Cytokines are a family of small glycosylated proteins involved in cell–cell signaling, cellular growth, differentiation, proliferation, chemotaxis, immunomodulation, immunoglobulin isotype switching, and apoptosis. The actions of cytokines are mediated through specific cytokine receptors on the surfaces of target cells. Although cytokines usually have effects on adjacent cells, they can act at a distance or have effects on the cell producing the cytokine itself. Many of these cytokines exhibit pleiotropy and have overlapping functions, making their individual roles in the pathogenesis of asthma and allergic disease difficult to unravel.

Until recently, T lymphocytes and eosinophils were considered to be the major sources of cytokines in allergic inflammation, but it is now recognized that cytokines are produced not only by other inflammatory cells but also by structural cells, including epithelial, endothelial and airway smooth muscle (ASM) cells, and fibroblasts (Figure 39-1). To date, more than 30 different cytokines have been described, and this list continues to grow (Table 39-1). Among these cytokines are T-cell–derived molecules that include so-called T-helper (Th)1 (interleukin-2 [IL-2], interferon-γ [IFN-γ], IL-12), Th2 (IL-4, IL-5, IL-9, IL-13, IL-17, IL-25), and Th3 or T regulatory cytokines [IL-10, transforming growth factor-β [TGF-β]], all of which are involved in the regulation of cell-mediated and humoral immunities. Although the distinction between Th1 and Th2 cells in humans is not as clear as in mice, there is overwhelming support in the literature for the notion that allergic inflammation is driven by an imbalance between Th1 and Th2 cytokines, favoring the Th2 arm of the immune response (see Figure 39-1). Other cytokines include proinflammatory cytokines (IL-1β, IL-6, IL-11, tumor necrosis factor-α [TNF-α], granulocyte–macrophage colony-stimulating factor [GM-CSF]) involved in innate host defense, antiinflammatory cytokines [IL-10, IFN-γ, IL-12, IL-18], growth factors platelet-derived growth factor [PDGF], TGF-β, fibroblast growth factor, epidermal growth factor), and chemotactic cytokines or chemokines (RANTES, monocyte chemoattractant protein-1 [MCP-1] to MCP-5, eotaxin, IL-8). For the purpose of this chapter, which is particularly focused on the role of cytokines and chemokines in asthma, cytokines and chemokines are broadly grouped on the basis of their functional activity and subdivided into (1) eosinophil-associated cytokines, (2) IgE-mediated cytokines, (3) remodeling-associated cytokines, and (4) immunomodulatory cytokines.

IgE-ASSOCIATED CYTOKINES

Asthma is clinically categorized into occupational, intrinsic, and atopic (allergic) forms; the vast majority of asthmatic patients have the atopic form. A central mediator in atopic asthma is IgE antibody, which is produced by sensitized allergen-specific B cells (Figure 39-2A). Allergens are antigens that elicit hypersensitivity or allergic reactions and that by themselves can increase IgE levels in the serum in susceptible subjects subsequent to stimulation. B cells, by presenting the allergen fragments in conjunction with the major histocompatibility complex (MHC), can activate specific Th2 helper cells to produce numerous cytokines, leading to further B-cell activation and antibody release. IgE antibodies bind to the high-affinity IgE receptor Fc epsilon receptor (FceRI), present on mast cells and basophils, sensitizing these cells to antigen exposure (see Figure 39-2A). Subsequently, crosslinking of adjacent IgE-FceRI complexes by allergens triggers the degranulation of cytoplasmic vesicles, containing histamine, and the de novo formation of eicosanoids and reactive oxygen species (Figure 39-2B). This results in smooth muscle contraction, mucous secretion, and vasodilatation, all of which are hallmarks of asthma. IgE-producing B cells play a critical role in allergic inflammation, and therefore factors responsible for their activation, namely IgE-associated cytokines such as IL-4, IL-9, and IL-13, are of considerable interest.

IL-4

IL-4 is vital for the regulation of growth, differentiation, activation, and function of B cells (see Figure 39-1). IL-4 exerts its activities through a specific cell surface receptor...
composed of the IL-4Rα chain and the gamma common (γc) chain. IL-4 increases expression of the antigen-presenting proteins, MHC class II molecules, on B cells, resulting in increased capacity of allergen presentation to Th2 cells.\(^2\) In the vasculature, IL-4 promotes expression of vascular cell adhesion molecule-1 (VCAM-1) on endothelium, thereby allowing for recruitment of eosinophils and other inflammatory cells, such as T cells, monocytes, and basophils, from the blood into sites of inflammation.\(^3\) IL-4 also induces isotype switching, a process leading to the production of IgE by B cells, and after switching occurs, IL-4 potentiates IgE production. Furthermore, IL-4 enhances the IgE-mediated response by up-regulating IgE receptors on inflammatory cells within the airway.\(^4\) Conversely, activation of IgE by IL-4 can be diminished by crossregulation from Th1 cytokines (see Figure 39-1). IFN-γ, a Th1 cytokine, can suppress isotype switch recombination to the IgE isotype in B cells activated by IL-4.\(^5\) Additionally, IFN-γ inhibits IL-4-induced expression of the low-affinity IgE receptor.\(^6\) IL-4Rα-deficient mice are more resistant to the development of features of asthma than are IL-4–deficient mice.\(^7\) However, these mice still develop airway hyperresponsiveness (AHR), pointing to the existence of other cytokines sharing partial sequence homology with IL-4.

**FIGURE 39-1** The Th1/Th2 paradigm. The two types of immune response are the cell-mediated immune response and the humoral or antibody-mediated immune response. Cytokines can be grouped on the basis of the types of T-helper (Th) cell that produce them and the types of immune response that they trigger. Th1 cells produce interleukin-2 (IL-2), interferon-γ (IFN-γ), and IL-12, which regulate cell-mediated inflammation, and Th2 cells produce IL-4, IL-5, IL-9, IL-13, IL-17, and IL-25, which regulate antibody-mediated inflammation. Bacteria and viruses can induce the innate immune system to produce cytokines that induce differentiation of Th0 cells to Th1; allergens induce Th2 cytokines, which promote Th0 cell differentiation to Th2. Th1 cytokines can crossregulate the production of Th2 cytokines and vice versa. T-regulatory cells (Treg) can regulate both arms of the immune system, through IL-10 and transforming growth factor-β (TGF-β). There is much evidence that allergic inflammation is driven by an imbalance between Th1 and Th2 cytokines, favoring the Th2 arm of the immune response.

IL-13

IL-13 has 70% sequence homology with IL-4 and binds a heterodimer composed of the IL-4Rα chain and an IL-13Rα chain.\(^8\) Like IL-4, IL-13 is produced by Th2 cells and is found in high concentrations within allergic tissues.\(^9,10\) Because of the redundancy in IL-4Rα binding, both IL-4 and IL-13 exhibit some degree of functional overlap. As with
IL-4, overexpression of IL-13 within the lungs results in IgE production, inflammation, mucus hypersecretion, eosinophilia, and up-regulation of VCAM-1. However, the unique nature of IL-13 is seen in its effects on airway sensitivity to contractile agonists, whereby blocking IL-13 prevents AHR in mice following antigen challenge. Accordingly, it is hypothesized that IL-13 is the primary factor involved in the expression and induction of allergen-induced AHR. Both IL-4 and IL-13 are critical in the induction and regulation of allergic asthma through their production of IgE. Interestingly, the effect of exogenous IL-13 is dependent on when it is given in relationship to allergen exposure as its administration after initial allergen sensitization in mice has no effect on serum IgE levels. The emerging paradigm is that IL-13 induces features of the allergic response through its actions on epithelial and smooth muscle cells rather than through traditional effector pathways involving eosinophils and IgE-mediated events.

IL-9

IL-9 also has actions relevant to IgE-dependent host responses. IL-9 is a Th2 cytokine, and its expression is regulated by a variety of mediators, in particular IL-2, which stimulates its production. Although IL-9 is produced by a variety of cell types, including mast cells, eosinophils, and neutrophils, the major source of this cytokine is the Th lymphocyte. Transgenic mice overexpressing IL-9 were found to have increased serum levels of all immunoglobulin isotypes, including IgE, and an associated accumulation of B cells in the lungs. In vitro, IL-9 enhances IgE production from Th1 cells, inhibition of IgE. IL-9 also stimulates protease production by mast cells and induces their expression of FcεRI. This suggests that, in addition to potentiating IgE production, IL-9 primes mast cells to respond to allergen challenge through increased cell surface expression of FcεRI and the production of proinflammatory mediators. Other than its effects on IgE-mediated immunity, IL-9 is capable of coordinating a multitude of responses associated with asthma, through direct, indirect, and synergistic means. IL-9 has been identified as a T-cell growth factor, capable of stimulating the proliferation of activated T cells. IL-9 transgenic mice demonstrate, in vivo, increased AHR, marked eosinophilia, mucous overproduction, and increased expression of...
FIGURE 39-2 Mast cell sensitization: IgE and B cells. A, Allergen presentation by B cells can activate specific Th2 helper cells to produce cytokines, leading to B-cell activation and IgE release. IgE antibodies can bind to the high-affinity IgE receptor FcεRI on mast cells and basophils, sensitizing these cells to allergen exposure. B, Crosslinking of adjacent IgE–FcεRI receptor complexes by allergens triggers the production and release of many inflammatory mediators, such as histamine and leukotrienes.

eotaxin and MCP-1, MCP-3, and MCP-5 in airway epithelial cells.  

**IL-25**
The newly described cytokine IL-25 (IL-17E) also seems to have a role in the regulation of IgE-mediated responses. IL-25 stimulates IgE synthesis and eosinophilia in mouse models of allergic inflammation by stimulating the release of IL-4 and IL-5 cytokines. As the roles of novel cytokines, such as IL-25, especially in the regulation of IgE-mediated inflammation, are clarified, it is evident that the cytokine network regulating inflammation is broad and complex. Nonetheless, insofar as these cytokines prove to be critical mediators of the inflammatory process, their stimulatory effects make them obvious targets in the treatment of allergic diseases.

**EOSINOPHIL-ASSOCIATED CYTOKINES**
Eosinophils are prominent in allergic airway disease, and the eosinophil is still considered by many to be the hallmark of asthma. Increased numbers of eosinophils in the bronchial mucosa, as well as the bronchoalveolar lavage (BAL) fluid and sputum, are consistent features of asthma, and BAL eosinophilia has even been linked to development of the late airway response (LAR) and asthma severity. Increased eosinophilia in asthmatic patients is observed not only in the large or central airways but also in the peripheral parts of the lungs. Although terminal differentiation of eosinophils occurs within the bone marrow, recent evidence indicates that they can also differentiate locally at the site of inflammation and that the presence of eosinophils within allergic mucosal tissue is not solely due to infiltration of mature cells.

Teleologically, eosinophils form part of the host defense against parasitic infestation. The biologic activity exerted by these cells is largely attributed to their release of prestored granular proteins, including eosinophil cationic protein, eosinophil peroxidase, and major basic protein (MBP). These potent cytotoxic proteins have been found in high concentrations in the sputum of asthmatic patients and are thought to play an important role in the epithelial damage seen in these patients. In addition to cytotoxic proteins, eosinophils can synthesize and release oxygen radicals, lipid mediators (leukotriene B₄, leukotriene C₄, platelet-activating factor), and numerous cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, TNF-α, GM-CSF) and
chemokines (IL-8, RANTES and macrophage inflammatory protein-1α [MIP-1α]). Recently, it has been suggested that eosinophils play a role in airway remodeling because they have the ability to synthesize and release fibrogenic cytokines, including TGF-β, IL-11, IL-17, and IL-25. Together, these eosinophil-associated cytokines are responsible for the tissue-destroying potency of this proinflammatory cell.

**IL-5**

IL-5 is the most important Th2 cytokine associated with eosinophils, and it can regulate most aspects of eosinophil behavior, including eosinophil growth, maturation, differentiation, survival, and activation (see Figure 39-1). Although IL-5 is produced by T-helper cells, cytotoxic T lymphocytes, and mast cells, eosinophils are the predominant source of this cytokine. Human eosinophils express IL-5 mRNA and release IL-5 protein in vitro, and allergen challenge in the bronchial segments results in increased IL-5 mRNA expression in eosinophils in BAL fluid, with a 300-fold increase in IL-5 protein concentrations. IL-5 plays a central role in accumulation and activation of eosinophils in the lungs, an effect readily seen in IL-5-overexpressing transgenic mice, which have lifelong eosinophilia. Moreover, it is a potent eosinophil chemoattractant, and it up-regulates integrin receptor expression on eosinophils, thereby promoting adherence of eosinophils to VCAM-expressing endothelial cells and eosinophil accumulation.

Studies with IL-5 monoclonal antibodies in animal models of allergic inflammation clearly support a role for IL-5 in allergic disease; however, similar studies in humans have proved disappointing. Although blocking IL-5 was effective in abolishing blood and sputum eosinophilia, it did not protect against the allergen-induced LAR or have any effect on baseline AHR in patients with mild asthma. These latest results bring into question the role of eosinophils in airway responsiveness in humans and suggest that alternative mechanisms and/or factors are responsible for airway narrowing in asthmatic patients.

**GM-CSF**

Although secondary in importance to IL-5, IL-3 and GM-CSF are also typically viewed as eosinophil-associated cytokines. IL-3 and GM-CSF are pluripotent hematopoietic growth factors that stimulate the formation of not only eosinophil lineages but also neutrophil, erythroid, and monocytic lineages. Increased expression of GM-CSF has been documented in the bronchial epithelium and in the eosinophils of asthmatic patients following endobronchial allergen challenge. GM-CSF is involved in the priming of eosinophils and accounts for increased eosinophil survival in the BAL fluid of asthmatic patients. In addition, GM-CSF may be involved in development of chronic eosinophilia and airway remodeling of asthma (see below) as insertion of the GM-CSF gene into the epithelium of rats caused eosinophil accumulation in their lungs and irreversible fibrosis.

**REMODELING-ASSOCIATED CYTOKINES**

It has been known for a long time that architectural and structural changes occur in the airways of asthmatic patients. These changes, which include collagen (types III and IV) and fibronectin deposition, increased thickness of subepithelial basement membrane, goblet cell hyperplasia, increased ASM mass and size, angiogenesis, and fibrosis,
collectively contribute to the phenomenon termed airway remodeling (Figure 39-3). Some of these changes were first described in postmortem airway sections from status asthmaticus victims in the 1960s, and, more recently, airway remodeling has been reported even in patients with mild asthma and in children with difficult asthma. The functional consequences of airway remodeling include persistent AHR and mucous hypersecretion, contributing to increased susceptibility to asthma exacerbations. The mechanisms involved in airway remodeling are poorly understood, but research done in the last 3 to 5 years suggests that the balance between matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs) may play a role in this process. Moreover, the increase in ASM content, along with a change in the phenotype of fibroblasts to contractile myofibroblasts, may explain the permanent reduction in airway caliber, which is steroid insensitive and typical in patients with severe forms of the disease. The predominant remodeling-associated cytokines include TGF-β, PDGF, IL-6, IL-11, IL-13, IL-17, and IL-25 (IL-17E).

**TGF-β**

TGF-β is a potent profibrotic cytokine. The major sources of TGF-β include fibroblasts, eosinophils, and epithelial cells. However, macrophages, monocytes, neutrophils, ASM cells, and lymphocytes are also known to produce this cytokine. TGF-β is detected in increased concentrations in baseline BAL fluid from asthmatic patients before allergen challenge, and the concentration increases even more after allergen challenge. Furthermore, TGF-β exerts an important influence on the turnover of extracellular matrix proteins. In tissue culture systems, TGF-β exhibits a pleiotropic nature, depending on the cell type, culture conditions, and presence of other cytokines. TGF-β induces proliferation and release of profibrotic and proinflammatory cytokines in fibroblasts and ASM cells, whereas in monocytes, lymphocytes, and epithelial cells, TGF-β inhibits cytokine proliferation and release. TGF-β is also a potent chemoattractant for many cell types, including monocytes, fibroblasts, and mast cells.

Recently, eosinophils have been recognized as one of the most abundant sources of TGF-β, not only in asthmatic airways but also in the nasal tissues of patients with nasal polyposis and in hypereosinophilic patients. In our laboratory, by use of in situ hybridization and immunocytochemistry, we have demonstrated TGF-β to be significantly elevated in both mildly and severely asthmatic patients in comparison with normal subjects and have shown levels of TGF-β expression to correlate with basement membrane thickness and disease severity in these patients. Approximately 65% of all TGF-β-positive cells were activated eosinophils, which were localized within the reticular lamina. The local production of TGF-β by eosinophils may be responsible for the subepithelial fibrosis observed in asthmatic patients. However, TGF-β can also inhibit eosinophil survival and function and may be involved in the repair process of airway epithelial cells. Such effects of TGF-β illustrate the complex actions of this cytokine in asthma.

**PDGF**

PDGF is not only a major mitogen but also a remodeling-associated cytokine. The ability of PDGF to stimulate proliferation of tissue structural cells, including fibroblasts, epithelial cells, and vascular smooth muscle cells, is well accepted, and this cytokine has been implicated in the alterations of lung function in several chronic lung diseases. Fibroblasts from asthmatic patients show enhanced responsiveness to PDGF, and it is known to activate fibroblasts to proliferate, secrete collagen, and contract collagen lattices. Eosinophils, once again, are the predominant cellular sources of this cytokine; however, platelets, macrophages, airway epithelial and endothelial cells, vascular smooth muscle cells, and fibroblasts themselves are known to secrete PDGF. PDGF can be induced by both mechanical and oxidative stress, as well as by exposure of cells to various cytokines, including IFN-γ, TNF-α, IL-1, and TGF-β. Although PDGF plays an important role in airway remodeling, it is thought that this growth factor is likely to be acting in concert with other remodeling cytokines, in particular TGF-β, to alter the structural makeup of the airway wall in asthmatic airways.

**IL-6**

IL-6 is another remodeling-associated cytokine. Originally, IL-6 was known for its antiviral activity and its growth-promoting effects on B cells. IL-6 is produced by macrophages, monocytes, T and B cells, fibroblasts, epithelial and endothelial cells, ASM cells, and eosinophils. This cytokine is consistently found in high concentrations in biologic fluids and tissues from both animal models of allergic disease and asthmatic patients, but its exact role in asthma remains unclear. IL-6 has the ability to stimulate T- and B-cell production of Th2 cytokines, thereby contributing to the generation and/or the perpetuation of Th2-driven inflammation. In addition, IL-6 is a potent stimulant of the acute-phase allergic response and has recently been shown to be a potent smooth muscle mitogen. Mice overexpressing IL-6 in their airways have subepithelial fibrosis, collagen deposition, and increased accumulation of α-actin–containing smooth muscle cells, without eosinophilia, mucous cell metaplasia, or AHR.

**IL-11 and IL-13**

IL-11 and IL-13 have recently received much attention as key remodeling-associated cytokines. The reason for this is that they are thought to not only cause fibrosis and collagen deposition but also induce myofibroblast hyperplasia, airway obstruction, and AHR. Much of what is known about the role of these cytokines in airway remodeling comes from studies using transgenic mice. Histologic analysis of mice in which IL-11 or IL-13 was constitutively overexpressed in the lungs showed airway wall thickening, enlarged alveoli, subepithelial and adventitial tissue fibrosis, collagen I and III deposition, and increased numbers of contractile and proinflammatory cells in comparison with littermate controls. In addition, IL-11 and IL-13 transgenic mice had baseline airway obstruction and were more responsive to methacholine challenge.
Sources of IL-11 and IL-13 include epithelial cells, fibroblasts, eosinophils, and smooth muscle cells. Recent evidence suggests that one mechanism by which IL-13 induces tissue fibrosis is by selectively stimulating and activating TGF-β production.47 We have recently found the results obtained in IL-11 transgenic mice to hold true for human asthma. Using immunocytochemistry and in situ hybridization, we have demonstrated increased expression of IL-11 mRNA and protein in the epithelial and subepithelial layers of the airway wall in patients with severe asthma but not in those with mild asthma or in healthy control subjects.41 Furthermore, IL-11 expression was inversely correlated with forced expiratory volume in 1 second (FEV₁) in patients with severe asthma, and the IL-11 mRNA-positive cells were localized to epithelial cells and MBP-positive eosinophils.42 One proposed mechanism by which IL-11 can induce these structural changes in the airways may be promotion of the synthesis of TIMP-1, the levels of which have been shown to be elevated in sputum and biopsy samples from asthmatic patients and to correlate with asthmatic airway obstruction.43 Although these studies clearly support a role for IL-11 in airway remodeling, other studies suggest that IL-11 levels may, in fact, be increased as a result of normal airway repair. IL-11 transgenic mice exhibited selective inhibition of antigen-induced airway and parenchymal eosinophilia, Th2 inflammation, Th2 cytokine production, and VCAM-1 gene expression.39 These conflicting data point to the dual nature of IL-11, which acts as both a cytokine that promotes healing and a cytokine capable of inducing local tissue fibrosis.

**IL-17 FAMILY**

The newly emerging remodeling-associated cytokines recently described in the literature include IL-17 and IL-17E (IL-25). These are potent proinflammatory cytokines. IL-17, or IL-17A, is produced exclusively by activated Th lymphocytes, whereas Th2 cells and mast cells secrete IL-25. Expression of IL-17 is markedly increased in asthmatic subjects.44 In mice, systemic overexpression of IL-17 induces neutrophilia via direct in vivo stimulation of IL-6 and IL-8, whereas overexpression of IL-25 results in increased Th2 cytokine gene expression (in particular, IL-4, IL-5, IL-10, and IL-13), increased mucous production, elevated serum levels of IgE and IgG₁, and tissue eosinophilia.24 These pathologic changes can be observed in several tissues and are not restricted to the lungs.

**IMMUNOMODULATORY CYTOKINES**

It is now generally accepted that adult atopic disease is characterized by expression of T-cell immunity to common airborne environmental allergens that is polarized toward the Th2 cytokine profile; Th1-skewed immunity is observed among nonatopic subjects. As a result, attempts to shift the balance from Th2 to Th1 immunomodulatory cytokines, such as IL-10, IL-12, and IFN-γ, may be important in the treatment of allergic inflammation.

**IL-10 FAMILY**

IL-10 is largely known as an inhibitory cytokine; however, it can have either immunosuppressive or immunostimulatory effects. IL-10 was originally identified as a product of murine Th2 cells, but in humans IL-10 is produced by Th0, Th1 and Th2 cells and also by activated monocytes, mast cells and macrophages.31 In normal lungs, alveolar macrophages are the major source of IL-10, but its expression is significantly reduced in asthmatic individuals.45,46 The effects of IL-10 are as follows: (1) it is capable of curtailing the effects of proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, MIP-1α) released during an allergic reaction; (2) it is able to inhibit eosinophil survival and migration by preventing the release of chemoattractants such as RANTES and IL-8 from human ASM cells; (3) it inhibits allergen-induced eosinophilia in sensitized mice and dampens their late-phase response to allergen challenge; (4) it down-regulates the IL-4–induced isotype switching of activated B cells,3 thus preventing IgE synthesis; (5) it may inhibit Th2-driven inflammation, but it is also known to inhibit the differentiation of Th1 cells, thereby preventing the release of IFN-γ and IL-2; (6) it has the ability to interfere with monocyte and macrophage function as it can inhibit MHC class II expression on the surfaces of antigen-presenting cells (APCs)3 and prevent superoxide and nitric oxide (NO) release from inflammatory cells.48

Included in the IL-10 family of cytokines are IL-19, IL-20, IL-22, and IL-24. Like IL-10, they are considered to be antiinflammatory cytokines, and are produced by a variety of cell types, including monocytes, keratinocytes, mast cells, and lymphocytes. However, unlike IL-10, these cytokines are unable to inhibit the effects of proinflammatory mediators involved in the allergic response. IL-19, IL-20, and IL-24 have not been extensively studied; however, studies on IL-22 have shown this cytokine to be involved in the induction of IgE-independent acute-phase response signals.49

**IFN-γ**

IFN-γ is the most important cytokine in cell-mediated immunity, controlling the balance of Th1/Th2 development (see Figure 39-1). IFN-γ is produced by Th1 cells and has an inhibitory effect on Th2 cells. IFN-γ inhibits allergic responses by preventing isotype switching of IgE and IgE production in B cells.50 The main sources of IFN-γ are the Th cells. However, it can also be produced by cytotoxic T cells and natural killer (NK) cells. In addition to its potent inhibitory effect on Th2 cells, IFN-γ stimulates de novo expression of MHC class II molecules on epithelial and endothelial cells and up-regulates their expression on macrophages–monocytes and dendritic cells. Importantly, IFN-γ stimulates monocytes, NK cells, and neutrophils to increase their cytokine production, phagocytosis, adhesion, respiratory burst, and NO production, thereby promoting cell-mediated cytotoxic responses at the site of inflammation.3

In sensitized and allergen-challenged mice, nebulized IFN-γ prevents allergen-induced increases in Th2 cytokine production, AHR, and lung eosinophilia.51 This has been proposed to occur via up-regulation of IL-10. In the BAL fluid of asthmatic patients, there is reduced T-cell production of IFN-γ, and this correlates closely with disease
severity. Clinical trials with IFN-γ in humans have proved disappointing as no significant improvement in lung function was observed in steroid-dependent asthmatic patients, despite reduced number of eosinophils in their blood.

**IL-12**

IL-12 is produced by APCs, including B cells, monocytes, macrophages, Langerhans cells, and dendritic cells, as well as neutrophils and mast cells. IL-12 promotes T-cell differentiation toward a Th1-mediated response by stimulating NK and T cells to produce IFN-γ while suppressing the expansion and differentiation of IL-4–secreting Th2 cells. The biologically active form of IL-12 is a heterodimer consisting of a p40 subunit and a p35 subunit, expressed by different genes. The effects of IL-12 have been extensively studied in small animal models of allergic inflammation, which have consistently demonstrated this cytokine to be involved in reduction of allergen-specific IgE production and abolition of AHR and airway eosinophilia. However, this effect of IL-12 is critically dependent on the timing of its administration. The most effective protection against allergen-induced inflammation is seen when IL-12 is administered early during the active sensitization process and can act in synergy with IL-18.

**CHEMOKINES**

Chemokines are small cytokines (8 to 10 kDa) that are primarily involved in attracting and regulating leukocyte trafficking into the tissues, in a process called chemotaxis, by binding specifically seven membrane-spanning G protein–coupled receptors (Figure 39-4). To date, more than 40 chemokines have been described, and they are classified into four subclasses according to their structure: CXC, CC, C, and CX3C. The two main groups are CXC (α-chemokines) and CC (β-chemokines). CXC chemokines include IL-8 and IFN-induced protein-10 (IP-10), which

![Diagram of Chemokines and Chemotaxis](image-url)

**FIGURE 39-4** Chemokines and chemotaxis. Cytokines and chemokines regulate the entry of inflammatory cells into sites of inflammation by increasing the expression of adhesion proteins on endothelial cells and on the inflammatory cell. This causes the rolling of the inflammatory cell on the vasculature, followed by firm adhesion and transmigration between the endothelial cells and through the matrix to the site of inflammation, following a chemokine gradient.
primarily target neutrophils, whereas eotaxin, RANTES, MCP-1 to MCP-4, MIP-1α, and MIP-1β are typical CC chemokines, targeting monocytes, T cells, and eosinophils (Table 39-2). For this reason, CC chemokines are thought to have the greatest relevance in the pathogenesis of asthma. Increased levels of chemokines in comparison with control subjects have been measured in both BAL fluid and biopsy samples from asthmatic patients.

Eotaxin and RANTES
Eotaxin and RANTES, acting in synergy with IL-5, are the most important eosinophil chemotactic agents in allergic inflammation. These chemokines are produced by the majority of inflammatory cells, and more recently their expression has been described in ASM cells and fibroblasts. Unlike RANTES, which binds many CC chemokine receptors (CCRs), including CCR1, CCR3, and CCR5, eotaxin binds specifically to CCR3, which is highly expressed on eosinophils and has selective chemotactic activity for eosinophils. In addition, eotaxin induces α5β1-integrin expression on eosinophils, allowing for firm adhesion of eosinophils to the endothelium and transmigration into the site of inflammation (see Figure 39-4). More importantly, eotaxin and RANTES are produced at high concentrations in the lungs of asthmatic patients.

MCPs and MIPs
MCP and MIP are monocyte–macrophage chemoattractants and activating factors. To date, four MCPs (MCP-1 to MCP-4) and two MIPs (MIP-1α and MIP-1β) have been described. Increased levels of MCP-1 and MCP-3 have been detected in BAL fluid of asthmatic patients, and increased expression of MCP-4 has been reported in the sputum. BAL fluid and bronchial mucosa, and small airways of asthmatic patients, as well as in the nose of patients with allergic rhinitis. MCP-1 binds CCR2, MCP-2 binds CCR3, MCP-3 binds CCR1 and CCR3, and MCP-4 binds CCR2, CCR3, and CCR5. MCP-1 immunoreactivity has been demonstrated in human eosinophils, whereas MCP-2, MCP-3, and, in particular, MCP-4 are also thought to be potent eosinophil chemoattractants. Furthermore, MCP-4 attracts not only eosinophils and monocytes but also lymphocytes and basophils. MIP-1α binds CCR1 and CCR5, whereas MIP-1β binds CCR5 exclusively.

Although the primary role of chemokines is chemotaxis, they have a variety of other functions. These include direct effects on T-cell differentiation. MIP-1α, MIP-1β, and RANTES can promote the development of IFN-γ-producing Th1 cells by stimulating IL-12 production by APCs. In contrast, MCP-1, MCP-2, MCP-3, and MCP-4 can increase T-cell production of IL-4 and decrease APC production of IL-12, resulting in a Th2 phenotype.

Cytokine and Chemokine Receptors
Cytokines function by binding to specific cytokine receptors, which may be either membrane bound or in soluble form. Cytokine receptors are grouped according to the degree of common structural homology of their extracellular regions. They include the immunoglobulin superfamily (IL-1R, IL-6R, and PDGFR), the cytokine receptor superfamily (IL-2R, IL-3R, IL-4R, IL-5R, IL-12R, and GM-CSFR), and the IFN receptor superfamily (IFN-γR and IL-10R) (Table 39-3). Some of the subunits of these receptors may be shared by more than one cytokine receptor, resulting in the formation of heterodimeric structures. Such examples include IL-3R, IL-5R, and GM-CSFR, which share the common GM-CSF receptor β-chain, and IL-2R, IL-4R, and IL-13R, which share the IL-2 receptor γ-chain. Receptors for IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, and GM-CSF have all been reported to show increased expression in asthma (see Table 39-3).

Some cytokine receptors exist also in a soluble form in the serum, and these are produced by alternative mRNA splicing, leading to proteins that lack the region of the receptor required for membrane anchoring. The soluble cytokine receptor interacts with its target cytokine, reducing the effective levels of cytokine available for target cells. In

Table 39-2 Chemokines Involved in Asthma Pathogenesis

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Expression</th>
<th>Major actions</th>
<th>Cellular source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eotaxin</td>
<td>Increased</td>
<td>Eosinophil chemoattractant</td>
<td>Epithelial cells, T cells, macrophages, eosinophils, endothelial cells</td>
</tr>
<tr>
<td>RANTES</td>
<td>Increased</td>
<td>Lymphocyte and eosinophil chemoattractant</td>
<td>Epithelial cells, T cells, ASM cells</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Increased</td>
<td>Monocyte chemoattractant, promotes Th2 response</td>
<td>Macrophage, monocytes, epithelial cells, eosinophils</td>
</tr>
<tr>
<td>MCP-2</td>
<td>Unknown</td>
<td>Monocyte and eosinophil chemoattractant, promotes Th2 response</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td>MCP-3</td>
<td>Increased</td>
<td>Eosinophil chemoattractant, promotes Th2 response</td>
<td>T lymphocytes, eosinophils</td>
</tr>
<tr>
<td>MCP-4</td>
<td>Increased</td>
<td>Eosinophil, monocyte, lymphocyte, and basophil chemoattractant, promotes Th2 response</td>
<td>Epithelium, macrophages, eosinophils, T cells</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>Increased</td>
<td>Eosinophil and basophil chemoattractant, stimulates IFN-γ production by Th1 cells</td>
<td>T cells, epithelial cells, eosinophils</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>Unknown</td>
<td>Stimulates IFN-γ production by Th1 cells</td>
<td>Monocytes, T cells, eosinophils</td>
</tr>
<tr>
<td>IL-8</td>
<td>Increased</td>
<td>Neutrophil chemoattractant, recruits primed eosinophils</td>
<td>Macrophages, eosinophils, epithelial cells, T cells, neutrophils, and fibroblasts</td>
</tr>
<tr>
<td>IP-10</td>
<td>Increased</td>
<td>Neutrophil chemoattractant</td>
<td>T cells, epithelial cells</td>
</tr>
</tbody>
</table>

ASM = airway smooth muscle; IFN-γ = interferon-γ; IL = interleukin; IP-10 = IFN-induced protein-10; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein.
Table 39-3  Cytokine and Chemokine Receptors in Asthma Pathogenesis

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Form</th>
<th>Expression</th>
<th>Cellular source</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1R</td>
<td>Soluble</td>
<td>Increased</td>
<td>Most inflammatory and structural cells</td>
</tr>
<tr>
<td>IL-2R</td>
<td>MB</td>
<td>Increased</td>
<td>CD4+ T cells</td>
</tr>
<tr>
<td>IL-3R</td>
<td>MB</td>
<td>Increased</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td>IL-4R</td>
<td>Soluble and MB</td>
<td>Increased</td>
<td>Epithelium, T and B cells</td>
</tr>
<tr>
<td>IL-5R</td>
<td>Soluble and MB</td>
<td>Increased</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>IL-6R</td>
<td>Soluble</td>
<td>Increased</td>
<td>Macrophages, T and B cells, epithelial cells</td>
</tr>
<tr>
<td>IL-10R</td>
<td>MB</td>
<td>Decreased</td>
<td>Lymphoid and myeloid cells</td>
</tr>
<tr>
<td>IL-12R</td>
<td>MB</td>
<td>Decreased</td>
<td>T cells and NK cells</td>
</tr>
<tr>
<td>IL-13R</td>
<td>Soluble and MB</td>
<td>Decreased</td>
<td>Unknown</td>
</tr>
<tr>
<td>IFN-γR</td>
<td>MB</td>
<td>Decreased</td>
<td>T and B cells, macrophages, dendritic cells</td>
</tr>
<tr>
<td>GM-CSFR</td>
<td>MB</td>
<td>Increased</td>
<td>Macrophages, eosinophils, fibroblasts</td>
</tr>
<tr>
<td>PDGF-R</td>
<td>MB</td>
<td>Increased</td>
<td>Fibroblasts and ASM cells</td>
</tr>
<tr>
<td>TGF-αR</td>
<td>Soluble and MB</td>
<td>Increased</td>
<td>Macrophages, T cells, epithelial cells, mast cells</td>
</tr>
<tr>
<td>CCR3</td>
<td>MB</td>
<td>Increased</td>
<td>Eosinophils, basophils, Th2 cells, mast cells</td>
</tr>
<tr>
<td>CCR4</td>
<td>MB</td>
<td>Increased</td>
<td>Th2 cells, eosinophils, mast cells, monocytes</td>
</tr>
<tr>
<td>CCR8</td>
<td>MB</td>
<td>Increased</td>
<td>Eosinophils, Th2 cells, and mast cells</td>
</tr>
</tbody>
</table>

ASM = airway smooth muscle; CCR = chemokine receptor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN-γ = interferon-γ; IL = interleukin; MB = membrane-bound; NK = natural killer; PDGF = platelet-derived growth factor; TNF-α = tumor necrosis factor-α.

murine experimental models of asthma, soluble IL-4R, which binds and sequesters free IL-4, reduces allergen-induced IgE production, AHR, and eosinophil accumulation.66 We have previously demonstrated that in patients with atopic and intrinsic asthma, there are not only increased levels of membrane-bound IL-5R mRNA but also increased soluble IL-5R mRNA levels in bronchial biopsy specimens and that this expression is directly correlated with FEV1.67 These results suggest that the soluble form of IL-5R may play an important role in the development of airflow obstruction. Studies such as these prompt interest in the potential use of receptors as therapeutic targets.

The chemokine receptors are a large family of G protein-coupled receptors. These have generated considerable interest because of the possibility of using receptor antagonists to block eosinophil trafficking and degranulation in asthma. CCR3, CCR4, and CCR8 are preferentially expressed by Th2 cells, mast cells, and eosinophils and therefore represent therapeutic targets for allergy and asthma. Of these, CCR3 has received the most attention. CCR3 not only binds eotaxin, RANTES, MCP-3, and MCP-4, but it is also considered to be the major CC chemokine receptor on eosinophils and basophils. Increased CCR3 expression has been found in bronchial biopsy specimens of asthmatic patients,68 and monoclonal antibody selective for CCR3 inhibits eosinophilia.69 CCR3 receptor is involved in eosinophil differentiation and is up-regulated by Th2 cytokines in CD34+ progenitor cells.68 For these reasons, the most recent research has been focused on identifying CCR3 antagonists that are capable of preventing allergic inflammation.70

**CYTOKINES, ASTHMA, AND INFECTION**

The reasons for the increased prevalence of allergic respiratory diseases in developed countries remain unclear. For many years, lower respiratory tract infections in early life have been recognized as primary triggers of asthma exacerbations in young children. Using both epidemiologic and virology data, prospective studies have convincingly shown that viral, not bacterial, respiratory infections precipitate reactive airway symptoms.72 It is now believed that the development of bacteria-induced, nonwheezing lower respiratory tract infection in childhood may protect against the development of atopy and asthma in later life. This line of thought comes from experimental evidence suggesting that the principal trigger for normal postnatal maturation of the immune system is the commensal microbial flora, particularly that of the gastrointestinal tract. Microbial exposure helps skew the immune response away from the allergic phenotype and toward the normal adult nonatopic immune response,73 and the longer the immune system takes to adapt postnatally to its functionally mature state, the greater the risk of allergic sensitization.74

**HYGIENE HYPOTHESIS**

The hygiene hypothesis suggests that decreasing levels of exposure to infections and/or commensal microbial stimuli in developed countries, particularly during the induction of primary Th1/Th2 responses to allergens in early life, may be responsible for the increased prevalence of asthma (Figure 39-5). There is ample epidemiologic evidence to support this hypothesis.75 The reported increase in atopy inversely correlates with a steady decline in the extent to which Western society is exposed to infectious diseases such as whooping cough, measles, tuberculosis, and influenza.76 The incidence of allergic diseases appears to increase with advancing socioeconomic development, they occur more frequently in industrialized countries than in developing areas,77 and the farming environment is thought to be protective against the development of allergies.78,79

Bacterial lipopolysaccharide (LPS), or endotoxin, has been suggested as a potential mediator of these effects. LPS is a major component of the outer membrane of ubiquitous gram-negative bacteria. It has been reported that LPS makes up a significant proportion of the weight of common house dust, and a significant correlation has been reported
between domestic LPS exposure and clinical severity of asthma in adults.\textsuperscript{80} The first direct in vivo evidence that environmental exposure to LPS early in life (before polarized Th cell responses are established) protected against allergen sensitization was reported by Gereda and colleagues, who demonstrated that the homes of allergen-sensitive infants (9 to 24 months of age) contained lower concentrations of LPS in house dust than those of nonsensitive infants, and the lower concentrations were associated with reduced proportions of IFN-γ–producing Th cells.\textsuperscript{81} In line with this hypothesis, three distinct LPS phenotypes in humans have been described (namely, sensitive, intermediate, and hyporesponsive), based on reduction in FEV\(_1\) following inhalation of increasing doses of LPS and in vitro production of IL-6 and IL-8 by peripheral blood monocytes and alveolar macrophages.\textsuperscript{82}

Experimental results in animal models of asthma have supported the hygiene hypothesis. They have shown that treatment with microbes (eg, BCG\textsuperscript{83} and Lactobacillus\textsuperscript{84}) or microbial products (LPS\textsuperscript{47,85} and CpG DNA\textsuperscript{86,87}) inhibits allergic sensitization, cosinophilic inflammation, and AHR in these animals. In a similar animal model of allergic disease, we previously showed that the timing of exposure and the dose were critical to the in vivo effect of LPS.\textsuperscript{85} That study demonstrated that whereas LPS exposure during early sensitization protects against increased production of IgE and consequent allergic inflammation, in marked contrast, LPS exposure after allergen challenge further exacerbates the allergic response as demonstrated by increased inflammation and reduced lung function.

**TLR4**

Toll is a receptor in *Drosophila* involved in antifungal immune responses. Toll-like receptors (TLRs) are a large family of evolutionarily conserved receptors originating from Toll, which sense invasion by microorganisms through the recognition of specific pathogen-associated molecule patterns and produce immediate innate responses (Figure 39-6). To date, 10 TLRs have been identified in humans and mice (TLR1 to TLR10).\textsuperscript{88} TLRs are single transmembrane domain receptors that have a cytoplasmic signaling portion homologous to IL-1R. Although the TLRs differ in their extracellular domain structure, similar cytoplasmic domains allow TLRs to use the same signaling molecules. All TLRs signal through an adaptor protein named myeloid differentiation factor 88 (MyD88) (see Figure 39-6). Following activation, MyD88 recruits the IL-1R/IL-1R–associated protein kinase (IRA) complex to the TLR; IRAK becomes

![Hygiene hypothesis](image-url)
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phosphorylated and is then able to activate the TNF receptor–associated factor 6 (TRAF6). This process leads to activation of c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK), and nuclear factor kappa B (NFκB) pathways, leading to a cascade of events, including the release of cytokines and activation of APCs.

Different TLRs recognize different ligands. TLR3 is a cell surface receptor for double-stranded RNA and hence may be implicated in viral recognition. TLR5 is specific for bacterial flagellin, whereas TLR9 is a receptor for unmethylated CpG motifs, which are abundant in bacterial DNA. In mammals, TLR4 is the principal receptor responsible for LPS-induced signal transduction. Recognition of LPS by TLR4 is aided by two accessory proteins: CD14 and MD-2 (see Figure 39-6). TLR4 is expressed at particularly high levels by cells of the innate immune system, such as monocytes, dendritic cells, macrophages, and endothelial cells. TLR4 expression is thought to be related to LPS sensitivity and it was recently demonstrated in murine macrophages that TLR expression and function decline with age.

More recently, we have shown the ability of bacterial LPS to prevent local allergen-induced allergic inflammation in the nasal mucosa of atopic children. This occurs through down-regulation of local Th2 cytokines and up-regulation of Th1 cytokines in the tissue proliferation and activation of TLR4+IL-10+ and CD25+ Th cells, and increased expression of the antiinflammatory cytokine IL-10. These events occur locally without systemic recruitment of inflammatory cells and are orchestrated through the TLR4-dependent pathway. TLR4 is an important bridge between innate and adaptive immunity, potentially driving the molecular mechanisms governing the hygiene hypothesis and helping to explain why reduced exposure to bacterial products may lead to delayed or skewed development of the immune system and more atopic disease.

CONCLUSIONS

Bronchial asthma is a complex, chronic disease of the airways that is characterized by reversible AHR, airway remodeling, and inflammation. Furthermore, these pathologic and physiologic changes occur even in patients with mild asthma and can be detected in asthmatic children. In the last decade, one of the most striking advances in the study of asthma has been the recognition that cytokines and chemokines play integral roles in orchestrating, perpetuating and amplifying the underlying processes in this disease. Future therapy for asthma may involve specific targeting of cytokine and chemokine receptors rather than global immunosuppression. Additionally, it has become clearer that bacterial products play a role in the maturation of early immune responses and can modulate allergic inflammation in young children. Only by understanding the principal regulatory mechanisms involved in asthma can we begin to provide a rational basis for novel drug design and make progress in identifying those individuals at risk for its development.
REFERENCES


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