and MMP9 (128).

Neural mechanisms

Neural mechanisms play an important role in the pathophysiology of allergic diseases, and there is a complex interplay between allergic inflammation and neural effects. Autonomic nervous control of the airways is complex; in addition to classical cholinergic and adrenergic mechanisms, non-adrenergic non-cholinergic (NANC) nerves and several neuropeptides have been identified in the nerves of the upper and lower respiratory tracts (129, 130). Several inflammatory mediators may act on prejunctional receptors on airway nerves to modulate the release of neurotransmitters. Thus, thromboxane and PGD₂ facilitate the release of acetylcholine from cholinergic nerves in canine airways, whereas histamine inhibits cholinergic neurotransmission at both parasympathetic ganglia and postganglionic nerves via histamine H₃-receptors.

Sensory nerves

Sensory nerves in the respiratory tract and skin play a key role in producing cough, sneezing, and itch, which are prominent symptoms of allergic disease. Inflammatory mediators may activate sensory nerves to produce these symptoms and may also initiate reflex effects. For example, bradykinin, histamine, and protons (acidity) are potent activators of unmyelinated C-fibers and produce coughing and sneezing when applied locally to the mucosal surface but also activate reflex effects, such as bronchoconstriction and mucus secretion via cholinergic neural pathways. Several inflammatory mediators, including PGE₂, bradykinin, and neurotrophins also sensitize sensory nerve endings in the airway epithelium or skin (hyperalgesia), so that the nerves become more easily activated by other stimuli or by stimuli that would not normally elicit any effects. Airway sensory nerves may also be involved in AHR, and one reason why animal models of asthma fail to show the high degree of AHR compared to human asthma is that the animals are usually anesthetized so that the sensory nerve component is absent. Transient potential receptor A1 (TRPA1) ion channels play a key role in the nociception in the airways and skin and may be activated by various irritants to

produce coughing, sneezing, and itching. TRPA1 expression is increased in asthma and with inflammatory cytokines, whereas blocking TRPA1 by gene deletion or a selective inhibitor reduces AHR and airway inflammation in a murine model of asthma (131).

Neuropeptides and neurogenic inflammation

Airway nerves may also release neurotransmitters that have proinflammatory effects. Thus, neuropeptides such as substance P (SP), neurokinin A, and calcitonin gene-related peptide (CGRP) may be released from sensitized inflammatory nerves in the airways or skin to increase and extend the ongoing inflammatory response (Fig. 5). There may also be a reduction in the activity of enzymes, such as neutral endopeptidase, that degrade neuropeptides like SP. The role of neurogenic inflammation, either via a classical axon reflex with antidromic release of neuropeptides from sensory nerve endings or from other nerves via a neural reflex pathway, is uncertain. It has been difficult to demonstrate the presence of neurogenic inflammation in the lower airways, but there is good evidence that it may be important in the nasal mucosa and skin (132, 133). Sensory nerves may be very closely associated physically with inflammatory cells, including mast cells and eosinophils. Neuropeptides released from sensory nerves in the skin may cause vasodilatation through the release of CGRP and mast-cell degranulation via the release of SP. Neuropeptides may also be released from inflammatory and structural cells involved in allergic diseases, and neuropeptide receptors may be expressed on inflammatory cells, so that the boundaries between neural and inflammatory cells has become less distinct. For example, during the late skin response to allergen, there is an infiltration of neutrophils expressing the sensory neuropeptide CGRP, which is a potent vasodilator (134).

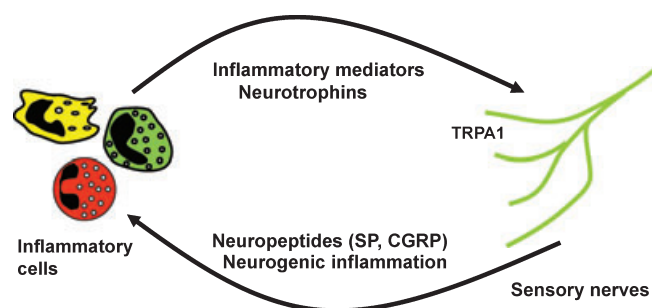


Fig. 5. Neural mechanism in asthma. There is a close interaction between asthmatic inflammation and neural mechanisms. SP, substance P; CGRP, calcitonin gene-related peptide; TRPA1, transient receptor potential A1 ion channel.

Neurotrophins

The role of neurotrophins in allergic diseases is increasingly recognized (135–137). Nerve growth factor (NGF), brain-derived neurotrophic factor, and neurotrophins 3 and 4/5 are nerve-related cytokines that play an important role in the function, proliferation, and survival of autonomic nerves. In sensory nerves, neurotrophins increase responsiveness and may also promote the expression of tachykinins. Neurotrophins may be produced by inflammatory cells, such as mast cells, lymphocytes, macrophages, and eosinophils, as well as structural cells, such as epithelial cells, fibroblasts, keratinocytes, and airway smooth muscle cells (138). Although neurotrophins have predominant effects on neuronal cells they may also act as growth factors for inflammatory cells, such as mast cells, as well as increasing chemotaxis and survival of eosinophils (139). NGF induces AHR in various animal models of asthma, including guinea pig, mice, and rats, and this is blocked by antibodies to NGF or its receptor TrkA (135, 140). In guinea pig, NGF enhances AHR by increasing the release of SP from sensory nerves (141) and increases sensory nerve activation (139). The role of neurotrophins in human allergic diseases is uncertain. However, NGF levels are increased in bronchoalveolar lavage fluid of asthmatic patients and there is a further increase after allergen challenge (142).

Stress and allergic inflammation

Psychological or social stress is a well known exacerbating factor in allergic diseases, especially asthma and atopic dermatitis, and may be explained by neuroimmune interactions (143). However, the mechanisms are far from clear. Activation of neuroendocrine and the sympathetic nervous system through cortisol and catecholamines secretion may influence the immune system, tipping the balance in favor of Th2 responses by reducing IL-12 release (144, 145). The increase in endogenous cortisol might be expected to benefit allergic disease since corticosteroids are highly effective in therapy, but it is possible that chronic stress induces a state of corticosteroid resistance (146). In a murine model of chronic psychosocial stress, there was a delay in resolution of inhaled allergen-induced lung inflammation in sensitized animals with increased Th2 cytokines. This was associated with *in vitro* evidence of a reduced anti-inflammatory response to corticosterone, suggesting that chronic stress may induce corticosteroid resistance (147). AHR has also demonstrated with psychological stress in other allergen-sensitized animal models (148). The fact that psychological factors may increase allergic

inflammation suggests that there may be psychological approaches to alleviating allergic diseases. The interaction between the central nervous system and allergic inflammation suggests that psychological treatments or alternative therapies, such as hypnosis and acupuncture, may provide clinical benefit, although this has yet to be demonstrated convincingly in controlled trials (149).

Transcription factors

The inflammation of asthma, allergic rhinitis, and atopic dermatitis is due to increased expression of multiple inflammatory proteins (cytokines, enzymes, receptors, adhesion molecules), which are regulated by transcription factors (150). Many transcription factors have now been implicated in the pathophysiology of allergic disease and have been considered as potential targets for new anti-inflammatory therapies. Proinflammatory transcription factors are involved in the regulation of multiple inflammatory genes and are activated in all inflammatory diseases; they probably play an important role in amplifying and perpetuating inflammation.

NF- κ B

NF- κ B can be activated by multiple stimuli, including allergens (151). NF- κ B is activated in asthmatic airways, particularly in epithelial cells and macrophages (152), and regulates the expression of several key genes that are overexpressed in asthmatic airways, including proinflammatory cytokines (IL-1 β , IL-6, TNF- α , GM-CSF), chemokines (CCL1, CCL5, CCL11, CCL17, CCL22), adhesion molecules (ICAM-1, VCAM-1), and inflammatory enzymes (cyclooxygenase-2 and NOS2). There is a common mechanism for inflammatory gene activation that involves activation of co-activator molecules by proinflammatory transcription factors, such as NF- κ B, at the start site of transcription to induce acetylation of core histones, around which DNA is wound in the chromosome. This unwinds DNA, opening up the chromatin structure, and allows gene transcription to proceed (153, 154). Activated inflammatory genes are then switched off by the nuclear enzyme histone deacetylase-2 (HDAC2), which is recruited by activated glucocorticoid receptors to the activated inflammatory gene complex (155).

T-cell transcription

Other transcription factors have a more specific effect as the transcript factor is restricted to specific cell types, such as T cells or epithelial cells. The transcription factor GATA3 (GATA-binding protein 3) is crucial for the differentiation of

uncommitted naive T cells into Th2 cells, and it also regulates the secretion of Th2 cytokines (156, 157) (Fig. 6). There is an increase in the number of GATA3⁺ T cells in the airways of asthmatic subjects compared with normal subjects (158, 159). Following simultaneous ligation of the T-cell receptor (TCR) and co-receptor CD28 by APCs, GATA3 is phosphorylated and activated by p38 MAPK. Activated GATA3 then translocates from cytoplasm to nucleus via the nuclear transporter protein importin- α , where it activates gene transcription (160). GATA3 expression in T cells is regulated by the transcription factor STAT6 (signal transducer and activator of transcription 6) via IL-4 receptor activation. By contrast, Th1 cells are regulated by T-bet, which regulates the expression of IFN- γ . T-bet expression is reduced in T cells from the airways of asthmatic patients compared with non-asthmatic patients (161). When phosphorylated, T-bet can associate with and inhibit the function of GATA3, by preventing it from binding to its DNA target sequences (162). T-bet-deficient mice show increased expression of GATA3 and production of Th2 cytokines, confirming that T-bet is an important regulator of GATA3 (161). GATA3 expression is also regulated by IL-27, a member of the IL-12 family, which downregulates GATA3 expression and upregulates T-bet expression, thereby favoring the production of Th1-type cytokines, which then act to further inhibit GATA3 expression (163). In turn, GATA3 inhibits the production of Th1-type cytokines by inhibiting STAT4,

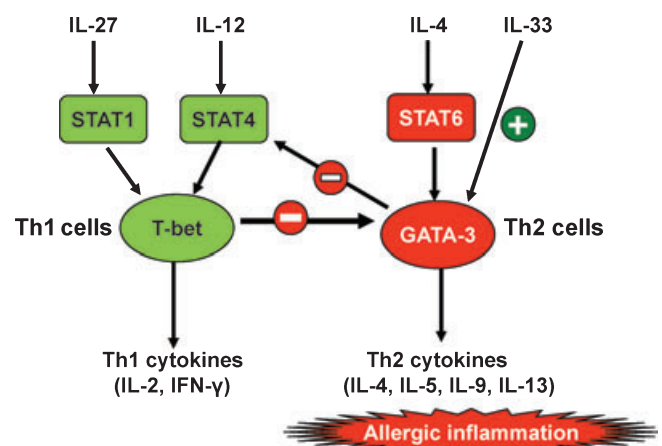


Fig. 6. GATA-3 in allergic inflammation. The transcription factor GATA3 (GATA-binding protein 3) is regulated by interleukin-4 (IL-4) via STAT-6 (signal transducer and activator of transcription 6) and regulates the expression of IL-4, IL-5, IL-9, and IL-13 from T-helper 2 (Th2) cells and also inhibits the expression of T-bet via inhibition of STAT4. IL-33 enhances the actions of GATA3. T-bet regulates Th1-cell secretion of IL-2 and interferon- γ (IFN- γ) and also has an inhibitory action on GATA3. T-bet is regulated by IL-12 via STAT4 and by IL-27 via STAT1. This demonstrates the complex interplay of cytokines and transcription factors in asthma.

the major transcription factor activated by the T-bet-inducing cytokine IL-12 (164). Nuclear factor of activated T cells (NFAT) is another T-cell-specific transcription factor and appears to enhance the transcriptional activation of GATA3 at the IL4 promoter (165). GATA3 nuclear translocation is rapidly and potently inhibited by corticosteroids through competition of the activated glucocorticoid receptor for nuclear import via importin- α , as well as by induction of MAPK phosphatase-1, which inhibits the phosphorylation of GATA3 by p38 MAPK (166).

Th17 cells are characterized by the expression of the transcription factor retinoic acid orphan receptor- γ t (ROR- γ t), whereas Tregs express FoxP3. However, some T cells may express different T-cell-regulating transcription factors. For example, a novel type of CD4⁺ cell that co-expresses GATA3 and ROR- γ t and coproduces Th2 cytokines and IL-17 have been described in mice and might play a key role in exacerbations, when increased eosinophils and neutrophils are found (167). Interestingly, this new subtype can be induced by exposing Th2 cells to the proinflammatory cytokines IL-1 β , IL-6, or IL-21, illustrating the plasticity of T-cell populations.

Jak-STAT

Expression of many of the key cytokines in allergy is regulated by STAT transcription factors that are activated by Janus-activated tyrosine kinases (Jaks) (168, 169). IL-4 and IL-13 stimulate Jak1 and Jak3 to phosphorylate STAT6, which activates GATA3 resulting in Th2 differentiation and cytokine expression. IL-12 activates Jak2 and Tyk2 to phosphorylate STAT4, which activates T-bet to drive differentiation into Th1 cells, so the balance between STAT4 and STAT6 plays a key role in Th-cell differentiation and function. In mice and human monocytes, IFN- γ activates STAT1, which in turn regulates suppressor of cytokine synthesis-1 (SOCS1) to suppress STAT6 and thus inhibit IL-4 expression. STAT5 appears to play an important signaling role in mast cells and mediates effects of IgE receptors and c-kit on proliferation, survival, and mediator release (170). The TSLP receptor signals through activation of STAT1, STAT3, and STAT5 via the activation of Jak1 and Jak2 (171). Several Jak inhibitors are now in development as immunomodulators to interfere with these Jak-STAT signaling pathways in allergic diseases (169). SOCS proteins may also be important therapeutic targets in allergy (172).

Epigenetic regulation

Epigenetic changes include post-translational modification of histones (acetylation, methylation, phosphorylation, ubiqui-

tinuation), small RNAs, and DNA methylation, all of which may alter the expression of genes involved in allergic inflammation (154, 173). Methylation of cytosine residues at CpG dinucleotide sequences in DNA may result in gene suppression, and these changes are long lasting and are involved in cell differentiation and may be heritable. Differentiation of naive T cells into Th1 cells is associated with methylation of the promoter region of IL4, whereas Th2 differentiation is associated with demethylation of this region (174). Demethylation of IL4 and IL13 allows binding of STAT6 and GATA3, whereas Th1 differentiation is also associated with demethylation of the IFNG gene and the binding of T-bet. Similar mechanisms may regulate the expression of Th2 cytokine genes in other cells, such as mast cells. In Tregs, the promoter region of FOXP3 becomes demethylated and therefore activated (175). DNA methylation may be affected by environmental influences, such as diet. In mice fed with a diet rich in folate (a methyl donor), there was increased allergic inflammation, IgE and AHR in the progeny associated with increased methylation and suppression of a RUNX3, a gene associated with suppression of IL4 and activation of FOXP3 (176).

Acetylation of core histones plays an important role in the regulation of inflammatory genes through the NF- κ B-activated acetyltransferase activity of coactivator molecules such as CREB-binding protein, p300, and PCAF, which result in hyperacetylation of histones and unwinding of DNA so that RNA polymerase 2 binding may occur leading to gene transcription (153, 177). This is reversed by HDACs, particularly HDAC2. In bronchial biopsies of asthmatic patients, there is evidence for increased histone acetyltransferase activity and reduced HDAC activity, thus favoring increased inflammatory gene transcription (178). HDAC activity is reduced in monocytes and macrophages from patients with severe asthma and associated with increased inflammatory gene expression (179, 180).

MicroRNAs are small single stranded non-coding RNAs that may influence the expression of groups of genes by repression of their translation. For example, selective blockade of miR-126 suppresses the Th2 response in sensitized mice by inhibiting GATA3 expression (181).

Anti-inflammatory mechanisms in allergy

Although most emphasis has been placed on inflammatory mechanisms in allergy, there are endogenous anti-inflammatory mechanisms that may be defective in allergic diseases, resulting in increased inflammatory responses (Fig. 7).

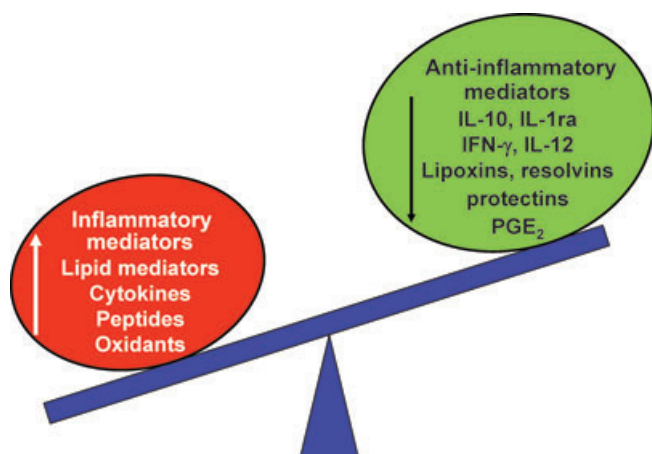


Fig. 7. Anti-inflammatory mediators in asthma. There may be an imbalance between increased proinflammatory mediators and a deficiency in anti-inflammatory mediators. IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; IFN- γ , interferon γ ; PGE₂, prostaglandin E₂.

Cortisol

Endogenous cortisol may be important as a regulator of the allergic inflammatory response, and nocturnal exacerbation of asthma may be related to the circadian fall in plasma cortisol. Blockade of endogenous cortisol secretion by metyrapone results in an increase in the late response to allergen in the skin (182). Cortisol is converted to the inactive cortisone by the enzyme 11 β -hydroxysteroid dehydrogenase-2, which is expressed in the epithelium and endothelium of asthmatic airways and may be a determinant of corticosteroid sensitivity (183). It is possible that this enzyme functions abnormally in asthma or may determine the severity of asthma.

Inhibitory cytokines

Various cytokines have anti-inflammatory actions (184). IL-1 receptor antagonist (IL-1ra) inhibits the binding of IL-1 to its receptors and therefore has a potential anti-inflammatory effects. In a murine model of allergic asthma adenovirus expressing IL-1ra has a protective effect (185). There is increased expression if IL-1ra in airway epithelial cells in asthma (186).

IL-12 promotes the differentiation of Th1 cells and thus the suppression of Th2 cells, resulting in a reduction in eosinophilic inflammation (187). IL-12 infusions in patients with asthma indeed inhibit peripheral blood eosinophilia (188). There is some evidence that IL-12 expression may be impaired in asthma (189). IL-27 is a member of the IL-12 family that promotes Th1 cell differentiation through a STAT1-dependent mechanism independently of IL-12 (190). It is produced by activated APCs and enhances Th1 function by downregulating

GATA3 expression and upregulating T-bet expression, thereby favoring the production of Th1-type cytokines, which then act to further inhibit GATA3 expression (163).

IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of many inflammatory proteins, including several cytokines (such as TNF- α , GM-CSF, IL-5, and several chemokines) that are overexpressed in allergic disease and also inhibits antigen presentation (191). There is a reduction in IL10 transcription and secretion from macrophages in individuals who have asthma (192). IL-10 is produced by a subset of Tregs and by macrophages (193). Specific allergen immunotherapy results in increased production of IL-10-producing Tregs (194). Several cytokines belong to the IL-10 superfamily, including IL-19, IL-22, IL-24, IL-26, IL-28, and IL-29, and have immunomodulatory or anti-infective properties, but how they are involved in allergic inflammation has not yet been defined (195).

Lipid anti-inflammatory mediators

Several lipid mediators may also have anti-inflammatory and immunosuppressive effects. PGE₂ has inhibitory effects on macrophages, epithelial cells, and eosinophils, and exogenous PGE₂ inhibits allergen-induced airway responses, and its endogenous generation may account for the refractory period after exercise challenge (196). However, it is unlikely that endogenous PGE₂ is important in most asthmatics, since non-selective COX inhibitors only worsen asthma in a minority of patients (aspirin-induced asthma).

Lipoxins are generated from arachidonic acid through cell-cell interaction via 5- and 15-lipoxygenase and inhibit the action of cys-LTs (197). Lipoxins are generated in asthma but are reduced in aspirin-sensitive and severe asthma (198). Resolvins are related anti-inflammatory lipid molecules that are generated from omega-3 fatty acids in the diet that are involved in the resolution of acute inflammation. In a murine model, resolvin E₁ promotes the resolution of allergic inflammation through inhibiting the effects of IL-23 and IL-6, which drive Th17 cells (199), and promotes the resolution of allergic inflammation (200). Protectins are related lipid mediators that are involved in the resolution of inflammation. Protectin D₁ accelerates the resolution of allergic inflammation in a murine model (201). Protectin D₁ is found in the exhaled breath condensate of normal subjects, but is reduced during exacerbations of asthma.

Indoleamine 2, 3-dioxygenase

Indoleamine 2, 3-dioxygenase (IDO), a tryptophan-degrading enzyme, is an important T-cell immunomodulator that

promotes immunological tolerance (202). IDO expression by DCs may suppress T-cell responses and promote tolerance to allergens through direct effects on T-cell function, which are mediated by tryptophan depletion, or toxic metabolites. IDO inhibits eosinophilic inflammation in murine models of asthma. IDO activity is reduced in asthma and restored by corticosteroid therapy through increased IL-10 secretion (203) and is reduced in DCs of house dust mite sensitive subjects by the allergen Der p1 (204). Interestingly, statins enhance the anti-inflammatory effects of corticosteroids in asthma through increasing IDO activity (205).

Therapeutic implications

Allergic inflammation is complex, involving many cells and mediators, as discussed above. This has identified many therapeutic targets. However, targeting individual mediators (such as individual cytokines) or cell types (such as eosinophils) is

unlikely to produce very effective therapies for allergic diseases, suggesting that treatments with a broader spectrum of action are needed. Corticosteroids are highly effective in treating allergic diseases and when applied topically largely avoid systemic side effects. Corticosteroids are effective, as they have multiple anti-inflammatory effects, including recruitment of HDAC2 to switch off activated inflammatory genes (155) and inhibition of GATA3 activity (166). It has proved very difficult to develop new therapies for asthma and other allergic disease that are as effective as corticosteroids (206). Specific immunotherapy may be effective when specific allergens are involved, and major progress has been made in understanding the cellular and molecular mechanisms so that more effective immunotherapy may be available in the future through enhancing Treg function (21). The recognition that epigenetic factors are likely to be important in regulating the inflammatory genes involved in allergic disease has also suggested that this may lead to new therapeutic approaches in the future (207, 208).

References

- Akhabir L, Sandford AJ. Genome-wide association studies for discovery of genes involved in asthma. *Respirology* 2011;**16**:396–406.
- Weidinger S, et al. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. *PLoS Genet* 2008;**4**:e1000166.
- Hamid Q, Tulic M. Immunobiology of asthma. *Annu Rev Physiol* 2009;**71**:489–507.
- Broide DH. Allergic rhinitis: pathophysiology. *Allergy Asthma Proc* 2010;**31**:370–374.
- Sicherer SH, Leung DY. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects in 2009. *J Allergy Clin Immunol* 2010;**125**:85–97.
- Leonardi-Bee J, Pritchard D, Britton J. Asthma and current intestinal parasite infection: systematic review and meta-analysis. *Am J Respir Crit Care Med* 2006;**174**:514–523.
- Flohr C, Quinnell RJ, Britton J. Do helminth parasites protect against atopy and allergic disease? *Clin Exp Allergy* 2009;**39**:20–32.
- Busse WW. The relationship of airway hyperresponsiveness and airway inflammation: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010;**138**:4S–10S.
- Reber L, Da Silva CA, Frossard N. Stem cell factor and its receptor c-Kit as targets for inflammatory diseases. *Eur J Pharmacol* 2006;**533**:327–340.
- Jensen BM, Akin C, Gilfillan AM. Pharmacological targeting of the KIT growth factor receptor: a therapeutic consideration for mast cell disorders. *Br J Pharmacol* 2008;**154**:1572–1582.
- Ali FR, Kay AB, Larche M. Airway hyperresponsiveness and bronchial mucosal inflammation in T cell peptide-induced asthmatic reactions in atopic subjects. *Thorax* 2007;**62**:750–757.
- Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Immunol Rev* 2008;**8**:183–192.
- Lambrecht BN, Hammad H. The role of dendritic and epithelial cells as master regulators of allergic airway inflammation. *Lancet* 2010;**376**:835–843.
- Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol* 2009;**10**:713–720.
- Yoshimoto T, et al. Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. *Nat Immunol* 2009;**10**:706–712.
- Nakanishi K. Basophils are potent antigen-presenting cells that selectively induce Th2 cells. *Eur J Immunol* 2010;**40**:1836–1842.
- Saenz SA, Noti M, Artis D. Innate immune cell populations function as initiators and effectors in Th2 cytokine responses. *Trends Immunol* 2010;**31**:407–413.
- Lloyd CM, Hessel EM. Functions of T cells in asthma: more than just T(H)2 cells. *Nat Rev Immunol* 2010;**10**:838–848.
- Soroosh P, Doherty TA. Th9 and allergic disease. *Immunology* 2009;**127**:450–458.
- Souwer Y, Szegedi K, Kapsenberg ML, de Jong EC. IL-17 and IL-22 in atopic allergic disease. *Curr Opin Immunol* 2010;**22**:821–826.
- Palomares O, Yaman G, Azkur AK, Akkoc T, Akdis M, Akdis CA. Role of Treg in immune regulation of allergic diseases. *Eur J Immunol* 2010;**40**:1232–1240.
- Leckie MJ, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness and the late asthmatic response. *Lancet* 2000;**356**:2144–2148.
- Haldar P, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;**360**:973–984.
- Shamri R, Xenakis JJ, Spencer LA. Eosinophils in innate immunity: an evolving story. *Cell Tissue Res* 2011;**343**:57–83.
- Halwani R, Al Muhsen S, Al Jahdali H, Hamid Q. Role of transforming growth factor- β in airway remodeling in asthma. *Am J Respir Cell Mol Biol* 2011;**44**:127–133.
- Birring SS, Berry M, Brightling CE, Pavord ID. Eosinophilic bronchitis: clinical features, management and pathogenesis. *Am J Respir Med* 2003;**2**:169–173.

27. Nair P, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;**360**:985–993.
28. Drolet JP, Frangie H, Guay J, Hajoui O, Hamid Q, Mazer BD. B lymphocytes in inflammatory airway diseases. *Clin Exp Allergy* 2010;**40**:841–849.
29. Cameron L, et al. Local synthesis of epsilon germline gene transcripts, IL-4, and IL-13 in allergic nasal mucosa after ex vivo allergen exposure. *J Allergy Clin Immunol* 2000;**106**:46–52.
30. Takhar P, et al. Class switch recombination to IgE in the bronchial mucosa of atopic and nonatopic patients with asthma. *J Allergy Clin Immunol* 2007;**119**:213–218.
31. Lindell DM, Berlin AA, Schaller MA, Lukacs NW. B cell antigen presentation promotes Th2 responses and immunopathology during chronic allergic lung disease. *PLoS ONE* 2008;**3**:e3129.
32. Kato A, Xiao H, Chustz RT, Liu MC, Schleimer RP. Local release of B cell-activating factor of the TNF family after segmental allergen challenge of allergic subjects. *J Allergy Clin Immunol* 2009;**123**:369–375.
33. Cundall M, Sun Y, Miranda C, Trudeau JB, Barnes S, Wenzel SE. Neutrophil-derived matrix metalloproteinase-9 is increased in severe asthma and poorly inhibited by glucocorticoids. *J Allergy Clin Immunol* 2003;**112**:1064–1071.
34. Jatakanon A, Uasaf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* 1999;**160**:1532–1539.
35. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006;**11**:54–61.
36. Alcorn JF, Crowe CR, Kolls JK. TH17 cells in asthma and COPD. *Annu Rev Physiol* 2010;**72**:495–516.
37. Doe C, et al. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest* 2010;**138**:1140–1147.
38. Novak N, Bieber T. Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 2003;**112**:252–262.
39. Beeh KM, Ksoll M, Buhl R. Elevation of total serum immunoglobulin E is associated with asthma in nonallergic individuals. *Eur Respir J* 2000;**16**:609–614.
40. Bachert C, Gevaert P, Zhang N, van Zele T, Perez-Novoa C. Role of staphylococcal superantigens in airway disease. *Chem Immunol Allergy* 2007;**93**:214–236.
41. Bachert C, Zhang N, Patou J, van Zele T, Gevaert P. Role of staphylococcal superantigens in upper airway disease. *Curr Opin Allergy Clin Immunol* 2008;**8**:34–38.
42. Bachert C, Gevaert P, Holtappels G, Johanson SG, Van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001;**107**:607–614.
43. Gould HJ, Takhar P, Harries HE, Chevreton E, Sutton BJ. The allergic march from *Staphylococcus aureus* superantigens to immunoglobulin E. *Chem Immunol Allergy* 2007;**93**:106–136.
44. Lee JY, et al. Role of staphylococcal superantigen-specific IgE antibodies in aspirin-intolerant asthma. *Allergy Asthma Proc* 2006;**27**:341–346.
45. Rocha-de-Souza CM, Berent-Maoz B, Mankuta D, Moses AE, Levi-Schaffer F. Human mast cell activation by *Staphylococcus aureus*: interleukin-8 and tumor necrosis factor alpha release and the role of Toll-like receptor 2 and CD48 molecules. *Infect Immun* 2008;**76**:4489–4497.
46. Barnes PJ. Intrinsic asthma: not so different from allergic asthma but driven by superantigens? *Clin Exp Allergy* 2009;**39**:1145–1151.
47. Fraser JD, Proft T. The bacterial superantigen and superantigen-like proteins. *Immunol Rev* 2008;**225**:226–243.
48. Herz U, et al. Airway exposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness—a model for non-allergic asthma. *Eur J Immunol* 1999;**29**:1021–1031.
49. Seiberling KA, et al. Superantigens and chronic rhinosinusitis: detection of staphylococcal exotoxins in nasal polyps. *Laryngoscope* 2005;**115**:1580–1585.
50. Zhang N, et al. Mucosal tissue polyclonal IgE is functional in response to allergen and SEB. *Allergy* 2011;**66**:141–148.
51. Muralimohan G, Rossi RJ, Guernsey LA, Thrall RS, Vella AT. Inhalation of *Staphylococcus aureus* enterotoxin A induces IFN-gamma and CD8 T cell-dependent airway and interstitial lung pathology in mice. *J Immunol* 2008;**181**:3698–3705.
52. Patou J, Gevaert P, van Zele T, Holtappels G, Van Cauwenberge P, Bachert C. *Staphylococcus aureus* enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol* 2008;**121**:110–115.
53. Umibe T, et al. Clonal expansion of T cells infiltrating in the airways of non-atopic asthmatics. *Clin Exp Immunol* 2000;**119**:390–397.
54. Ou LS, Goleva E, Hall C, Leung DY. T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. *J Allergy Clin Immunol* 2004;**113**:756–763.
55. Cardona ID, Goleva E, Ou LS, Leung DY. *Staphylococcus aureus* enterotoxin B inhibits regulatory T cells by inducing glucocorticoid-induced TNF receptor-related protein ligand on monocytes. *J Allergy Clin Immunol* 2006;**117**:688–695.
56. Li LB, Goleva E, Hall CF, Ou LS, Leung DY. Superantigen-induced corticosteroid resistance of human T cells occurs through activation of the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK-ERK) pathway. *J Allergy Clin Immunol* 2004;**114**:1059–1069.
57. Zehn D, Bevan MJ, Fink PJ. Cutting edge: TCR revision affects predominantly Foxp3 cells and skews them toward the Th17 lineage. *J Immunol* 2007;**179**:5653–5657.
58. Eyerich K, et al. IL-17 in atopic eczema: linking allergen-specific adaptive and microbial-triggered innate immune response. *J Allergy Clin Immunol* 2009;**123**:59–66.
59. Szczeklik A, Nizankowska E, Serafin A, Dyczek A, Duplaga M, Musial J. Autoimmune phenomena in bronchial asthma with special reference to aspirin intolerance. *Am J Respir Crit Care Med* 1995;**152**:1753–1756.
60. Sun RS, Chen XH, Liu RQ, Cheng TM, Ran XZ, Yang T. Autoantibodies to the high-affinity IgE receptor in patients with asthma. *Asian Pac J Allergy Immunol* 2008;**26**:19–22.
61. Nahm DH, et al. Identification of cytokeratin 18 as a bronchial epithelial autoantigen associated with nonallergic asthma. *Am J Respir Crit Care Med* 2002;**165**:1536–1539.
62. Nahm DH, Lee KH, Shin JY, Ye YM, Kang Y, Park HS. Identification of alpha-enolase as an autoantigen associated with severe asthma. *J Allergy Clin Immunol* 2006;**118**:376–381.
63. Kwon B, Lee HA, Choi GS, Ye YM, Nahm DH, Park HS. Increased IgG Antibody-Induced Cytotoxicity Against Airway Epithelial Cells in Patients with Nonallergic Asthma. *J Clin Immunol* 2009;**29**:517–523.
64. Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev* 1998;**50**:515–596.
65. Wilson SJ, Lau L, Howarth PH. Inflammatory mediators in naturally occurring rhinitis. *Clin Exp Allergy* 1998;**28**:220–227.
66. Leung DY, Soter NA. Cellular and immunologic mechanisms in atopic dermatitis. *J Am Acad Dermatol* 2001;**44**:S1–S12.
67. van Ganse E, Kaufman L, Derde MP, Yernaullt JC, Delaunois L. Effects of antihistamines in adult asthma: a meta-analysis of clinical trials. *Eur Respir J* 1997;**10**:2216–2224.
68. Peters-Golden M, Henderson WR Jr. Leukotrienes. *N Engl J Med* 2007;**357**:1841–1854.

69. Boyce JA. Eicosanoids in asthma, allergic inflammation, and host defense. *Curr Mol Med* 2008;**8**:335–349.
70. Luster AD, Tager AM. T-cell trafficking in asthma: lipid mediators grease the way. *Nat Rev Immunol* 2004;**4**:711–724.
71. Gelfand EW, Dakhama A. CD8+ T lymphocytes and leukotriene B4: novel interactions in the persistence and progression of asthma. *J Allergy Clin Immunol* 2006;**117**:577–582.
72. Cho KJ, et al. Blockade of airway inflammation and hyperresponsiveness by inhibition of BLT2, a low-affinity leukotriene B4 receptor. *Am J Respir Cell Mol Biol* 2010;**42**:294–303.
73. Oguma T, Asano K, Ishizaka A. Role of prostaglandin D(2) and its receptors in the pathophysiology of asthma. *Allergol Int* 2008;**57**:307–312.
74. Pettipher R, Hansel TT, Armer R. Antagonism of the prostaglandin D2 receptors DP1 and CRTH2 as an approach to treat allergic diseases. *Nat Rev Drug Discov* 2007;**6**:313–325.
75. Schuligoi R, et al. CRTH2 and D-type prostanoid receptor antagonists as novel therapeutic agents for inflammatory diseases. *Pharmacology* 2010;**85**:372–382.
76. Grant GE, Rokach J, Powell WS. 5-Oxo-EETE and the OXE receptor. *Prostaglandins Other Lipid Mediat* 2009;**89**:98–104.
77. Montuschi P, Barnes PJ, Roberts LJ. Isoprostanes: markers and mediators of oxidative stress. *FASEB J* 2004;**18**:1791–1800.
78. Feltenmark S, et al. Eoxins are proinflammatory arachidonic acid metabolites produced via the 15-lipoxygenase-1 pathway in human eosinophils and mast cells. *Proc Natl Acad Sci U S A* 2008;**105**:680–685.
79. Barnes PJ. Cytokine networks in asthma and chronic obstructive pulmonary disease. *J Clin Invest* 2008;**118**:3546–3556.
80. Oh CK, Geba GP, Molfino N. Investigational therapeutics targeting the IL-4/IL-13/STAT-6 pathway for the treatment of asthma. *Eur Respir Rev* 2010;**19**:46–54.
81. Busse WW, Ring J, Huss-Marp J, Kahn JE. A review of treatment with mepolizumab, an anti-IL-5 mAb, in hypereosinophilic syndromes and asthma. *J Allergy Clin Immunol* 2010;**125**:803–813.
82. Wenzel SE, et al. A randomized, double-blind, placebo-controlled study of TNF- α blockade in severe persistent asthma. *Am J Respir Crit Care Med* 2009;**179**:549–558.
83. Liu YJ. Thymic stromal lymphopoietin: master switch for allergic inflammation. *J Exp Med* 2006;**203**:269–273.
84. Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* 2010;**11**:289–293.
85. Ying S, et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 2005;**174**:8183–8190.
86. Mou Z, et al. Overexpression of thymic stromal lymphopoietin in allergic rhinitis. *Acta Otolaryngol* 2009;**129**:297–301.
87. Zhang Z, et al. Thymic stromal lymphopoietin overproduced by keratinocytes in mouse skin aggravates experimental asthma. *Proc Natl Acad Sci USA* 2009;**106**:1536–1541.
88. Barlow JL, McKenzie AN. IL-25: a key requirement for the regulation of type-2 immunity. *Biofactors* 2009;**35**:178–182.
89. Kurowska-Stolarska M, Hueber A, Stolarski B, McInnes IB. Interleukin-33: a novel mediator with a role in distinct disease pathologies. *J Intern Med* 2011;**269**:29–35.
90. Yagami A, et al. IL-33 mediates inflammatory responses in human lung tissue cells. *J Immunol* 2010;**185**:5743–5750.
91. Kurowska-Stolarska M, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol* 2009;**183**:6469–6477.
92. Prefontaine D, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;**125**:752–754.
93. Pushparaj PN, et al. The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci U S A* 2009;**106**:9773–9778.
94. Hsu CL, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PLoS ONE* 2010;**5**:e11944.
95. Wells TN, Power CA, Shaw JP, Proudfoot AE. Chemokine blockers—therapeutics in the making? *Trends Pharmacol Sci* 2006;**27**:41–47.
96. Gutierrez-Ramos JC, Lloyd C, Kapsenberg ML, Gonzalo JA, Coyle AJ. Non-redundant functional groups of chemokines operate in a coordinate manner during the inflammatory response in the lung. *Immunol Rev* 2000;**177**:31–42.
97. Erin EM, Williams TJ, Barnes PJ, Hansel TT. Eotaxin receptor (CCR3) antagonism in asthma and allergic disease. *Curr Drug Targets Inflamm Allergy* 2002;**1**:201–214.
98. Vijayanand P, et al. Chemokine receptor 4 plays a key role in T cell recruitment into the airways of asthmatic patients. *J Immunol* 2010;**184**:4568–4574.
99. Montuschi P, et al. Increased 8-Isoprostane, a marker of oxidative stress, in exhaled condensates of asthmatic patients. *Am J Respir Crit Care Med* 1999;**160**:216–220.
100. Paredi P, Kharitonov SA, Barnes PJ. Elevation of exhaled ethane concentration in asthma. *Am J Respir Crit Care Med* 2000;**162**:1450–1454.
101. Barnes PJ. Reactive oxygen species in asthma. *Eur Respir Rev* 2000;**10**:240–243.
102. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;**84**:731–765.
103. Barnes PJ, et al. Exhaled nitric oxide in pulmonary diseases: a comprehensive review. *Chest* 2010;**138**:682–692.
104. Kharitonov SA, Barnes PJ. Clinical aspects of exhaled nitric oxide. *Eur Respir J* 2000;**16**:781–792.
105. Kharitonov SA, Rajakulasingam K, O'Connor BJ, Durham SR, Barnes PJ. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 1997;**99**:58–64.
106. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. *Am J Respir Crit Care Med* 2001;**163**:1693–1772.
107. Hansel TT, et al. A selective inhibitor of inducible nitric oxide synthase inhibits exhaled breath nitric oxide in healthy volunteers and asthmatics. *FASEB J* 2003;**17**:1298–1300.
108. Singh D, et al. Selective inducible nitric oxide synthase inhibition has no effect on allergen challenge in asthma. *Am J Respir Crit Care Med* 2007;**176**:988–993.
109. Lundberg JON, et al. High nitric oxide production in human paranasal sinuses. *Nature Med* 1995;**1**:370–373.
110. Polosa R, Blackburn MR. Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. *Trends Pharmacol Sci* 2009;**30**:528–535.
111. Muller T, et al. A potential role for P2X7R in allergic airway inflammation in mice and humans. *Am J Respir Cell Mol Biol* 2011;**44**:456–464.
112. Idzko M, et al. Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 2007;**13**:913–919.
113. Basoglu OK, Pelleg A, Essilfie-Quaye S, Brindici C, Barnes PJ, Kharitonov SA. Effects of aerosolized adenosine 5'-triphosphate vs adenosine 5'-monophosphate on dyspnea and airway caliber in healthy nonsmokers and patients with asthma. *Chest* 2005;**128**:1905–1909.
114. Tschopp J, Schroder K. NLRP3 inflammation activation: the convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol* 2010;**10**:210–215.
115. Tschumperlin DJ, Drazen JM. Chronic effects of mechanical force on airways. *Annu Rev Physiol* 2006;**68**:563–583.

116. Hassan M, et al. Airway smooth muscle remodeling is a dynamic process in severe long-standing asthma. *J Allergy Clin Immunol* 2010;**125**:1037–1045.
117. Cox G. Bronchial thermoplasty for severe asthma. *Curr Opin Pulm Med* 2011;**17**:34–38.
118. Araya J, Nishimura SL. Fibrogenic reactions in lung disease. *Annu Rev Pathol* 2010;**5**:77–98.
119. Bergeron C, Al Ramli W, Hamid Q. Remodeling in asthma. *Proc Am Thorac Soc* 2009;**6**:301–305.
120. Aceves SS, Ackerman SJ. Relationships between eosinophilic inflammation, tissue remodeling, and fibrosis in eosinophilic esophagitis. *Immunol Allergy Clin North Am* 2009;**29**:197–xiv.
121. Paredi P, Barnes PJ. The airway vasculature: recent advances and clinical implications. *Thorax* 2009;**64**:444–450.
122. Paredi P, Kharitonov SA, Barnes PJ. Correlation of exhaled breath temperature with bronchial blood flow in asthma. *Respir Res* 2005;**6**:15.
123. Siddiqui S, et al. Vascular remodeling is a feature of asthma and nonasthmatic eosinophilic bronchitis. *J Allergy Clin Immunol* 2007;**120**:813–819.
124. Walters EH, Reid D, Soltani A, Ward C. Angiogenesis: a potentially critical part of remodelling in chronic airway diseases? *Pharmacol Ther* 2008;**118**:128–137.
125. Fahy JV, Dickey BF. Airway mucus function and dysfunction. *N Engl J Med* 2010;**363**:2233–2247.
126. Chen G, et al. SPDEF is required for mouse pulmonary goblet cell differentiation and regulates a network of genes associated with mucus production. *J Clin Invest* 2009;**119**:2914–2924.
127. Davis CW, Lazarowski E. Coupling of airway ciliary activity and mucin secretion to mechanical stresses by purinergic signaling. *Respir Physiol Neurobiol* 2008;**163**:208–213.
128. Burgel PR, Nadel JA. Roles of epidermal growth factor receptor activation in epithelial cell repair and mucin production in airway epithelium. *Thorax* 2004;**59**:992–996.
129. Joos GF, De Swert KO, Schelfhout V, Pauwels RA. The role of neural inflammation in asthma and chronic obstructive pulmonary disease. *Ann NY Acad Sci* 2003;**992**:218–230.
130. Barnes PJ. Neurogenic inflammation in the airways. *Respir Physiol* 2001;**125**:145–154.
131. Caceres AI, et al. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc Natl Acad Sci U S A* 2009;**106**:9099–9104.
132. Lacroix JS, Landis BN. Neurogenic inflammation of the upper airway mucosa. *Rhinology* 2008;**46**:163–165.
133. Cevikbas F, Steinhoff A, Homey B, Steinhoff M. Neuroimmune interactions in allergic skin diseases. *Curr Opin Allergy Clin Immunol* 2007;**7**:365–373.
134. Kay AB. Calcitonin gene-related peptide- and vascular endothelial growth factor-positive inflammatory cells in late-phase allergic skin reactions in atopic subjects. *J Allergy Clin Immunol* 2011;**127**:232–237.
135. Freund-Michel V, Frossard N. The nerve growth factor and its receptors in airway inflammatory diseases. *Pharmacol Ther* 2008;**117**:52–76.
136. Raap U, Braunstahl GJ. The role of neurotrophins in the pathophysiology of allergic rhinitis. *Curr Opin Allergy Clin Immunol* 2010;**10**:8–13.
137. Borroni RG, Truzzi F, Pincelli C. The skin neurotrophic network in health and disease. *Actas Dermosifiliogr* 2009;**100**(Suppl.):70–74.
138. Fox AJ, Patel HJ, Barnes PJ, Belvisi MG. Release of nerve growth factor by human pulmonary epithelial cells: role in airway inflammatory diseases. *Eur J Pharmacol* 2001;**424**:159–162.
139. Nassenstein C, Kutschker J, Tumes D, Braun A. Neuro-immune interaction in allergic asthma: role of neurotrophins. *Biochem Soc Trans* 2006;**34**:591–593.
140. Watson JJ, et al. TrkA5: a novel therapeutic agent for treatment of inflammatory pain and asthma. *J Pharmacol Exp Ther* 2006;**316**:1122–1129.
141. de Vries A, et al. Airway hyper-responsiveness in allergic asthma in guinea-pigs is mediated by nerve growth factor via the induction of substance P: a potential role for trkA. *Clin Exp Allergy* 2006;**36**:1192–1200.
142. Kassel O, et al. Local increase in the number of mast cells and expression of nerve growth factor in the bronchus of asthmatic patients after repeated inhalation of allergen at low-dose. *Clin Exp Allergy* 2001;**31**:1432–1440.
143. Veres TZ, Rochlitz S, Braun A. The role of neuro-immune cross-talk in the regulation of inflammation and remodelling in asthma. *Pharmacol Ther* 2009;**122**:203–214.
144. Montoro J, et al. Stress and allergy. *J Investig Allergol Clin Immunol* 2009;**19**(Suppl.):40–47.
145. Arndt J, Smith N, Tausk F. Stress and atopic dermatitis. *Curr Allergy Asthma Rep* 2008;**8**:312–317.
146. Haczku A, Panettieri RA Jr. Social stress and asthma: the role of corticosteroid insensitivity. *J Allergy Clin Immunol* 2010;**125**:550–558.
147. Bailey MT, et al. Social stress enhances allergen-induced airway inflammation in mice and inhibits corticosteroid responsiveness of cytokine production. *J Immunol* 2009;**182**:7888–7896.
148. Quarcoo D, Pavlovic S, Joachim RA. Stress and airway reactivity in a murine model of allergic airway inflammation. *Neuroimmunomodulation* 2009;**16**:318–324.
149. Frew AJ, Plummeridge MJ. Alternative agents in asthma. *J Allergy Clin Immunol* 2001;**108**:3–10.
150. Barnes PJ. Transcription factors in airway diseases. *Lab Invest* 2006;**86**:867–872.
151. Barnes PJ, Karin M. Nuclear factor- κ B: a pivotal transcription factor in chronic inflammatory diseases. *New Engl J Med* 1997;**336**:1066–1071.
152. Hart LA, Krishnan VL, Adcock IM, Barnes PJ, Chung KF. Activation and localization of transcription factor, nuclear factor- κ B, in asthma. *Am J Respir Crit Care Med* 1998;**158**:1585–1592.
153. Barnes PJ, Adcock IM, Ito K. Histone acetylation and deacetylation: importance in inflammatory lung diseases. *Eur Respir J* 2005;**25**:552–563.
154. Adcock IM, Ford P, Barnes PJ, Ito K. Epigenetics and airways disease. *Respir Res* 2006;**7**:21.
155. Barnes PJ. How corticosteroids control inflammation. *Br J Pharmacol* 2006;**148**:245–254.
156. Ho IC, Pai SY. GATA-3 - not just for Th2 cells anymore. *Cell Mol Immunol* 2007;**4**:15–29.
157. Barnes PJ. Role of GATA-3 in allergic diseases. *Curr Mol Med* 2008;**8**:330–334.
158. Caramori G, et al. Expression of GATA family of transcription factors in T-cells, monocytes and bronchial biopsies. *Eur Respir J* 2001;**18**:466–473.
159. Nakamura Y, et al. Gene expression of the GATA-3 transcription factor is increased in atopic asthma. *J Allergy Clin Immunol* 1999;**103**:215–222.
160. Maneechotesuwan K, et al. Regulation of Th2 cytokine genes by p38 MAPK-mediated phosphorylation of GATA-3. *J Immunol* 2007;**178**:2491–2498.
161. Finotto S, et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 2002;**295**:336–338.
162. Hwang ES, Szabo SJ, Schwartzberg PL, Glimcher LH. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. *Science* 2005;**307**:430–433.
163. Yoshimoto T, Yoshimoto T, Yasuda K, Mizuguchi J, Nakanishi K. IL-27 suppresses Th2 cell development and Th2 cytokines

- production from polarized Th2 cells: a novel therapeutic way for Th2-mediated allergic inflammation. *J Immunol* 2007;**179**:4415–4423.
164. Usui T, et al. T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. *J Exp Med* 2006;**203**:755–766.
 165. Avni O, Lee D, Macian F, Szabo SJ, Glimcher LH, Rao A. T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol* 2002;**3**:643–651.
 166. Maneechotesuwan K, et al. Suppression of GATA-3 nuclear import and phosphorylation: a novel mechanism of corticosteroid action in allergic disease. *PLoS Med* 2009;**6**:e1000076.
 167. Wang YH, et al. A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J Exp Med* 2010;**207**:2479–2491.
 168. Pernis AB, Rothman PB. JAK-STAT signaling in asthma. *J Clin Invest* 2002;**109**:1279–1283.
 169. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. *Immunol Rev* 2009;**228**:273–287.
 170. Morales JK, Falanga YT, Depczynski A, Fernando J, Ryan JJ. Mast cell homeostasis and the JAK-STAT pathway. *Genes Immun* 2010;**11**:599–608.
 171. Wohlmann A, Sebastian K, Borowski A, Krause S, Friedrich K. Signal transduction by the atopy-associated human thymic stromal lymphopoietin (TSLP) receptor depends on Janus kinase function. *Biol Chem* 2010;**391**:181–186.
 172. Knisz J, Rothman PB. Suppressor of cytokine signaling in allergic inflammation. *J Allergy Clin Immunol* 2007;**119**:739–745.
 173. Schwartz DA. Epigenetics and environmental lung disease. *Proc Am Thorac Soc* 2010;**7**:123–125.
 174. Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and IL4 locus accessibility. *Annu Rev Immunol* 2006;**24**:607–656.
 175. Lal G, et al. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. *J Immunol* 2009;**182**:259–273.
 176. Hollingsworth JW, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. *J Clin Invest* 2008;**118**:3462–3469.
 177. Ito K, Barnes PJ, Adcock IM. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits IL-1b-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol* 2000;**20**:6891–6903.
 178. Ito K, et al. Expression and activity of histone deacetylases (HDACs) in human asthmatic airways. *Am J Respir Crit Care Med* 2002;**166**:392–396.
 179. Cosio BG, et al. Histone acetylase and deacetylase activity in alveolar macrophages and blood monocytes in asthma. *Am J Respir Crit Care Med* 2004;**170**:141–147.
 180. Hew M, et al. Relative corticosteroid insensitivity of peripheral blood mononuclear cells in severe asthma. *Am J Respir Crit Care Med* 2006;**174**:134–141.
 181. Mattes J, Collison A, Plank M, Phipps S, Foster PS. Antagonism of microRNA-126 suppresses the effector function of Th2 cells and the development of allergic airways disease. *Proc Natl Acad Sci U S A* 2009;**106**:18704–18709.
 182. Herrscher RF, Kasper C, Sullivan TJ. Endogenous cortisol regulates immunoglobulin E-dependent late phase reactions. *J Clin Invest* 1992;**90**:593–603.
 183. Orsida BE, Krozowski ZS, Walters EH. Clinical relevance of airway 11beta-hydroxysteroid dehydrogenase type II enzyme in asthma. *Am J Respir Crit Care Med* 2002;**165**:1010–1014.
 184. Barnes PJ, Lim S. Inhibitory cytokines in asthma. *Mol Medicine Today* 1998;**4**:452–458.
 185. Wang CC, et al. Adenovirus expressing interleukin-1 receptor antagonist alleviates allergic airway inflammation in a murine model of asthma. *Gene Ther* 2006;**13**:1414–1421.
 186. Sousa AR, Lane SJ, Nakhosteen JA, Lee TH, Poston RN. Expression of interleukin-1 beta (IL-1b) and interleukin-1 receptor antagonist (IL-1ra) on asthmatic bronchial epithelium. *Am J Respir Crit Care Med* 1996;**154**:1061–1066.
 187. Wills-Karp M. IL-12/IL-13 axis in allergic asthma. *J Allergy Clin Immunol* 2001;**107**:9–18.
 188. Bryan S, et al. Effects of recombinant human interleukin-12 on eosinophils, airway hyperreactivity and the late asthmatic response. *Lancet* 2000;**356**:2149–2153.
 189. Naseer T, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroids. *Am J Respir Crit Care Med* 1997;**155**:845–851.
 190. Owaki T, et al. A role for IL-27 in early regulation of Th1 differentiation. *J Immunol* 2005;**175**:2191–2200.
 191. Pretolani M, Goldman M. IL-10: a potential therapy for allergic inflammation? *Immunol Today* 1997;**18**:277–280.
 192. John M, et al. Inhaled corticosteroids increase IL-10 but reduce MIP-1a, GM-CSF and IFN-g release from alveolar macrophages in asthma. *Am J Respir Crit Care Med* 1998;**157**:256–262.
 193. Hawrylowicz CM. Regulatory T cells and IL-10 in allergic inflammation. *J Exp Med* 2005;**202**:1459–1463.
 194. Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007;**120**:707–713.
 195. Commins S, Steinke JW, Borish L. The extended IL-10 superfamily: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. *J Allergy Clin Immunol* 2008;**121**:1108–1111.
 196. Pavord ID, Tattersfield AE. Bronchoprotective role for endogenous prostaglandin E2. *Lancet* 1995;**344**:436–438.
 197. Levy BD. Lipoxins and lipoxin analogs in asthma. *Prostaglandins Leukot Essent Fatty Acids* 2005;**73**:231–237.
 198. Planaguma A, et al. Airway lipoxin A4 generation and lipoxin A4 receptor expression are decreased in severe asthma. *Am J Respir Crit Care Med* 2008;**178**:574–582.
 199. Haworth O, Cernadas M, Yang R, Serhan CN, Levy BD. Resolvin E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation. *Nat Immunol* 2008;**9**:873–879.
 200. Aoki H, et al. Protective effect of resolvin E1 on the development of asthmatic airway inflammation. *Biochem Biophys Res Commun* 2010;**400**:128–133.
 201. Levy BD, et al. Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. *J Immunol* 2007;**178**:496–502.
 202. Le AV, Broide DH. Indoleamine-2,3-dioxygenase modulation of allergic immune responses. *Curr Allergy Asthma Rep* 2006;**6**:27–31.
 203. Maneechotesuwan K, Supawita S, Kasetsinsombat K, Wongkajornsilp A, Barnes PJ. Sputum indoleamine-2, 3-dioxygenase activity is increased in asthmatic airways by using inhaled corticosteroids. *J Allergy Clin Immunol* 2008;**121**:43–50.
 204. Maneechotesuwan K, et al. Der p 1 suppresses indoleamine 2, 3-dioxygenase in dendritic cells from house dust mite-sensitive patients with asthma. *J Allergy Clin Immunol* 2009;**123**:239–248.
 205. Maneechotesuwan K, Ekjitrakul W, Kasetsinsombat K, Wongkajornsilp A, Barnes PJ. Statins enhance the anti-inflammatory effects of inhaled corticosteroids in asthmatic patients through increased induction of indoleamine 2, 3-dioxygenase.

- J Allergy Clin Immunol 2010;**126**:754–762.
206. Barnes PJ. New drugs for asthma: is there any progress? Trends Pharmacol Sci 2010;**31**:335–343.
207. Barnes PJ. Targeting the epigenome in the treatment of asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc 2009;**6**:693–696.
208. Szyf M. Epigenetics, DNA methylation, and chromatin modifying drugs. Annu Rev Pharmacol Toxicol 2009;**49**:243–263.