

Review Article

Advances in the pathophysiology of bronchial asthma

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ABSTRACT

Asthma is a complex disorder associated with the activation of T lymphocytes and with eosinophil infiltration within the airways. A substantial amount of current research involves the interaction among inflammatory cells as a result of the production of a wide array of T helper (h) 2 cytokines. Recent advances in the pathophysiologic mechanisms of asthma point to the importance of transcription factors of cytokines that underlie the development of Th2-type responses. The study of transcription factors has begun to reveal mechanisms of dysregulated gene regulation in asthmatic diathesis.

Key words: apoptosis, bronchial asthma, eosinophils, Fas ligand, GATA-3, inflammation, STAT6, T cells, Th2 cytokines.

INTRODUCTION

Bronchial asthma is a chronic inflammatory disease of the airways that is characterized clinically by paroxysmal airway constriction and increased responsiveness of the airways to inhaled bronchoconstrictor stimuli.¹ Earliest studies from post-mortem cases of asthmatic individuals who had died from status asthmatics^{2,3} revealed occlusive mucus plugs within the bronchi, tissue eosinophilia, extensive epithelial desquamation, hypertrophy/hyperplasia of the airway smooth muscle and a thickening of the reticular basement membrane. The introduction of flexible fiberoptic bronchoscopy has made it possible to elucidate the pathology of the airways of asymptomatic or even mild asthmatic subjects. These studies on bronchial biopsies have revealed that shedding of the

epithelial cell layer, inflammatory cell infiltration and the deposition of collagen along the reticular basement membrane are observed even in the most mildly affected individuals.^{4–6}

In addition, through the development of immunocytochemical markers for inflammatory cells, it has been possible to examine their contribution to the pathophysiology of asthma. While substantial attention has been directed towards eosinophil and T cells, interactions between multiple cell types, including basophils, epithelial cells and B lymphocytes, are proving to add further complexity to asthma pathogenesis. A substantial amount of current research involves the interaction among inflammatory cells by cytokines. In particular, a subgroup of cytokines that exhibit chemotactic activity for various blood cells have been implicated in the recruitment of inflammatory cell types to the airways. These are categorized as chemokines and these chemokines coordinate the presence of airway inflammation and, thus, may prove to be critical in the development of asthma. Furthermore, studies into several specific transcription factors of cytokines has begun to reveal mechanisms of dysregulated gene regulation in asthmatic diathesis, providing a new approach for asthma therapy. This paper will focus on recent advances in the cellular and immunologic mechanisms that have been identified as contributing to asthma diathesis.

T LYMPHOCYTES

Lymphocytes amplify and coordinate adaptive immune responses against foreign antigens. These responses are usually self-limiting, without harmful damage to the host. However, the presence of lymphocytes within the lungs of asthmatic individuals appears to promote inappropriate inflammatory cell infiltration and antibody secretion. An increased number of lymphocytes, as determined by morphologic criteria, is a frequent finding within the bronchial mucosa of patients with all forms of asthma,

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from newly diagnosed to severe.^{2,7} These lymphocytes appear activated, showing irregular, atypical morphology⁷ and surface expression of CD25.⁸ These findings suggest the fundamental role of lymphocytes in the pathogenesis of asthma. The advent of techniques involving monoclonal antibodies (mAbs) and the detection of mRNA have been valuable in revealing the precise mechanisms by which these cells operate in the pathophysiology of asthma.

To date, T lymphocytes have been broadly categorized according to their cell surface markers, into two distinct subsets corresponding to particular effector functions. T cells expressing the CD4 antigen are referred to as T helper (Th) cells, whereas those expressing the CD8 antigen are termed T cytotoxic/suppressor cells (TC/S). These CD8⁺ cells orchestrate the cell-mediated response and interact with endogenously processed antigen presented in conjunction with major histocompatibility complex (MHC) class I. In contrast, CD4⁺ lymphocytes are capable of recognizing foreign antigens processed in association with MHC class II on the surface of professional antigen-presenting cells, such as dendritic cells. Therefore, these CD4⁺ cells have attracted considerable attention in the pathogenesis of asthma because of their ability to drive antigen-specific inflammatory responses and regulate immunoglobulin production. They modulate these functions through the secretion of specific cytokines and chemokines.

Although T lymphocytes appear critical in the development and maintenance of chronic allergic inflammation, there is no consistent trend for a selective accumulation of either CD4⁺ or CD8⁺ cells in atopic asthma. Some studies report either no increase in either CD4⁺ or CD8⁺ cells within the airway mucosa,⁹ a trend towards an increase in the number of CD4⁺ or CD8⁺ cells⁸ or a selective increase in CD8⁺ cells.¹⁰ In non-atopic (intrinsic) asthma, CD4⁺ cell numbers were reported to be elevated within the lungs.¹¹ Those lymphocytes within the airways are shown to be activated, as determined by their expression of the interleukin (IL)-2 receptor (CD25).^{8,10} Whether this activated state is observed for both CD4⁺ and CD8⁺ cells¹² or whether it is restricted to CD3⁺ cells in general¹³ remains to be clarified. Furthermore, the activation of these cell types has been shown to be different in atopic and non-atopic asthma.¹⁴ These findings suggest that the difference in the type and severity of asthma may account for such disparity in the activation status of lymphocytes.

Activation of CD4⁺ cells has been shown to be present among inflammatory infiltrates at baseline asthma, after

antigen challenge and in acute exacerbation of asthma. The CD4⁺ cells in bronchoalveolar lavage fluid (BALF) of asthmatics became more activated following antigen challenge.¹⁵ However, allergen challenge is not associated with an increase in the numbers of CD3⁺ or CD4⁺ cells in the bronchial mucosa of asthmatics.¹⁶ In BALF, in the early phase after allergen challenge, there may be a loss of cells expressing the CD4 and CD8 cell marker, suggesting that these cells are selectively retained in the bronchial mucosa.¹⁷ Studies using ovalbumin (OVA)-sensitized Brown Norway rats have shown that administration of antigen (Ag)-primed CD8⁺ T cells suppresses the late allergic reaction (LAR) after antigen challenge in contrast with CD4⁺ cells.¹⁸ Thus, it has been suggested that CD4⁺ lymphocytes promote the development of a LAR, whereas CD8⁺ cells prevent this reaction. However, a number of reports have shown the potential of CD8⁺ cells to promote airway inflammation in asthma. The CD8⁺ cells in the bronchial mucosa and BAL-derived T cell clones are capable of producing cytokines implicated in the pathophysiologic features of asthma, such as IL-4, IL-5 and granulocyte-macrophage colony stimulating factor (GM-CSF).^{19,20} Moreover, it has been suggested that CD8⁺ T cells may precipitate a worsening of asthma by mediating cellular defense mechanisms against acute viral infections, including cytotoxic action on epithelial cells and the activation of macrophages. Whatever their actions, CD8⁺ T cells comprise the minority of CD3⁺ lymphocytes found within the bronchial mucosa and, thus, it is likely that they provide only a modulatory influence over CD4⁺-driven inflammation. Indeed, while both CD4 and CD8 T lymphocytes in BALF from asthmatic subjects expressed CD25, only the numbers of activated CD4⁺ cells correlated with numbers of bronchoalveolar lavage (BAL) eosinophils and disease severity.¹²

$\gamma\delta$ T CELLS

The T cell receptor consists of a disulfide-linked heterodimer that, in the majority of T cells, is made up of α and β chains. An alternative receptor consisting of γ and δ chains is expressed on a subset of T cells. These $\gamma\delta$ T cells are only rarely found within bronchial biopsy specimens from asthmatic and normal subjects²¹ and the role of T cells expressing $\gamma\delta$ in asthma is unclear. However, it has been shown that the proportion of $\gamma\delta$ T lymphocytes is higher in BALF from asthmatic patients than controls and that these cells appeared to be allergen

specific.²² The finding that the number of circulating $\gamma\delta$ cells is reduced in asthmatic subjects²³ suggests preferential recruitment from the blood stream to the lung. Using gene-disrupted mice, $\gamma\delta$ T cells have been shown to be essential for initial IL-4 production, IL-4-dependent IgE and IgG1 responses and for Th2-mediated airway inflammation to peptide antigens.²⁴ By releasing IL-4, these $\gamma\delta$ T cells may actively participate in the initiation of Th2 immune responses.

EOSINOPHILS

Elevated numbers of eosinophils in the airways is a consistent feature of asthma that has been recognized for many years for both atopic and intrinsic asthma.^{5,10,25–27} Post-mortem studies have revealed massive infiltration of eosinophils around the bronchi in patients who died of asthma.^{2,28–30} Within membrane-bound granules, eosinophils store cytotoxic mediators, such as major basic protein (MBP), eosinophilic cationic protein (MCP), eosinophil peroxidase (MPO) and eosinophil-derived neurotoxin (EDN). These products have consistently been found in bronchiolar washings from both symptomatic and asymptomatic asthmatic subjects.^{10,27,31,32} In animals, MBP has been shown to provoke increased sensitivity to methacholine³³ and inhaled antigen,³⁴ possibly via damage to the epithelium. Major basic protein may also act as an allosteric inhibitor of muscarinic M_2 receptors, resulting in enhanced vagally mediated bronchoconstriction.³⁵ Thus, once within the airways, eosinophils are believed to play an important role in the pathophysiology of asthma through the release of these mediators. Eosinophils also have the capacity to express a large variety of cytokines and chemokines including IL-1, -2, -3, -5, -6, -10 and -12 and transforming growth factor (TGF)- β , tumor necrosis factor (TNF)- α , GM-CSF, macrophage inflammatory protein (MIP)-1 α and RANTES. In terms of the host defense, these cells are now recognized as not only contributing to the physical damage sustained by the invading pathogen, but also to the immunoregulatory mechanisms in action.

What is interesting from a clinical viewpoint is the relationship between eosinophils within the lungs and changes in allergen-induced lung function. The increase in the number of eosinophils in BALF has been shown to accompany allergen-induced early and late airway responses.^{31,36–38} However, it is widely recognized that the presence of eosinophils within the airways 24 h after allergen challenge does not discriminate between single

and dual responders. Nevertheless, their presence within the airways 3–4 h after challenge is associated with the development of the LAR and the subsequent increase in airway responsiveness.^{37,38} This seems to suggest that it is not eosinophils already present in the tissue that are critical to the LAR, but rather their acute appearance within the airways.

It is currently accepted that allergen-induced eosinophil accumulation within lungs of asthmatics is attributed to mature eosinophil migration from the circulation. Eosinophils and basophils develop from CD34⁺ pluripotent progenitor cells, which proliferate and differentiate within the bone marrow in response to IL-3, IL-5 and GM-CSF. For eosinophils, the terminal differentiation of committed precursors normally occurs in the bone marrow under the influence of IL-5. However, almost 15 years ago, a population of eosinophil–basophil progenitors was found within the circulation and the levels of these cells were increased in atopic individuals.³⁹ The number of these eosinophil–basophil progenitors was later shown to increase during exacerbation of asthma⁴⁰ and after allergen exposure.⁴¹ More recently, it has been demonstrated that CD34⁺ hematopoietic cells, the earliest identified precursor cell of eosinophils and basophils, circulate in increased numbers in atopic subjects.⁴² These CD34⁺ cells bear the IL-5 receptor and display an increased responsiveness to IL-5, suggesting that they are primed towards the development of eosinophils.⁴³

Not only are there increased numbers of circulating eosinophil–basophil progenitors in atopic asthmatic individuals, but their production within the bone marrow can also be upregulated in response to allergen inhalation.⁴⁴ Moreover, it has been observed that there are increased numbers of CD34⁺ cells as well as mature and immature eosinophils in the bone marrow of atopic individuals, regardless of asthmatic status.⁴² Interestingly, cells within the bone marrow of dual responders differ from those of single responders by their increased sensitivity to IL-5, indicating they are already primed for eosinophil–basophil differentiation. Indeed, there is an increase in the proportion of CD34⁺ cells expressing the α subunit of the IL-5 receptor on their surface.⁴³

Correspondingly, several studies have shown the involvement of these CD34⁺ cells in asthmatic airways. Increased numbers of CD34⁺ cells within the airways have been found in both asthmatic and non-asthmatic individuals, denoting the presence of tissue progenitor cells as a hallmark of atopy.⁴⁵ However, the numbers of CD34⁺ cells expressing α IL-5 receptor mRNA were

increased only in asthmatic individuals, suggesting that local differentiation takes place in the mucosa. An *ex vivo* challenge study in nasal mucosal tissue has shown the possibility of local differentiation eosinophils *in situ*.⁴⁶ Whether or not the same mechanism plays a role in the lungs of asthmatic subjects remains to be elucidated. Once the concept of local differentiation is addressed, it may then be possible to determine whether these cells are actually proliferating *in situ*. It is possible that these cells are the source of the actively dividing, presumably hematopoietic, cells that appear in BALF within 24 h of allergen challenge.⁴⁴

DISREGULATED CELL DEATH MAY CONTRIBUTE TO THE PERSISTENCE OF ALLERGIC INFLAMMATION

The above discussion focused on the recruitment and activation of inflammatory cells in the pathophysiology of asthma. However, defects in the elimination of these inflammatory cells are believed to facilitate the persistence of chronic allergic inflammation. Indeed, there is increasing evidence that programmed cell death of certain inflammatory cells is impaired in asthmatic subjects. A decreased number of apoptotic eosinophils is a feature of asthmatic airways^{47,48} and peripheral blood T cells of asthmatic subjects fail to undergo a normal degree of apoptosis.⁴⁹

Among the molecules that are associated with apoptosis, Bcl-2 is well known as the anti-apoptotic molecule and the Fas–Fas ligand system is known to promote apoptosis. Under normal conditions, activated T cells express the Fas antigen, which elicits activation-induced apoptosis and limits the consequences of a persistent immune response.

In asthmatic airways, the number of Bcl-2⁺ cells was higher than in normal subjects and correlated with the number of T lymphocytes and the severity of asthma,⁴⁷ suggesting the involvement of Bcl-2 in allergic inflammation. However, it is controversial whether or not the Fas receptor is upregulated in asthmatic inflammation. Krug *et al.* have shown that, in asthmatic and control subjects, almost all T cells in the BALF expressed Fas antigen without changes after saline or allergen challenge and the number of Fas ligand-expressing T cells is small before allergen challenge.⁵⁰ Interestingly, after allergen challenge, almost all T cells found within BALF of asthmatic patients express the Fas ligand.⁵⁰ Similarly, a small population of eosinophils in the bronchial mucosa express

Fas ligand,⁵¹ whereas eosinophils in the peripheral blood fail to express this molecule.^{51,52} This suggests that the Fas–Fas ligand system may be activated in inflammatory cells as a consequence of local activation. Because the increase of Fas ligand in T cells does not correspond to the increase in the numbers of apoptotic lymphocytes, these findings were interpreted as suggesting that this upregulation reflects antigen-induced T cell activation rather than cell death. On the contrary, Spinozzi *et al.* have reported that T cells in BALF from asthmatic subjects do not express surface Fas receptors nor do they contain Fas mRNA.⁵³ Interestingly, Th2-type cytokines, such as IL-4, IL-5 and GM-CSF, have a dose-dependent and specific inhibitory effect on Fas mRNA of T cells from asthmatic subjects.⁵⁴ Further study is needed to elucidate the mechanism and role of apoptosis in the pathogenesis of asthma.

CYTOKINE EXPRESSION IN ASTHMA

Cytokines are small, usually glycosylated proteins that mediate communication between cells, facilitating various functions such as chemoattraction, proliferation and differentiation of cells and immunoglobulin isotype switching. Recently, bronchial asthma has been recognized as a chronic inflammatory disease of the airways and cytokines have been shown to play an important role in the regulation of the inflammatory process (Fig. 1). The earliest reports showed the preferential expression of Th2-type cytokines in asthmatic airways. The number of cells expressing mRNA for IL-2, IL-3, IL-4, IL-5 and GM-CSF increased in asthmatic airways,^{55,56} whereas no increase in the number of interferon (IFN)- γ mRNA-positive cells was observed. The specific cytokines studied to date in asthma, with their actions and cellular sources, are summarized elsewhere.⁵⁷

The CD4⁺ Th cells have been further subdivided into distinct phenotypes based on their profile of cytokine production: Th1 and Th2.⁵⁸ The Th2-type cells produce IL-4 and IL-5 and have been shown to differentiate from naïve Th0 cells under the influence of IL-4. Conversely, Th1-type cells express IFN- γ and IL-2 and are induced from Th0-type cells by the presence of IL-12. Thus, our current understanding is that the cytokines present at the time of antigen exposure are one of the major determinants directing Th cells towards either a Th1- or Th2-type response. However, the cellular sources of IL-4 and IL-12 that induce the polarization of T cell cytokine production remain unclear. The Th1-type cytokines are important

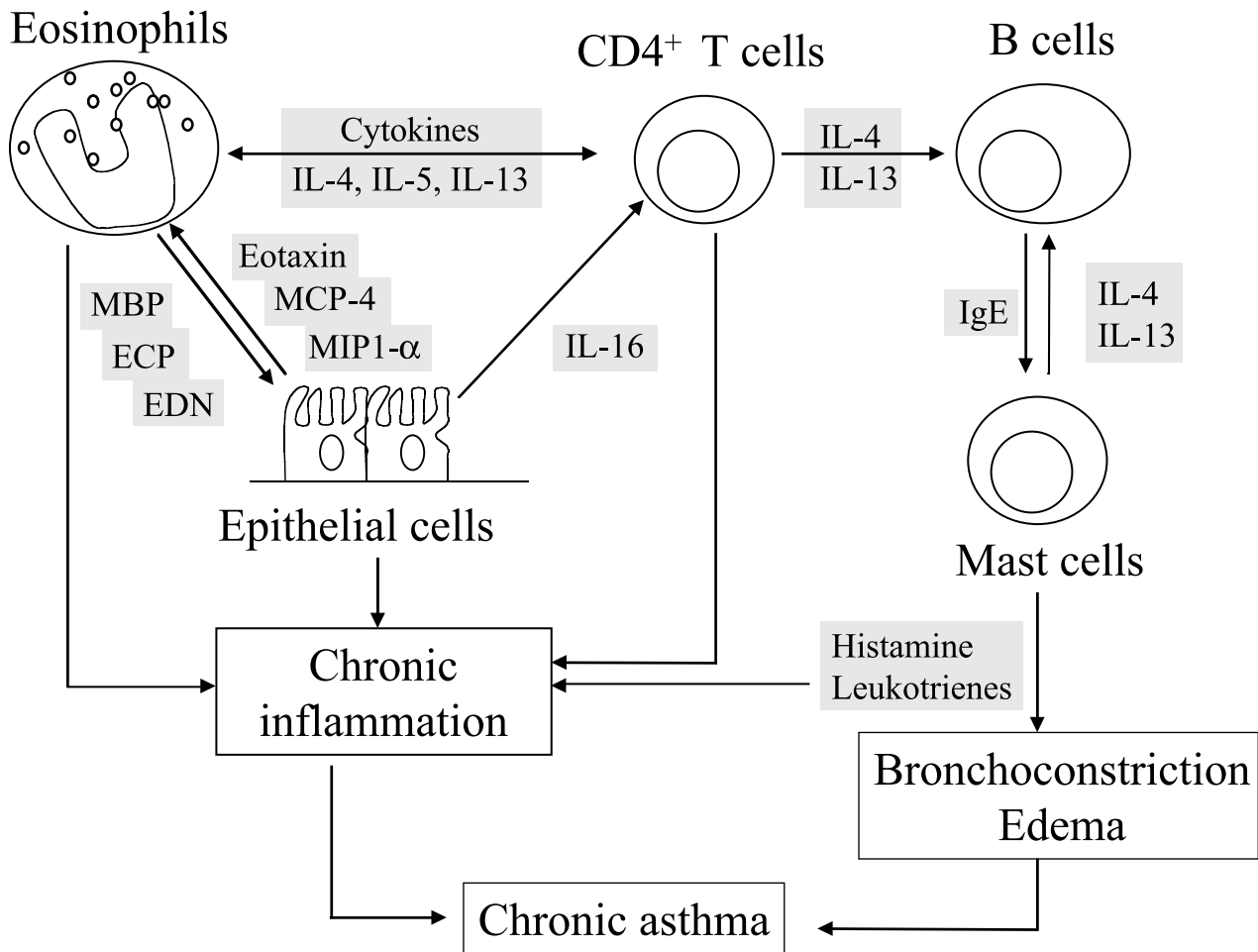


Fig. 1 Role of the cytokine and chemokine network in the pathogenesis of chronic asthma. MBP, major basic protein; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; MCP, eosinophilic cationic protein; MIP1- α , macrophage inflammatory protein-1 α ; IL, interleukin.

regulators of cell-mediated immune responses, whereas Th2-type cytokines mediate predominantly humoral and eosinophilic responses. Alterations of the ratio of Th1 to Th2 responses are important determinants of susceptibility to viral and parasitic infections, allergies, antitumor responses and autoimmunity. Observations of cytokine expression in asthmatic airways are compatible with a dominant Th2 response and, interestingly, both atopic and non-atopic asthmatic subjects have a common immunopathologic feature in terms of the increase in Th2-type cytokines and IgE-positive cells.^{59,60}

The Th2-type cytokines even increased after allergen challenge within the asthmatic airways.^{15,16} Moreover, treatment of asthmatic patients with steroids results in a decrease in the numbers of IL-4 and IL-5 mRNA-positive cells and an increase in the numbers of IFN- γ

mRNA-positive cells.⁶¹ Since the original observations of Th2-type cytokines in asthma, there has been a tremendous expansion in the numbers of cytokines characterized and there seem to be several crucial cytokines in the pathogenesis of bronchial asthma.

Within asthmatic airways, IL-4 is presumed to be critical for the development of Th2-type cells and inhibits the expression of the IL-12 receptor β_2 subunit on T cells.⁶² This cytokine is also critical in the switching of B cells to favor IgE production.⁶³ Interleukin-4 has also been shown to promote goblet cell metaplasia, mucus hypersecretion and upregulation of vascular cell adhesion molecule (VCAM)-1 expression in pulmonary endothelial cells, resulting in the recruitment of eosinophils. Furthermore, IL-4 can promote the growth of eosinophils and basophils.⁶⁴ All these observations suggest the importance

of this cytokine in eosinophilic inflammation in asthmatic airways. In fact, inhalation of IL-4 causes the development of sputum eosinophilia and an increased responsiveness of the airways.⁶⁵ The major cellular sources of IL-4 mRNA within airways of both atopic and non-atopic asthmatic subjects are CD4⁺ cells^{56,66} and, to a lesser extent, CD8⁺ cells, eosinophils and mast cells.

Interleukin-5, as well as IL-3 and GM-CSF, is critical in the production of eosinophils within the bone marrow.⁶⁷ In addition to its hematopoietic properties, IL-5 is a weak eosinophil chemoattractant and will prime these cells for recruitment by other chemotactic agents, such as eotaxin.⁶⁸ Interleukin-5 mRNA expression is closely related to lung function in atopic asthmatics.⁶⁹ Remarkably, the numbers of IL-5 receptor α mRNA-positive cells have been shown to be associated with the resident eosinophil infiltrate.^{55,61} Furthermore, the alternative splicing of α IL-5 receptor mRNA transcripts results in two isoforms (membrane bound and soluble form) and the differential regulation of these two isoforms may influence the eosinophil response and the accompanying changes in lung function.⁷⁰ Explanted airways provide a useful means of examining tissue reactions in the absence of inflammatory cell recruitment. Using this technique, it has been shown that allergen challenge results in an increase in the number of IL-5 mRNA-positive cells, suggesting that at least some of the cytokine production is from resident inflammatory cells.⁷¹ The release of IL-5 from T cells following allergen challenge in mice has recently been postulated to induce maturation, proliferation and, possibly, release of eosinophil precursors from the bone marrow, so inducing a systemic amplification of the immune response.⁷²

Although activated CD4⁺ cells represent the major subset of CD3⁺ cells within the asthmatic airways, CD8⁺ cells can also secrete IL-4 and IL-5.^{73,74} Resting peripheral blood CD8⁺ cells from atopic asthmatic patients can secrete increased levels of IL-4 compared with those from non-atopic control subjects.⁷⁵ The CD8⁺ cells expressing IL-4 and IL-5 mRNA have also been identified in bronchial biopsies from both atopic and non-atopic asthmatics.²⁰ These CD8⁺ Th2-type cells can contribute to the regulation of other T cells, provide B cell help and influence the production of immunoglobulins.

Interleukin-9 stimulates the proliferation of activated T cells, enhances the production of IgE by B cells and promotes the proliferation and differentiation of both mast cells and hematopoietic progenitor cells. The major cellular source of this cytokine is Th2-type T cells. The locus of

the IL-9 gene has been revealed to be associated with airway hyperresponsiveness and elevated levels of serum IgE levels;^{76,77} thus, IL-9 is presumed as being a predisposing factor towards the development of asthma. We have recently demonstrated that IL-9 is increased in asthma compared with chronic bronchitis and controls and have shown that T cells are the major sites of production.⁷⁸

Interleukin-12 stimulates the growth and activation of T cells, natural killer cells and their production of IFN- γ . Interleukin-12 is secreted by dendritic cells, B cells and macrophages. The principal actions of IL-12 in atopic disorders are to inhibit IgE synthesis and to promote the differentiation of Th1-type cells.⁷⁹ In asthmatic airways, the expression of IL-12 mRNA is decreased, but can be increased after treatment with steroids.⁸⁰ The β_2 subunit of the IL-12 receptor is a cell surface marker of Th1-type cells and this is remarkably reduced in the lungs of patients with asthma.^{81,82}

Interleukin-13 is similar to IL-4 in many ways, sharing the IL-4 receptor α subunit for its high-affinity receptor formation. Interleukin-13 downregulates the transcription of IFN- γ and IL-12 and, so, may modulate the cytokine environment at the time of antigen presentation. Interleukin-13, as well as IL-4, induces IgE production and the expression of VCAM-1 on endothelial cells and activates eosinophils by inducing the expression of CD69. Interleukin-13 has been shown to have a close association with the pathophysiology of asthma. The expression of IL-13 mRNA has been reported to increase in atopic and non-atopic asthma^{80,83} and IL-13 mRNA-positive and IL-13-immunoreactive cells are increased 24 h after segmental allergen challenge.^{84,85} Wills-Karp *et al.*⁸⁶ showed that administration of IL-13 was sufficient to induce airway hyperresponsiveness (AHR) and administration of soluble IL-13 receptor α_2 completely reversed IL-13-mediated AHR in A/J mice. Moreover, both the mRNA and protein for the α -specific subunit of the IL-4 receptor increased in atopic asthmatic patients compared with atopic non-asthmatic individuals.⁸⁷ However, the IL-13 receptor α_1 , IL-13 receptor α_2 , IL-4 receptor α and γ chain are proposed to form four types of IL-13 receptor complexes⁸⁸ and the combination of IL-13 receptor α_1 and IL-4 receptor α forms a high-affinity IL-13 receptor complex. The expression and role of these receptor complexes in allergic diseases remains to be determined.

Interleukin-16 acts through the CD4 receptor to induce cellular migration and growth and, therefore, is a chemoattractant specific for CD4⁺ cells. This cytokine

also induces CD25 and MHC class II expression on monocytes, suggesting the immunomodulatory role of this cytokine. In asthmatic subjects, the level of IL-16 has been shown to be increased within BALF after specific allergen challenge,⁸⁹ possibly the consequence of histamine release from mast cells.⁹⁰ The expression of this cytokine is increased, even in stable asthmatics, and is localized primarily in the epithelium.⁹¹ In non-epithelial inflammatory cells, immunohistochemical studies have shown that the majority of IL-16 immunoreactivity is colocalized with CD3⁺ T cells and MBP⁺ eosinophils, with tryptase-positive mast cells making a minor contribution.⁹²

CHEMOKINE EXPRESSION IN ASTHMA

Chemokines are small 8–10 kDa proteins that facilitate the movement of inflammatory cells. They are subdivided into four families on the basis of the relative position of their cysteine residues: CXC, CC, C, and CX3C chemokine families. The CC chemokines, such as RANTES, eotaxin-1, eotaxin-2, eotaxin-3 and monocyte chemoattractant protein (MCP)-2, MCP-3 and MCP-4, are the most potent chemokines for the recruitment of eosinophils, T cells and monocytes. Stimulated T cell chemoattractant protein-1 is a newly identified CC chemokine with chemoattractant activity for Th2-type cells.⁹³ The other CC chemokines, such as exodus-1, exodus-2, exodus-3, alternative macrophage activation-associated CC chemokine-1 and leukotactin-1, await their roles to be elucidated.

Eotaxin is the chemoattractant specific for eosinophils that was originally found in BALF of allergen-challenged guinea pigs.⁹⁴ Several reports have shown increased numbers of cells expressing eotaxin in biopsy samples taken from the bronchial mucosa of patients with atopic asthma. Both eotaxin mRNA and protein were found within the airway epithelium and inflammatory cells, including macrophages, eosinophils, mast cells and T cells. Structural cells within the airways, such as the smooth muscle⁹⁵ and fibroblast populations,⁹⁶ have also been described as potential sources of eotaxin within the airways. This supports the concept that structural cells, as well as inflammatory cells, participate in the immune response through the production of chemokines and cytokines. It is of note that the expression of eotaxin has been shown to be associated with eosinophil infiltration into the airways and decreased lung function after allergen challenge in asthmatic subjects. Brown *et al.*⁹⁷ demonstrated an increase in eotaxin mRNA as early as

2 h after allergen challenge in the airways of asthmatic subjects. This increase was localized mainly to bronchial epithelial cells, endothelial cells, macrophages, T cells, eosinophils and mast cells. Allergen-induced release of eotaxin correlated with the numbers of total and activated eosinophils and the level of airflow obstruction 4 h after allergen exposure. CCR3 is shown to be increased within the airways of asthmatic subjects⁹⁸ and cells other than eosinophils express the eotaxin receptor CCR3, such as basophils and Th2-type lymphocytes. However, the relative contribution of eotaxin in the recruitment of these cells to the airways remains to be determined.

In addition to eotaxin, other chemokines, such as RANTES, MIP-1 α , MCP-3, MCP-4 and MCP-5, also participate in the recruitment of eosinophils to sites of inflammation. Indeed, the number of cells expressing mRNA for eotaxin, eotaxin-2, RANTES, MCP-3, MCP-4 and CCR3 was increased significantly in both non-atopic and atopic asthmatic subjects.⁹⁸

RANTES has been shown to promote the infiltration of eosinophils and T cells. Antigen challenge of the airways is also associated with increase in RANTES expression and accompanied by eosinophil and T cell infiltration.⁹⁹ Furthermore, seasonal increases in eosinophil chemotactic activity are induced by IL-5 and RANTES.¹⁰⁰ Expression of RANTES has been shown to be increased within the airways of atopic and non-atopic asthmatic.^{98,101} The expression of this cytokine has been localized to airway smooth muscle cells and submucosal T cells.

Monocyte chemoattractant protein-4 is another eosinophil-associated chemokine shown to be upregulated at sites of allergic inflammation. We have recently shown the upregulation of the expression of this chemokine in the large and small airways of asthmatic individuals as well as eotaxin.¹⁰² The roles of other eosinophil-associated chemokines, such as MIP-1 α , MCP-1 and MCP-3, have not yet been clarified.

TRANSCRIPTION FACTORS IN ASTHMA

During the past decade, there have been tremendous advances in the understanding of the mechanisms of gene regulation. This has revealed much about the mechanisms controlling cytokine production and T cell differentiation. Transcription factors are central to the control of gene transcription. Once transcription factors are activated by phosphorylation, translocation to the nucleus occurs. Then, the activated transcription factors bind to specific recognition sites of the DNA, which are

usually located in the upstream promoter region to facilitate or inhibit mRNA production. For example, IL-4 engagement with its receptor leads to phosphorylation of signal transducers and activators of transcription (STAT)-6 by Jak1 and Jak3. This causes dimerization and nuclear translocation of STAT proteins and results in the activation of IL-4 regulated genes, such as IL-4R, IgE, FcR and MHC class II molecules.

In the differentiation of helper T cells, a change in the configuration of chromatin is postulated to cause the accessibility of specific transcription factors, leading to the preferential production of specific cytokines. Agarwal and Rao proposed a sequential two-step model to explain specific cytokine gene expression in Th1 and Th2 cell subsets.¹⁰³ They showed that naïve T cells have a closed chromatin configuration around the IL-4/IL-5/IL-13 and IFN- γ gene loci. Once cells are induced to differentiate by antigen and IL-4, STAT-6 is activated to yield specific demethylation around the IL-4/IL-5/IL-13 gene locus, resulting in a characteristic open chromatin structure (Fig. 2). This step is accompanied by the concomitant induction of Th2-specific transcription factors, such as GATA-3 and c-Maf. After these Th2-specific transcription factors bind to DNA, these tissue-specific transcription factors synergize with more widely expressed factors, such as activating protein (AP)-1 and NF-AT, to induce transcription of Th2 genes. It appears that the open chromatin

configuration and the expression of tissue-specific transcription factors are maintained in differentiated Th2 cells. Effector/memory cells are thought to be in a state with open chromatin structure and high levels of GATA-3 and c-Maf expression. During subsequent antigenic stimulation of the memory/effector cells, transcription factors, such as AP-1, NF- κ B, nuclear factor of activated T cells (NF-ATc) and CCAAT/enhancer-binding proteins (C/EBP β), are rapidly induced to synergize with pre-existing factors, such as GATA-3 and c-Maf, causing active transcription of the IL-4/IL-5/IL-13 gene locus in Th2 cells.

Both AP-1 and NF- κ B are particularly important in the pathophysiology of asthma because they are responsible for the generation of a wide range of cytokines implicated in the asthma process, including IL-1 β , TNF- α , IL-2, IL-6, GM-CSF, MCP-1 and RANTES. In addition, steroids are thought to exert their effect through the sequestration of AP-1¹⁰⁴ and steroids have also been shown to induce inhibitory protein- κ B (I- κ B), which prevents the nuclear translocation of NF- κ B and leads to the inhibition of the production of NF- κ B-dependent inflammatory cytokines.¹⁰⁵

To date, there have been few studies examining the expression of transcription factors in asthma. There has been a report of an increased expression of c-fos, a component of the transcription factor activated protein (AP-1) in the epithelium of asthmatic airways.¹⁰⁶ The expression

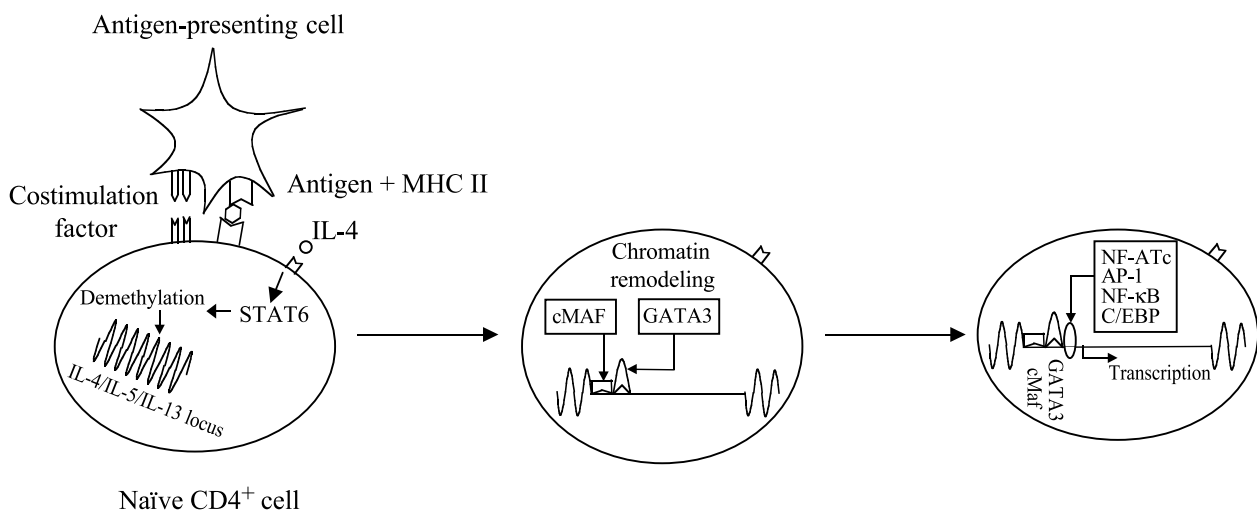


Fig. 2 Role of transcription factors in the differentiation of T helper (Th) 2 cells. Antigen stimulation and interleukin (IL)-4 receptor engagement activates signal transducers and activators of transcription (STAT)-6, followed by the demethylation of the IL-4/IL-5/IL-13 locus. This demethylation causes chromatin remodelling, which allows the Th2-specific transcription factors, such as cMaf and GATA-3, access to the IL-4/IL-5/IL-13 locus. Thereafter, more widely expressed and transiently induced transcription factors, such as nuclear factor of activated T cells (NF-ATc), activating protein (AP)-1, nuclear factor (NF)- κ B and CCAAT/enhancer-binding proteins (C/EBP), bind to the promoter, resulting in the transcription of IL-4, IL-5 and IL-13 genes. MHC, major histocompatibility complex.

of NF- κ B has been demonstrated to be upregulated in asthmatic patients compared with non-asthmatic control subjects.¹⁰⁷ It has been shown that GATA-3 is pivotal to the expression of IL-4 and IL-5^{108,109} and we have indicated that GATA-3 mRNA is highly expressed in asthmatic airway tissue compared with non-asthmatic control subjects.¹¹⁰ These results also support the significant role of GATA-3 in allergic disorders by biasing T cells in favor of Th2-type cytokine production. Targeting this transcription factor may provide a novel means of regulating the local inflammatory milieu within the lungs of asthmatic individuals.

Unlike GATA-3, c-Maf directly activates the IL-4 promoter, but does not appear to regulate the expression of all Th2 cytokine genes.¹¹¹ Nuclear factor-AT has been shown to directly activate the IL-4 promoter. Whereas NF-ATc^{-/-} chimeric mice displayed impaired IL-4 production, NF-ATp^{-/-} mice showed exaggerated and prolonged IL-4 production and constitutive nuclear localization of NF-ATc.¹¹² Thus, unlike NF-ATc, NF-ATp can behave as a negative regulator of Th2 responses *in vivo*. Another molecule that appears to be a repressor of the Th2 response is BCL-6. Targeted depletion of the BCL-6 gene led to eosinophilic inflammation and elevated IgE levels.^{113,114} The expression of these transcription factors and the precise role of these in the pathophysiology of asthma remain to be understood. Targeting transcription factors may provide a novel means of regulating the local inflammatory milieu within the lungs of asthmatic individuals.

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