

Molecular mechanisms in allergy and clinical immunology

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Molecular pathology of allergic disease I: Lower airway disease

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Asthma is a complex disorder associated with eosinophil infiltration and the activation of T lymphocytes within the airways. Recent advances in the pathophysiologic mechanisms of asthma point to the importance of eosinophil-basophil progenitor cells and a family of transcription factors that underlie the development of T_H2-type responses. Further research is needed to address the development of chronic inflammatory changes, the role of profibrotic cytokines, and especially their reliance on eosinophils in the lungs. (*J Allergy Clin Immunol* 2000;105:20-36.)

Key words: Bronchial asthma, eosinophils, T lymphocytes, cytokines, chemokines

Bronchial asthma is a chronic inflammatory disease of the airways that is recognized clinically by paroxysmal airway obstruction and an increased responsiveness to bronchoconstrictor stimuli. Recent estimates of worldwide prevalence have suggested that there has been a real increase in the number of individuals with asthma over the past 5 decades. Currently we do not fully understand the factors responsible for the development of asthma in a given individual, nor are we entirely certain of the underlying pathophysiologic mechanisms. This review will examine some recent advances in the cellular and immunologic mechanisms that have been identified as contributing to the asthmatic diathesis. Only by further investigations into the pivotal cell types and mediators responsible can novel therapeutic agents be designed to improve the treatment of patients with asthma and possibly to prevent its development.

IMMUNOPATHOGENESIS OF BRONCHIAL ASTHMA

The histopathologic features of asthma

The earliest pathologic studies of the asthmatic lung came from postmortem studies of patients who had died

Abbreviations used

AP-1:	Activator protein-1
BAL:	Bronchoalveolar lavage
IL-5R:	IL-5 receptor
LAR:	Late airways response
MBP:	Major basic protein
MCP:	Monocyte chemotactic protein
MIP- α :	Macrophage inflammatory protein-1 α
MMP:	Matrix metalloproteinase
mRNA:	Messenger RNA
NF- κ B:	Nuclear factor- κ B
T _C S:	T cytotoxic/suppressor cells
TGF- β :	Transforming growth factor- β
T _H :	T helper cells
TIMP:	Tissue inhibitor of matrix metalloproteinase
VCAM-1:	Vascular cell adhesion molecule-1

from status asthmaticus.^{1,2} These studies described evidence of gross pathologic changes in the airways, notably the overinflation of the lungs and loss of elastic recoil. This could be attributed to the widespread dissemination of viscid mucus plugs within the airways, containing desquamated epithelium, macrophages, and products of activated eosinophils. There was also evidence of smooth muscle hypertrophy or hyperplasia, thickening of the "basement membrane," goblet cell hyperplasia, and a local eosinophilic infiltrate of the bronchial wall. Similar findings were also observed in biopsy material taken at the time of surgery in children with asthma during periods of remission.³ However, the confirmation that these changes could be observed even in mild asthma and in newly diagnosed asthma did not occur until the mid-1980s and was made possible by the advent of bronchoscopy.⁴⁻⁶ Being able to directly sample the airways of living asthmatic subjects showed that even in the absence of frank desquamation epithelial fragility was present within the asthmatic airways with a widening of the intercellular spaces and the opening of tight junctions.⁷ In areas of epithelial desquamation, the basal cells are usually still attached to the basement membrane, demonstrating a preferential cleavage line between the basal and superficial cell layers. However, epithelial detachment can also be observed in biopsy specimens from healthy subjects, and studies on sections from sur-

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gically resected lungs of patients with mild asthma did not demonstrate epithelial detachment⁸ (Fig 1).

Inflammation has always been a critical component of the asthmatic diathesis. This is particularly so recently with the emergence of sophisticated molecular biology tools to investigate the phenotype and profile of cytokine production of particular cell types. Indeed, the fundamentals of asthma research rests on the descriptive pathology of the airways with use of the latest probes and antibodies as they become available. Although each inflammatory cell has its proponents, asthma is believed to be driven by the persistence of a subset of chronically activated memory T cells that home to the lungs. T cell-derived cytokines are in turn thought to orchestrate the eosinophil as an effector mechanism to rid the airways of potentially noxious antigens. Whether this scenario actually takes place within the asthmatic airways requires further investigation; however, undoubtedly these cell types are critically involved in the immune dysfunction observed in this disorder. This review will focus on the role of T cells and eosinophils in the pathogenesis of asthma and will highlight the importance of cytokines and chemokines in regulating the inflammatory response. This does not undermine the importance of other inflammatory cells and mediators.

Lymphocytes

Lymphocytes coordinate and mediate the adaptive immune responses that the body elicits in response to foreign antigens. These responses are usually self-limiting and do not directly injure the host. However, the presence of lymphocytes within the lungs of asthmatic individuals appears to direct inappropriate inflammatory cell infiltration and antibody secretion. An increased number of lymphocytes, as determined on morphologic criteria, is a frequent finding within the bronchial mucosa of patients with all forms of asthma, from newly diagnosed to severe.^{1,9,10} These lymphocytes appear "activated,"^{11,12} suggesting that they are a cardinal feature of the disorder. However, a lymphocytic infiltration of the airways has been observed in a variety of disorders that bear no similarity to asthma, and the interest was really only piqued in this cell type by the advent of techniques involving mAbs, which allowed T-cell subsets to be identified (Fig 1).

To date, T lymphocytes have been broadly subdivided into 2 distinct subsets according to their cell surface markers and distinct effector functions. T cells expressing the CD4 antigen are involved in humoral immunity and are termed helper T cells (T_H), whereas those expressing the CD8 antigen are referred to as T cytotoxic/suppressor cells (T_{CS}). These CD8⁺ cells orchestrate the cell-mediated response and interact with endogenously processed antigen presented in conjunction with 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. In doing so, these CD4⁺ cells have attracted considerable attention in the

pathogenesis of asthma because of their ability to drive antigen-specific inflammatory responses and regulate Ig production.

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 airways mucosa,¹² a trend toward an increase in the number of CD4⁺ and CD8⁺ cells,¹¹ or a selective increase in CD8⁺ T cells.¹³ CD4⁺ cell numbers were, however, reported to be elevated within the lungs of individuals with intrinsic asthma.¹⁴ What is consistently found is that those lymphocytes within the airways are activated, as determined by their expression of the IL-2 receptor (CD25).^{11,13} Whether this activated state is observed for both CD4⁺ and CD8⁺ cells¹⁵ or is restricted to CD3⁺ cells in general¹⁶ remains to be clarified. The reasons for such disparity in the experimental findings are possibly related to the severity of asthma.

In contrast to the findings at baseline, an increased number of lymphocytes within the airways of asthmatic subjects after allergen challenge is consistently reported.^{17,18} In atopic asthmatic subjects, allergen challenge results in the selective recruitment of CD4⁺ cells in BAL fluid at 6 and 48 hours in those asthmatic subjects previously demonstrated to undergo a late airways response (LAR).^{17,19} However, CD8⁺ cells are also found in increased numbers within the BAL fluid after allergen provocation,²⁰ and the recruitment of these cells has been associated with the prevention of a subsequent LAR.¹⁹

Thus the evidence presented so far appears to suggest that CD4⁺ cells are principally involved in the development of a LAR, whereas the recruitment of CD8⁺ cells may abrogate this latter fall in lung function. Alternatively, these cell types may be purely markers of the underlying processes. In light of recent findings, the view that CD4⁺ cells are proinflammatory and CD8⁺ cells suppressive in nature may be a vast oversimplification. There appears to be reason to suspect that CD8⁺ cells in the bronchial mucosa and BAL-derived T-cell clones are capable of producing cytokines implicated in the pathophysiologic features of asthma.^{21,22} 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 a cytotoxic action on epithelial cells and the activation of macrophages. Whatever their actions, CD8⁺ T cells comprise the minority of CD3⁺ T lymphocytes found within the bronchial mucosa, and thus it is likely they provide only a modulatory influence over the CD4⁺-driven inflammation.

Whatever drives the activation of T cells, it is defects in their programmed cell death that are believed to facilitate these chronic inflammatory responses to persist. Indeed, very low numbers of apoptotic lymphocytes and eosinophils are a feature of asthmatic airways.^{23,24} Under normal conditions activated T cells express the Fas antigen, which elicits activation-induced apoptosis and lim-

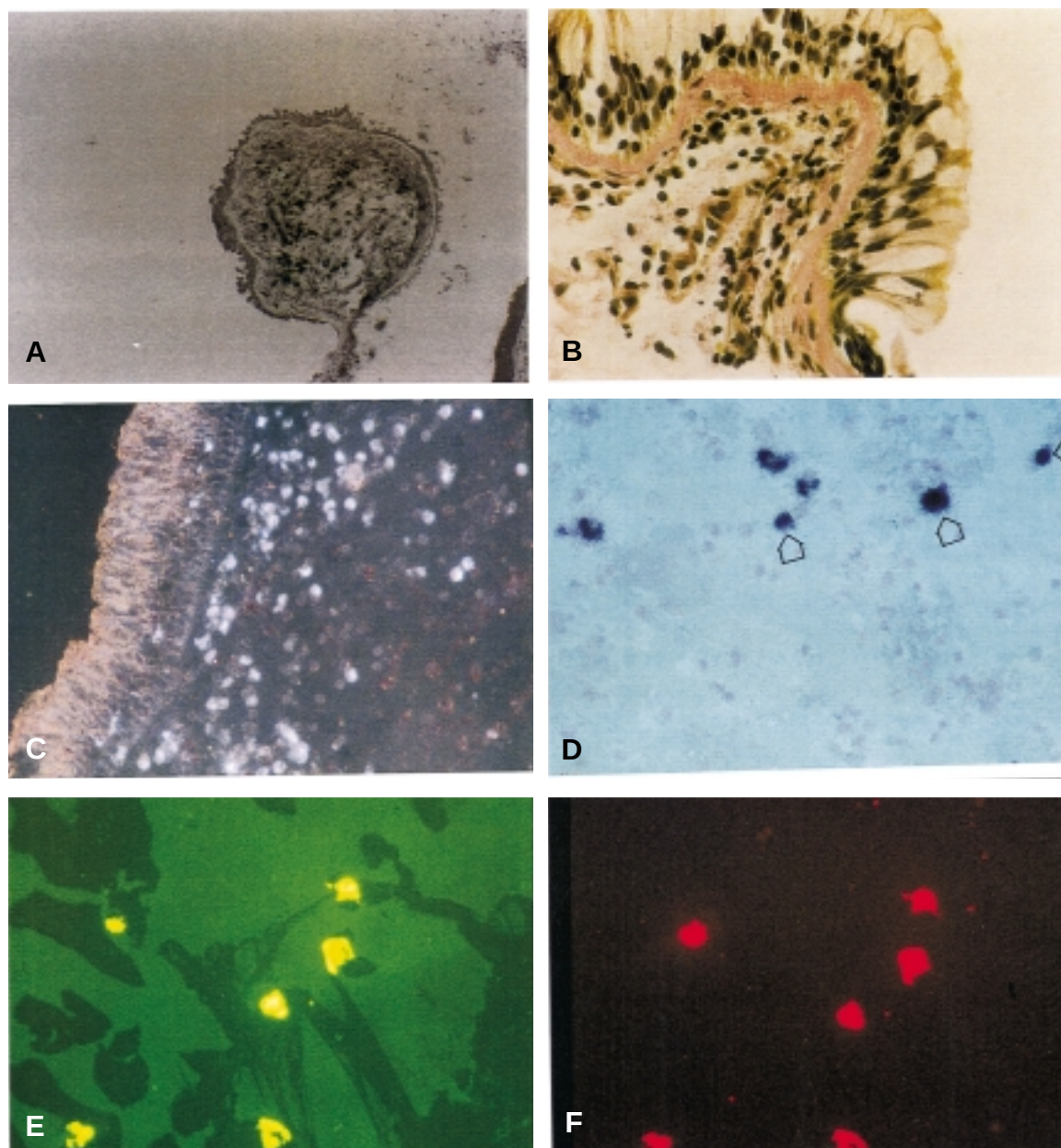


FIG 1. Histologic and molecular pathologic features of bronchial asthma. Low-power view of bronchial biopsy specimen that has undergone in situ hybridization for IL-5 messenger RNA (mRNA) is shown (**A**). Note relatively well-preserved histologic features and large number of IL-5 mRNA-positive cells that can be seen as dark aggregations of silver grains. Representative example (**B**) of bronchial biopsy specimen taken from asthmatic subject demonstrating marked inflammatory cell infiltrate a thickening of lamina reticularis (subepithelial fibrosis). Epithelium in this specimen is well preserved. **C**, Dark-field illumination of in situ hybridization for IL-5 mRNA in bronchial biopsy specimen from asthmatic subject. IL-5 mRNA-positive cells are observed as collections of silver grains and have been colocalized to CD3+ T lymphocytes. IL-4 mRNA-positive cells are shown as dark aggregations of silver grains in light-field illumination in bronchoalveolar lavage (BAL) cells from patients with asthma (**D**). Again, the majority of these cells have been shown to be T lymphocytes. **E** and **F**, Fluorescent micrographs of simultaneous in situ hybridization, IL-5 mRNA, and immunocytochemistry for CD3+, respectively, demonstrating that the majority of IL-5 mRNA-positive cells are lymphocytes.

its the consequences of a persistent immune response. Mucosal T cells observed in bronchial biopsy specimens from asthmatic patients displayed both an inability to express Fas antigen on their cell surfaces and had an

increased expression of the antiapoptotic gene, *bcl-2*.²³ Interestingly, various cytokines involved in the pathologic mechanisms of asthma (IL-4, IL-5, and GM-CSF) have a dose-dependent and specific inhibitory effect on

Fas mRNA that increases the persistence and survival of these inflammatory cells.²⁵ The influence of these cytokines may dwindle once the T cells leave the submucosa and enter the airway lumen because almost all the T cells found within the BAL fluid of asthmatic patients express the Fas antigen.²⁶ Interestingly, there is an increase in the expression of the Fas ligand after allergen challenge in asthmatic patients; this is observed on both CD4⁺ and CD8⁺ T cells. Because there was no corresponding increase in the numbers of apoptotic lymphocytes, these findings were interpreted as suggesting that this up-regulation reflects antigen-induced T-cell activation rather than cell death. Although this suggests a possible functional deficit in the apoptotic pathway, this does not appear to be the case and it is likely that defects in accessory cell regulation of T-cell function are responsible.²⁷

The above discussion focused on the majority of T cells within the airways that are positive for the $\alpha\beta$ subunit of the T-cell receptor. The role of T cells expressing $\gamma\delta$ in asthma is uncertain; however, these cells are only rarely found within bronchial biopsy specimens from asthmatic subjects.²⁸ Nevertheless, increased numbers of $\gamma\delta$ T cells (either CD4⁺ or CD4⁻CD8⁻) have been reported within the BAL fluid of patients with asthma.²⁹ They appeared to be allergen specific because they proliferated in response to the appropriate allergen and underwent apoptosis in the presence of corticosteroids. These cells are possibly preferentially recruited from the bloodstream because reduced numbers of circulating $\gamma\delta$ T cells has been reported in both atopic and nonatopic asthma.³⁰

Eosinophils

There is a considerable body of evidence suggesting there is a continuing eosinophilic inflammatory infiltrate within the lungs of asthmatic individuals and that this takes place under the regulation of lymphocyte-derived products. Infiltration of the airways with eosinophils has been described in both mild atopic and nonatopic asthma^{5,31} and large numbers of these cells surround the bronchi in postmortem examinations of patients dying from asthma.^{1,3,32,33} These cells are not purely innocent bystanders because their granular products can consistently be demonstrated in bronchoalveolar washings^{9,13,34,35} from both symptomatic and asymptomatic asthmatic subjects. There are also observations supporting the increased presence of the eosinophils within asthmatic lungs and their activation in situ after allergen challenge to the airways. Eosinophilic inflammation of the airways, as assessed by BAL, has been described not only accompanying allergen-induced early and late airway responses^{17,18,34,36,37} but also after exposure of patients with occupational asthma to various inciting agents.³⁵ Moreover, examination of repeated bronchial biopsy specimens has demonstrated that after allergen challenge these activated eosinophils migrate from the submucosa through the basement membrane to the epithelial lining.³⁷ Although the prominent BAL eosinophilia is consequent to allergen-induced release of

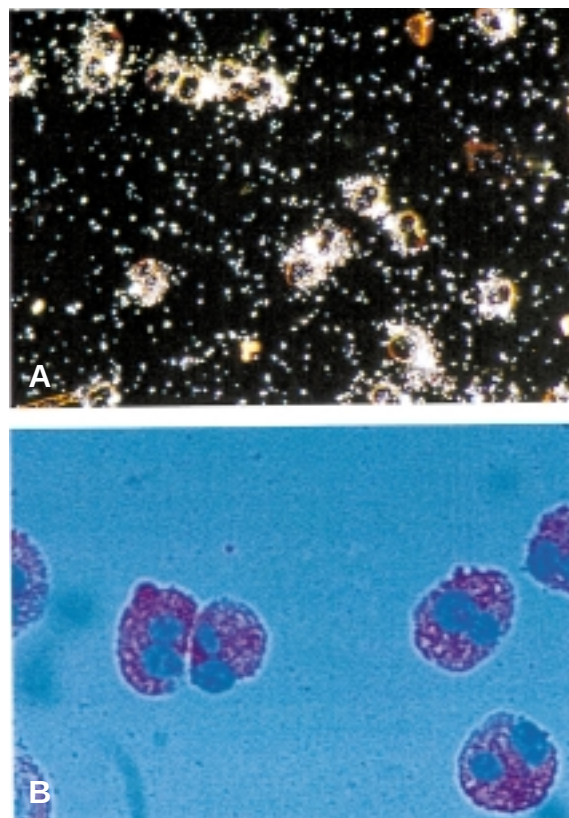


FIG 2. Peripheral blood eosinophils are a potent source of cytokines. The majority of eosinophils from peripheral blood of healthy subjects expresses IL-12 mRNA as detected with use of in situ hybridization and dark-field illumination (**A**). IL-12 mRNA-positive signals are seen as silver grains overlying individual cells. Peripheral blood eosinophils from asthmatic subjects also have the capacity to stain positive for IL-6 immunoreactivity (**B**). Positive immunoreactivity is visualized as red cytoplasmic staining.

inflammatory mediators, it has also been observed during low-dose allergen challenge, suggesting it is independent of the preceding bronchoconstriction.³⁸ Once within the airways, eosinophils are believed to liberate several proinflammatory granular products including major basic protein (MBP), eosinophil cationic protein, and eosinophil peroxidase. These elicit a profusion of actions that are relevant to the pathophysiologic features of asthma, including the induction of cellular cytotoxicity, the allosteric modulation of muscarinic M₂ receptors, mast cell and basophil degranulation, and the stimulation of mucus secretion. Eosinophils also release a tremendous variety of cytokines including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-11, IL-12, transforming growth factor- β (TGF- β), TNF- α , GM-CSF, macrophage inflammatory protein-1 α (MIP-1 α), and RANTES (Fig 2). 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

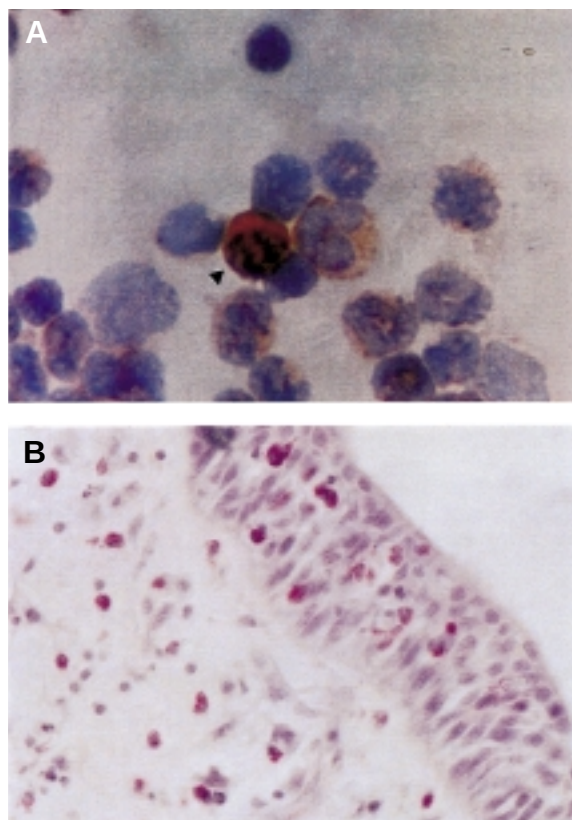


FIG 3. IL-5-immunoreactivity within bone marrow and locally differentiating MBP-positive eosinophils in nasal explants. Immunocytochemistry for IL-5-immunoreactive protein in bone marrow of Balb/c mice 6 hours after antigen challenge (**A**). Number of cells expressing IL-5 immunoreactive protein is increased as a result of antigen exposure in previously sensitized animals and is predominantly localized to CD3⁺ lymphocytes. **B**, Number of MBP-positive eosinophils within nasal explants 6 hours after allergen challenge. In absence of de novo inflammatory cell recruitment, MBP-positive cells are believed to differentiate from locally residing progenitor cells that express CD34 cell marker.

changes in allergen-induced lung function. It is widely recognized that the presence of eosinophils within the airways 24 hours after allergen challenge does not discriminate between single and dual responders. However, their presence within the airways 3 to 4 hours after the challenge is associated with the development of the LAR and the subsequent increase in airways responsiveness.^{36,37} This seems to suggest that it is not the eosinophil itself that is critical to the LAR but its presence acutely within the airways. This has invariably been ascribed to cellular recruitment, possibly by the release of an eosinophil-specific chemotactic agent such as eotaxin. Needless to say, there is always the alternative hypothesis that remains to be disproved, and this asserts that these eosinophils may be derived from locally differentiating progenitor cells.

The hypothesis of inflammatory cells that complete their maturation within the tissue appears contrary to our established wisdom on the development of the hematopoietic system. Eosinophils and basophils devel-

op 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 here under the influence of IL-5. However, almost 15 years ago it was shown that a population of eosinophil-basophil progenitor cells was to be found within the circulation and that the levels of these cells were increased in atopic individuals.³⁹ Since this time, increases in the numbers of circulating eosinophil-basophil progenitors have been shown to occur during acute asthmatic symptoms⁴⁰ and as a result of deliberate⁴¹ or seasonal allergen exposure.⁴² More recently it has been demonstrated that CD34⁺ hematopoietic cells, the earliest identified precursor cell of eosinophils and basophils, circulates in increased numbers in atopic subjects.⁴³ These CD34⁺ cells bear the IL-5 receptor (IL-5R)⁴⁴ and display an increased responsiveness to IL-5, suggesting they are primed toward the development of eosinophils.

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 up-regulated in response to allergen inhalation.⁴⁵ 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.⁴⁶ Interestingly, the 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 production. This may be the result of the specific induction of a population of more committed eosinophil-basophil progenitors or an up-regulation of IL-5R on the surface of these cells. Indeed, there is an increase in the proportion of CD34⁺ cells expressing the α subunit of the IL-5 receptor on the surface of these cells.⁴⁴ This is consistent with the association between IL-5 production and the LAR noted previously after allergen challenge.⁴⁷ Strong evidence for the involvement of these cells in asthma comes from work recently performed on bronchial biopsy specimens. This study found increased numbers of CD34⁺ cells within the airways of both asthmatic and atopic individuals denoting the presence of tissue progenitors as a hallmark of atopy. However, the numbers of CD34⁺ cells expressing α IL-5 receptor mRNA were increased only in asthmatic individuals, suggesting that local mucosal differentiation takes place.⁴⁶ Studies with ex vivo challenge of bronchial biopsy specimens will determine whether lung eosinophils are similar to those found in the nasal mucosa⁴⁸ in this capacity. Once the concept of local differentiation is addressed, then it may be possible to determine whether these cells are actually proliferating in situ. Possibly these cells are the source of the actively dividing, presumably hematopoietic, cells that appear in the BAL fluid within 24 hours of allergen challenge⁴⁵ (Fig 3).

As was observed for T lymphocytes, the numbers of apoptotic eosinophils are reduced within the airways of

asthmatic individuals compared with patients with other eosinophilic disorders of the lung, such as chronic bronchitis.²³ Several cytokines have been shown to increase eosinophil survival, including GM-CSF⁴⁹ and IL-5.⁵⁰ The release of GM-CSF was implicated in the increased eosinophil survival observed when eosinophils were cocultured with myofibroblasts.⁵¹ Whether this mechanism operates *in vivo* requires further investigation. What is apparent is that one possible therapeutic action of anti-asthma medication is to augment numbers of apoptotic cells.^{24,52} This reduces the overall tissue burden of eosinophils as seen by the reduction in the numbers of activated eosinophils in sputum and within the airways. Designing drugs aimed at selectively enhancing eosinophil apoptosis may be a possible strategy in reducing the chronic inflammation.

The presence of eosinophils is not restricted to the upper airways. There is increasing evidence that cell infiltration in particular with eosinophils also occurs in peripheral airways and parenchyma.⁸

CYTOKINE EXPRESSION IN ASTHMA

Cytokines are small, usually glycosylated proteins involved in cell-to-cell signaling. They are one of the fundamental units of the specific immune response and may act in either an autocrine, paracrine, or endocrine manner. Cytokines provide communication between cells facilitating such diverse functions as growth, chemoattraction, immunoglobulin isotype switching, and cellular proliferation and differentiation. Many of the cytokines recognized to date exhibit pleiotropy and there is often considerable redundancy in their actions, making their role in the pathogenesis of various disease states difficult to establish. Like other inflammatory mediators, cytokines act on high-affinity cell surface receptors. It is through the regulation of receptor number or the production of membrane-bound versus soluble forms of the receptor that the actions of cytokines are often modulated.

Because asthma is an immunologically mediated disorder, cytokines have been shown to play a unique role in the regulation of the inflammatory process occurring within the lungs. They not only coordinate the inflammation but also determine its character and influence its chronicity. The earliest attempts to determine which cytokines were present in asthma showed an increase in the numbers of cells expressing mRNA for IL-2, IL-3, IL-4, IL-5, and GM-CSF.^{53,54} In contrast, there was no increase in the numbers of IFN- γ mRNA-positive cells. This profile of cytokine expression fitted the T-helper T_H2 subset described previously in the mouse that preferentially expresses IL-4 and IL-5.⁵⁵ These T_H2 -type cells have been shown to differentiate from naive T_H0 -type cells under the influence of IL-4. Conversely, T_H1 -type cells express IFN- γ and IL-2 and are induced from T_H0 -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 T-helper cells toward either a T_H1 - or a T_H2 -

type response. The cellular sources of IL-4 and IL-12 that induce the polarization of T-cell cytokine production are as yet unclear. Moreover, this area has become considerably more complex by the recent suggestion that different types of dendritic cells primed with IFN- γ or PGE₂ can provoke the development of T_H1 - or T_H2 -type cells, respectively.⁵⁶ Interestingly, both atopic and nonatopic asthmatic subjects appear to have a common immunopathologic mechanism in the increase in IL-4, IL-5, and IgE-positive cells^{57,58} (Fig 1).

Although activated CD4⁺ cells represent the major subset of CD3⁺ T cells within the airways, it has been demonstrated that CD8⁺ cells can also secrete IL-4 and IL-5.^{59,60} Resting peripheral blood CD8⁺ cells from atopic asthmatic patients secrete increased levels of IL-4 compared with those from nonatopic control subjects.⁶¹ Moreover, CD8⁺ cells expressing IL-4 and IL-5 mRNA have been identified in bronchial biopsy specimens from both atopic and nonatopic individuals.²² Like T_H2 -type cells, these CD8⁺ T_{C2} -type cells can regulate the activation and cytokine production of other T cells, provide B-cell help, and influence the production of Igs. The factors influencing the skewing of cytokine production in CD8⁺ cells are currently unknown, as is the mechanism by which exogenous antigens are processed in combination with MHC class I antigens before CD8⁺ T-cell stimulation.

T_H2 -type cytokines are not only present at baseline within the asthmatic airways but are also increased after allergen challenge.^{62,63} 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.⁶⁴ Since the original observations on T_H2 -type cytokines in asthma, there has been a tremendous expansion in the numbers of cytokines characterized to date, and for the majority their expression has been investigated in asthma (Table I). Instead of reviewing the contribution of each of these cytokines to the asthmatic diathesis, this review will concentrate on recent advances in cytokine biology in asthma.

Within asthmatic airways, IL-4 is postulated to be critical for the development of T_H2 -type cells and inhibits the expression of the IL-12 receptor β_2 subunit on T cells.⁶⁵ It is a critical cytokine in the switching of B cells to favor IgE production,⁶⁶ although this effect can be enhanced by various other cytokines including IL-5, IL-10, and TNF- α .⁶⁷ In relation to the pathogenesis of asthma, the release of IL-4 within the airways also promotes goblet cell metaplasia, mucus hypersecretion, and the recruitment of eosinophils by up-regulating VCAM-1 expression in the pulmonary endothelial cells. The latter action along with ability of IL-4 to promote the growth of eosinophils and basophils⁶⁸ may underlie the observation that inhalation of IL-4 causes the development of sputum eosinophilia and an increased responsiveness of the airways.⁶⁹ The major cellular sources of IL-4 mRNA within the airways of both atopic and nonatopic asthmatic subjects are CD4⁺ T cells,^{54,70} and to a lesser extent CD8⁺ T cells, eosinophils, and mast cells.

TABLE I. Summary of cellular sources and actions of cytokines pertinent to asthma

Cytokine	Expression in asthma	Major actions	Cellular sources
IL-1 β	Increased	Epithelial activation, mucin secretion, and growth factor for T _H 2 cells	Macrophages, epithelium
IL-2	Normal/increased	T-cell proliferation	T cells (T _H 0), eosinophils
IL-3	Increased	Proliferation and differentiation of myeloid progenitors	T cells (T _H 1/T _H 2), mast cells
IL-4	Increased	IgE production, eosinophil recruitment (by VCAM-1 induction), T _H 2-type switching	T cells (T _H 0/T _H 2), mast cells, eosinophils
IL-5	Increased	Eosinophil differentiation, activation and survival	T cells (T _H 2), mast cells, eosinophils
IL-6	Increased*	IgE production, airway smooth muscle proliferation and hypertrophy	Macrophages, eosinophils, T cells (T _H 2), epithelial cells, mast cells
IL-7	Unknown	Lymphocyte proliferation, B-cell growth factor	
IL-8	Increased	Neutrophil chemoattractant, recruits primed eosinophils	Epithelial cells, eosinophils
IL-9	Increased	Mast cell proliferation and differentiation, T-cell growth factor	T cells (T _H 2)
IL-10	Increased†	Inhibits macrophage and lymphocyte activation, reduces eosinophil survival	Lymphocytes (T _H 1/T _H 2), macrophages
IL-11	Increased‡	Airway remodeling, inhibition of macrophage activation	Eosinophils, epithelial cells, lung stromal cells, airway smooth muscle
IL-12	Decreased	Induces T _H 1-type differentiation, inhibits IgE synthesis, stimulates IFN- γ production by T cells	Macrophages, lymphocytes
IL-13	Increased	Promotes IgE production and VCAM-1 expression, IFN- γ and IL-12 down-regulation	CD4 and CD8 T cells, basophils
IL-14	Unknown	B-cell growth factor	
IL-15	Unknown	T-cell growth factor	
IL-16	Increased	CD4 T-cell chemoattractant	Epithelial cells
IL-17	Unknown	Macrophage activation	
IL-18	Decreased	Induces IFN- γ production from T _H 1 cells, activates NK cells and monocytes	Macrophages
IFN- γ	Normal	IgE inhibition, activates epithelial cells, endothelial cells, alveolar macrophages and monocytes	Lymphocytes
GM-CSF	Increased	Eosinophil survival, proliferation and differentiation of myeloid progenitors	Epithelial cells, T lymphocytes (T _H 1/T _H 2)
TNF- α	Increased	Granulocyte recruitment, primes inflammatory cells, fibroblast proliferation	Macrophages, epithelial cells
TGF- β	Increased	Induction of fibroblast collagen deposition, reduces T-cell proliferation	Activated eosinophils, macrophages, fibroblasts, epithelial cells
SCF	Increased	Mast cell proliferation and differentiation	Macrophages, epithelial cells
PDGF	Increased	Fibroblast, smooth muscle and epithelial cell proliferation	Eosinophils, epithelial cells
IGF-1	Normal	Mitogen for fibroblasts and smooth muscle cells	Epithelial cells, smooth muscle

VCAM-1, Vascular cell adhesion molecule-1; NK, natural killer; SCF, stem cell factor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor.

*Reported to be increased in serum.

†Increased only in nonatopic subjects.

‡Increased in severe asthma.

IL-13 is similar to IL-4 in many ways, and not surprisingly they share common receptor subunits, although notably IL-13 does not act on T cells. IL-13 is described as a potent modulator of monocyte and B-cell function. It decreases the transcription of IFN- γ and IL-12 and so may modulate the cytokine environment at the time of

antigen presentation. Like IL-4, IL-13 also stimulates IgE production and the expression of VCAM-1 on endothelial cells and activates eosinophils by inducing the expression of CD69. An increased expression of IL-13 mRNA has been reported in atopic and nonatopic asthma.^{71,72} Moreover, increased numbers of IL-13

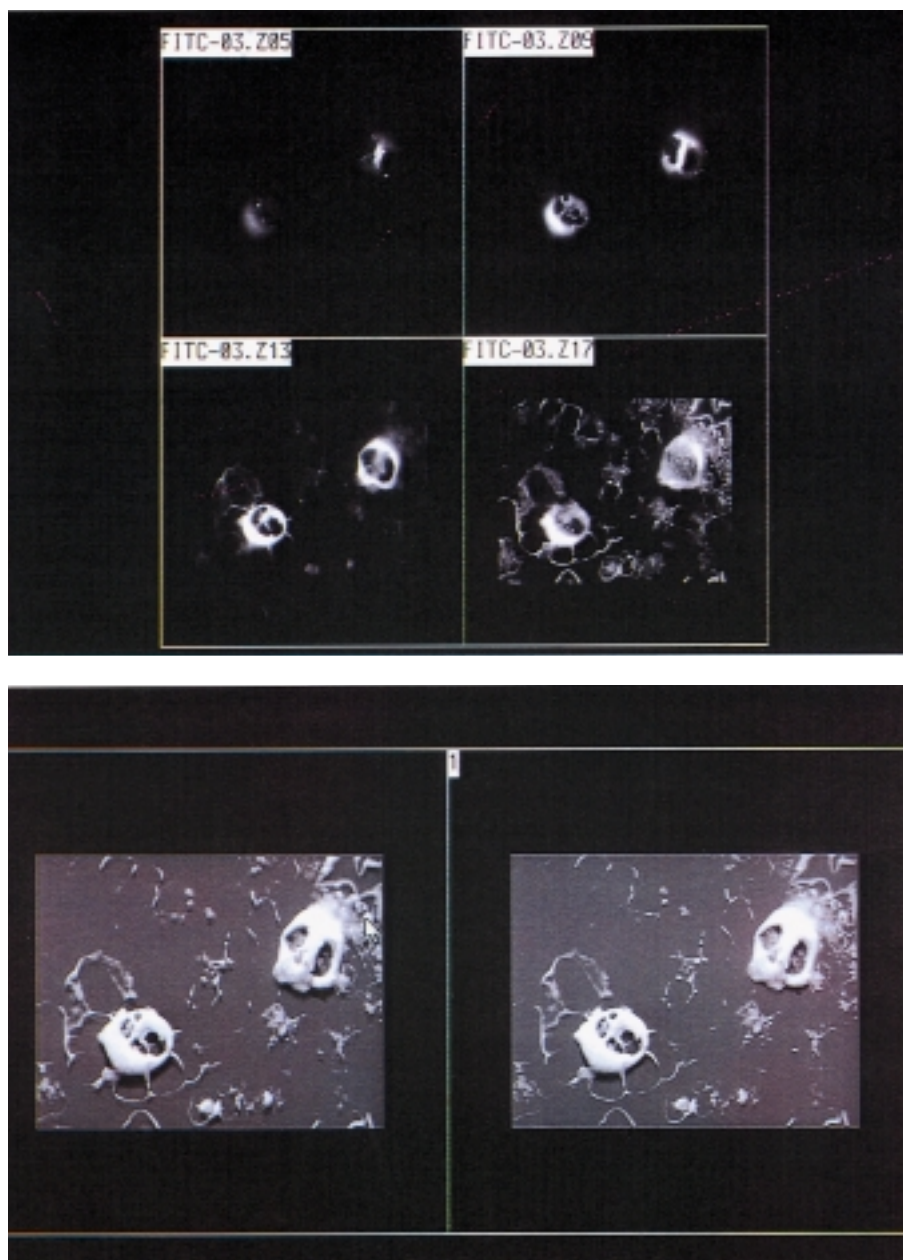


FIG 4. Confocal microscopy of IL-5R mRNA detected by FITC-labeled probe showing serial sections through eosinophils and 3-dimensional imaging. Expression of IL-5R was up-regulated in both atopic and nonatopic asthmatic subjects.

mRNA and immunoreactive protein cells are detected 24 hours after segmental allergen challenge.^{73,74} Investigations in the expression of the IL-4/IL-13 receptor in asthma have shown that both the mRNA and protein for the α -specific subunit of the IL-4 receptor is increased in atopic asthmatic patients compared with atopic nonasthmatic individuals.⁷⁵

The role of IL-3, GM-CSF, and IL-5 in the production of eosinophils within the bone marrow has previously been discussed. 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.⁷⁶ IL-5 will also stimulate the production of IgA from plasma cells, which are extremely efficient at degranulating eosinophils when bound by antigen. This cytokine has a distinguished role in asthma in that there has been a consistent relationship between the numbers of IL-5 mRNA-positive cells and the resident eosinophil infiltrate.^{53,64} In addition, the numbers of IL-5R α mRNA-positive cells are directly and closely related to lung function in a population of asthmatic patients including those with no atopic component to their disorder⁷⁷ (Fig 4). Explanted airways provide a useful means of examining tissue reactions in the absence of

de novo inflammatory cell infiltration. With use of this technique it has been shown that allergen challenge resulted in an increase in the numbers of IL-5 mRNA-positive cells, suggesting that at least some of the cytokine production is from resident inflammatory cells.

IL-9, originally identified as a T-cell growth factor, has stimulated interest because of its preferential production by T_H2 -type T cells. This cytokine 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. Genetic linkage has associated genes in the vicinity of IL-9 as predisposing toward the development of asthma and elevated levels of serum total IgE levels.⁷⁸ Moreover, the IL-9 receptor has also been linked to airway hyperresponsiveness from genetic studies. We have recently demonstrated that IL-9 is increased in asthma compared with chronic bronchitis and controls and showed that T cells are the major site of production (Fig 5).

IL-12 was initially described as a cytokine capable of stimulating cytotoxic responses from T cells and was soon discovered to induce IFN- γ synthesis in resting PBMCs. This cytokine is secreted by dendritic cells, B cells, and macrophages and these are in a prime position to influence T-helper cell differentiation. The principal actions of IL-12 in atopic disorders are to inhibit IgE synthesis and to promote the differentiation of T_H1 -type cells.⁷⁹ IL-12 also stimulates the growth and activation of T cells and natural killer cells and their production of IFN- γ . There is a decreased expression of IL-12 mRNA in asthma,⁷¹ and this is increased after treatment with steroids. A cell surface marker of T_H1 -type cells, the β_2 subunit of the IL-12 receptor, is notably reduced in the lungs of patients with asthma.^{80,81}

IL-16, previously known as lymphocyte chemoattractant factor, is a chemotactic agent specific for $CD4^+$ cells. Indeed, IL-16 acts through the $CD4^+$ receptor to induce cellular migration and growth. It also exhibits immunoregulatory effects in inducing CD25 and MHC class II expression. In asthma, increased levels of IL-16 have been found within the BAL fluid after specific allergen challenge,⁸² and this is possibly the result of the release of histamine from mast cells.⁸³ Even in the absence of allergen, patients with mild atopic asthma also demonstrated an increased expression of this cytokine, which in particular is localized to the epithelium⁸⁴ (Fig 1). In asthmatic subjects the majority of IL-16 immunoreactivity is associated with $CD3^+$ T cells and MBP^+ eosinophils, with tryptase⁺ mast cells making a minor contribution.⁸⁵

CHEMOKINE EXPRESSION IN ASTHMA

Chemokines are small (8–10 kD) secreted factors that facilitate the movement of inflammatory cells. More than 40 different chemokines have been recognized to date, and 4 distinct chemokine families can be distinguished on the basis of the arrangement of their cysteine residues. These are the CXC, CC, C, and CX_3C chemokine families. These families also differ in their biologic activity

according to the various kinds of effector cells they act on. Thus eosinophils, T cells, and monocytes are most responsive to chemokines belonging to the CC family such as RANTES, eotaxin-1, eotaxin-2, eotaxin-3 and monocyte chemoattractant protein (MCP)-2, MCP-3, and MCP-4. The newly identified chemoattractant stimulated T-cell chemoattractant protein-1 is a novel CC chemokine with chemoattractant activity for T_H2 -type cells.⁸⁶ The effects of other CC chemokines, such as exodus-1, exodus-2, exodus-3, alternative macrophage activation-associated CC chemokine-1, and leukotactin-1 have not been investigated to date.

Of the CC chemokines, eotaxin has commanded by far the majority of the attention because of the potency and specificity of its actions on eosinophils. This chemokine was originally described as a novel eosinophil chemoattractant found within the BAL fluid of allergen-challenged guinea pigs.⁸⁷ After the cloning of human eotaxin in 1996,⁸⁸ there have been extensive investigations into cellular sources of this chemokine and its regulation within the respiratory mucosa. Perhaps unsurprisingly, there have been several reports of increased numbers of cells transcribing the eotaxin gene and expressing its immunoreactivity in biopsy samples taken from the bronchial mucosa of patients with atopic asthma.⁷⁶ Both eotaxin mRNA and protein were found within the airway epithelium and submucosal inflammatory cells, including macrophages, eosinophils, mast cells, and T cells. It is of note that structural cells within the airways, including the smooth muscle and fibroblast populations, have also been described as potential sources of eotaxin within the airways.^{89,90} This reinforces the concept that chemokines as well as cytokines can be expressed by a wide variety of cells within the airways, including those not previously recognized as participating in immunologic reactions (Fig 6).

The expression of eotaxin after allergen challenge is of interest, particularly with regard to the kinetics of eosinophil infiltration into the airways and their association with the LAR. This was recently investigated by Brown et al,⁹¹ who demonstrated an increase in eotaxin mRNA as early as 2 hours after allergen challenge. This increase was localized mainly to bronchial epithelial cells, endothelial cells, macrophages, T cells, eosinophils, and mast cells. What is promising from the viewpoint of using specific eotaxin antagonists in the treatment of allergic disorders is that the allergen-induced release of eotaxin correlated with the numbers of total and activated eosinophils and the level of airflow obstruction 4 hours after allergen exposure.⁹¹ Although cells other than eosinophils express the eotaxin receptor CCR3, such as basophils and T_H2 -type lymphocytes, the relative contribution of eotaxin in the recruitment of these cells to the airways remains to be determined. CCR3 is increased within the airways of atopic asthmatic subjects.²²

Although it is pertinent to discuss the role of eotaxin in the recruitment of eosinophils to sites of inflammation, it is obvious from both animal and clinical studies that addi-

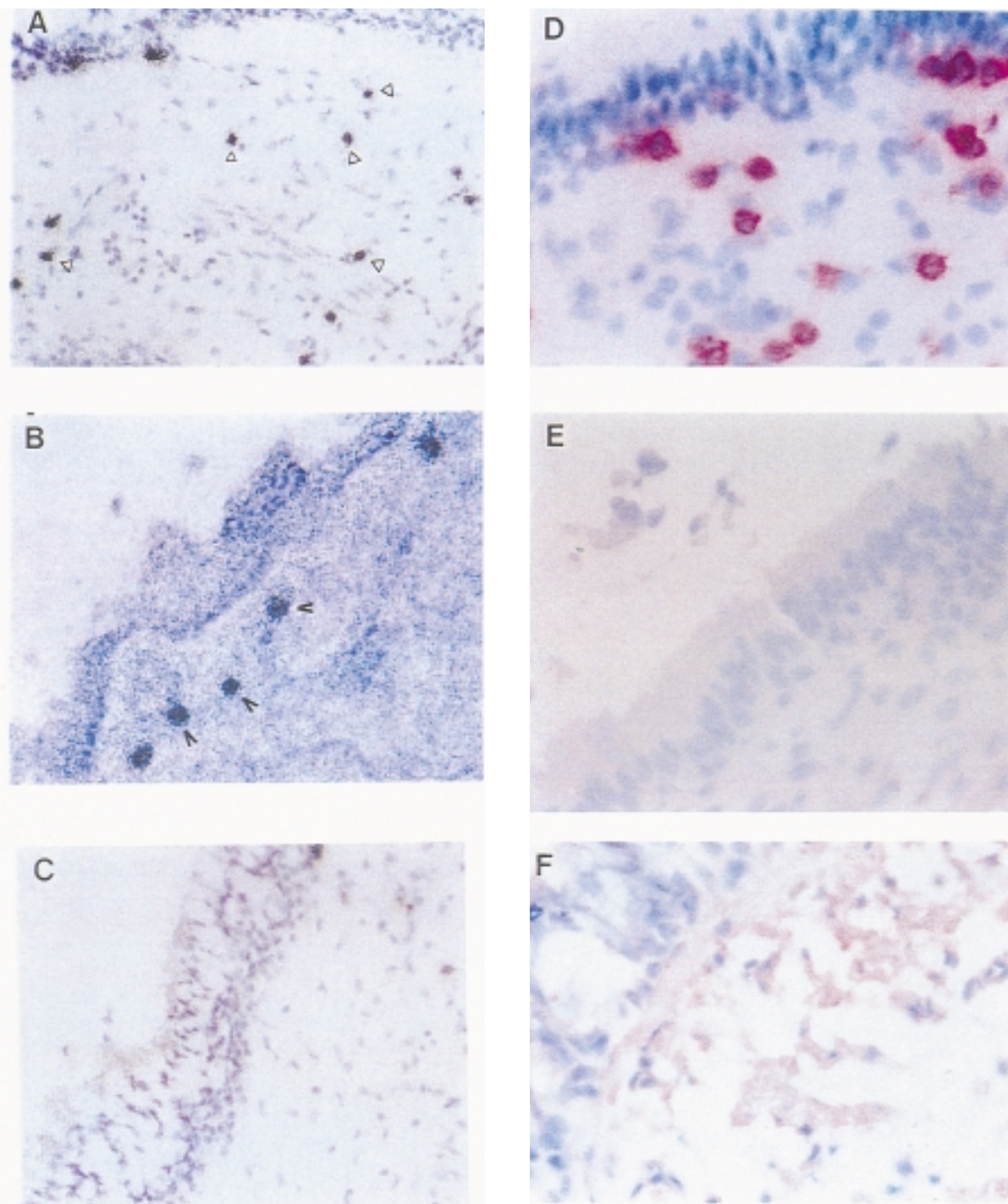


FIG 5. Expression of IL-9 mRNA and immunoreactive protein in asthma and sarcoidosis, a nonallergic lung disease. Representative examples of in situ hybridization of IL-9 mRNA with use of sulfur 35-labeled complementary RNA probes in bronchial biopsy specimens from asthmatic subjects. (**A**, Original magnification $\times 200$; **B**, original magnification $\times 400$). **C**, Expression of IL-9 mRNA in bronchial biopsy specimen from patient with sarcoidosis. (Original magnification $\times 400$). IL-9 immunoreactivity is shown in atopic asthmatic subject (**D**), in patient with sarcoidosis (**E**), and in nonatopic nonasthmatic control subject (**F**).

tional chemotactic agents are operating. These include chemokines such as RANTES, MIP-1 α , MCP-3, MCP-4, and MCP-5. RANTES, a product of activated T cells, was shown to promote the infiltration of eosinophils and

T cells and has been consistently shown to be increased within the airways of atopic and nonatopic asthmatic patients.⁹² The expression of this chemokine has been localized to the airway smooth muscle and submucosal T

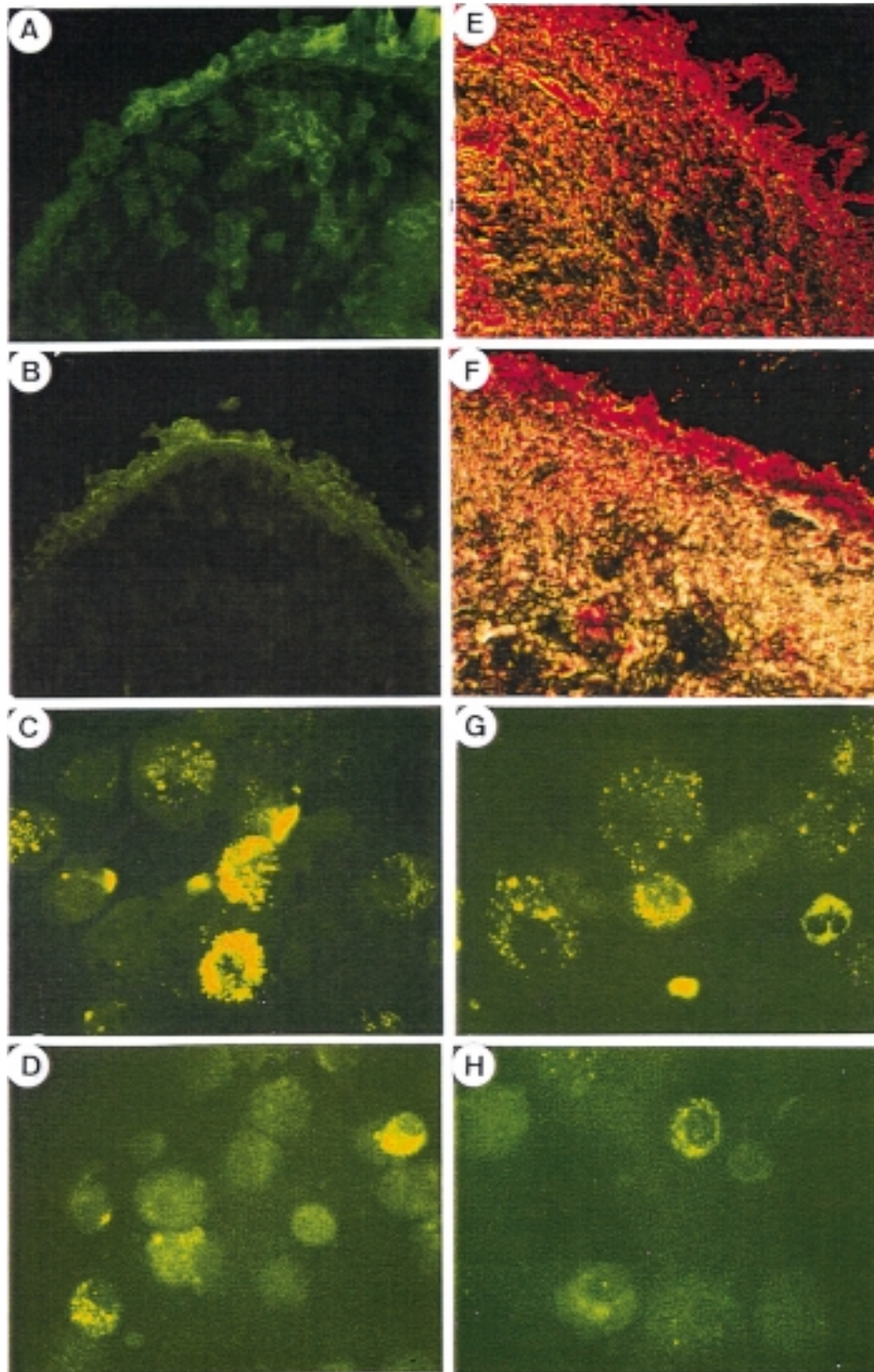


FIG 6. Expression of eotaxin mRNA and immunoreactive protein in asthma. Cellular distribution of eotaxin mRNA (**A** to **D**) and immunoreactive protein (**E** to **H**) within the airways (**A**, **B**, **E**, **F**) and BAL cells (**C**, **D**, **G**, **H**) of asthmatic patients (**A**) compared with healthy control subjects (**B**). Staining was observed exclusively in the airway's epithelium and in many infiltrating cells in asthmatic biopsy specimens. Representative cytospin preparations of BAL cells obtained from asthmatic patient (**C**) and nonasthmatic control subject (**D**) hybridized with FITC-labeled antisense riboprobe to eotaxin. Immunostaining for eotaxin-immunoreactive protein within the airways (**E**, **F**) and BAL cells of asthmatic (**G**) and nonasthmatic control (**H**) subjects, respectively. Tissue sections and BAL cells were fixed and stained with rabbit polyclonal anti-eotaxin antibody. Alkaline phosphatase-conjugated goat antirabbit IgG and fast red substrate or FITC-conjugated swine antirabbit IgG and fast red substrate or FITC-conjugated swine antirabbit IgG were used to visualize eotaxin immunoreactivity.

cells. Antigen challenge of the airways is also associated with an increase in RANTES expression and accompanied by eosinophil and T-cell infiltration.⁹³ Furthermore, seasonal increases in eosinophil chemotactic activity is provided by IL-5 and RANTES.⁹⁴ MCP-4 is another chemokine that we have recently described as being present in the large and small airways of asthmatic individuals.⁹⁵ The profile of eotaxin and MCP-4 expression was consistent, with both mediators playing a role in the recruitment of eosinophils throughout the airway tree. Moreover, there may be additional chemoattractants acting because only ~50% of the eosinophil-chemotactic activity in BAL fluid from atopic asthmatic subjects was accounted for by a combination of eotaxin, RANTES, and MCP-4.⁷⁶ These may include MIP-1 α , MCP-1, and MCP-3.⁷⁰

In the context of eosinophil recruitment to the airways and the visualization of CD34⁺/ α IL-5R cells within the airways,⁴⁶ it is important to address the recruitment of these progenitor cells. The only known chemokine identified to date with any activity on CD34⁺ cells is stromal cell-derived factor-1.⁹⁶ Although the acquisition of receptors for eotaxin is an early event in eosinophil development, its significance remains to be elucidated.⁴⁴ Obviously this may prove to be a very fruitful area of further investigation.

TRANSCRIPTION FACTORS IN ASTHMA

Within the past decade there have been major insights into the mechanisms by which genes are regulated, and this research is beginning to be applied in deciphering the control of cytokine production in asthma. Central to the control of gene transcription are transcription factors, proteins that act to facilitate or inhibit RNA production. These transcription factors operate by binding to specific recognition sites, which are usually located in the upstream promoter region of the gene. Once the upstream elements controlling the phosphorylation state of the transcription factors are activated, there is the resultant translocation of the transcription factor to the nucleus. Specific DNA binding by transcription factors results in the activation or repression of gene expression and is mirrored by a change in the formation of mRNA and protein. Knowledge regarding the localization of transcription factors (cytoplasmic or nuclear) is important in assessing their state of activation. Despite advances in the recognition of T_H2-type cytokine expression in allergic disorders, the transcription factors underlying the production and cellular effects of these cytokines remain to be elucidated.

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.⁹⁷ In addition, greater numbers of cells expressing nuclear factor- κ B (NF- κ B) have been demonstrated in asthmatic patients compared with nonasthmatic control subjects.⁹⁸ Both AP-1 and NF- κ B are particularly important to the asth-

matic patient 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, one of the mechanisms by which steroids are thought to act is by the sequestration of AP-1.⁹⁹ Moreover, steroids have also been shown to induce I κ B, a molecule that prevents the translocation of NF- κ B to the nucleus, and thus they attenuate the activation of NF- κ B-dependent inflammatory cytokines.¹⁰⁰

From the viewpoint of designing agents to prevent the expression of T_H2-type cytokines, a great deal of interest has been stimulated by the discovery of GATA-3. This transcription factor has been shown to be selectively expressed in T_H2- (but not T_H1)-type cytokine-producing T cells.^{101,102} Blockade of GATA-3 in T_H2-type cells inhibited the expression of IL-4 and IL-5, whereas its ectopic expression in T_H1-type cells resulted in the activation of T_H2-type cytokine promoters. These results suggest that this transcription factor is necessary and sufficient for the expression of IL-4 and IL-5. Recent results from our own laboratory have indicated that there is a high expression of GATA-3 mRNA in asthmatic biopsy specimens compared with nonasthmatic control specimens.¹⁰³ These results suggest there is a significant role for GATA-3 in allergic disorders by biasing T cells in favor of T_H2-type cytokine production. Targeting this transcription factor may provide a novel means of regulating the local inflammatory milieu within the lungs of asthmatic individuals.

AIRWAY FIBROSIS AND REMODELING IN ASTHMA

The chronic sequelae of asthma have come under intense investigation recently, particularly with the identification of many profibrotic cytokines that exhibit an enhanced expression within the airways of asthmatic patients (Table II). These structural changes are thought to result in the irreversible component of the airway obstruction seen in asthma and the persistent airway hyperresponsiveness. Although these inflammatory mediators may undoubtedly be contributing to the remodeling of the airways, whether they actually are responsible for alterations in airway function remains to be addressed. Although it is tempting to speculate these are somehow connected, until long-term trials of therapies designed to reverse these structural abnormalities are undertaken there will be no certainty in this.

Subepithelial fibrosis is an almost invariable finding within the asthmatic airways. It has attributed to the deposition of collagen types III and V, fibronectin, tenascin, and proteoglycans.^{6,104} This thickening of this reticular lamina is thought to be an early and fundamental change within the airways and can be observed even in newly diagnosed asthma¹⁰ (Fig 7). The locality of this thickening suggests it is not attributable to epithelial cell dysfunction but is rather the result of fibroblast and myofibroblast activation. Myofibroblasts are fibroblasts

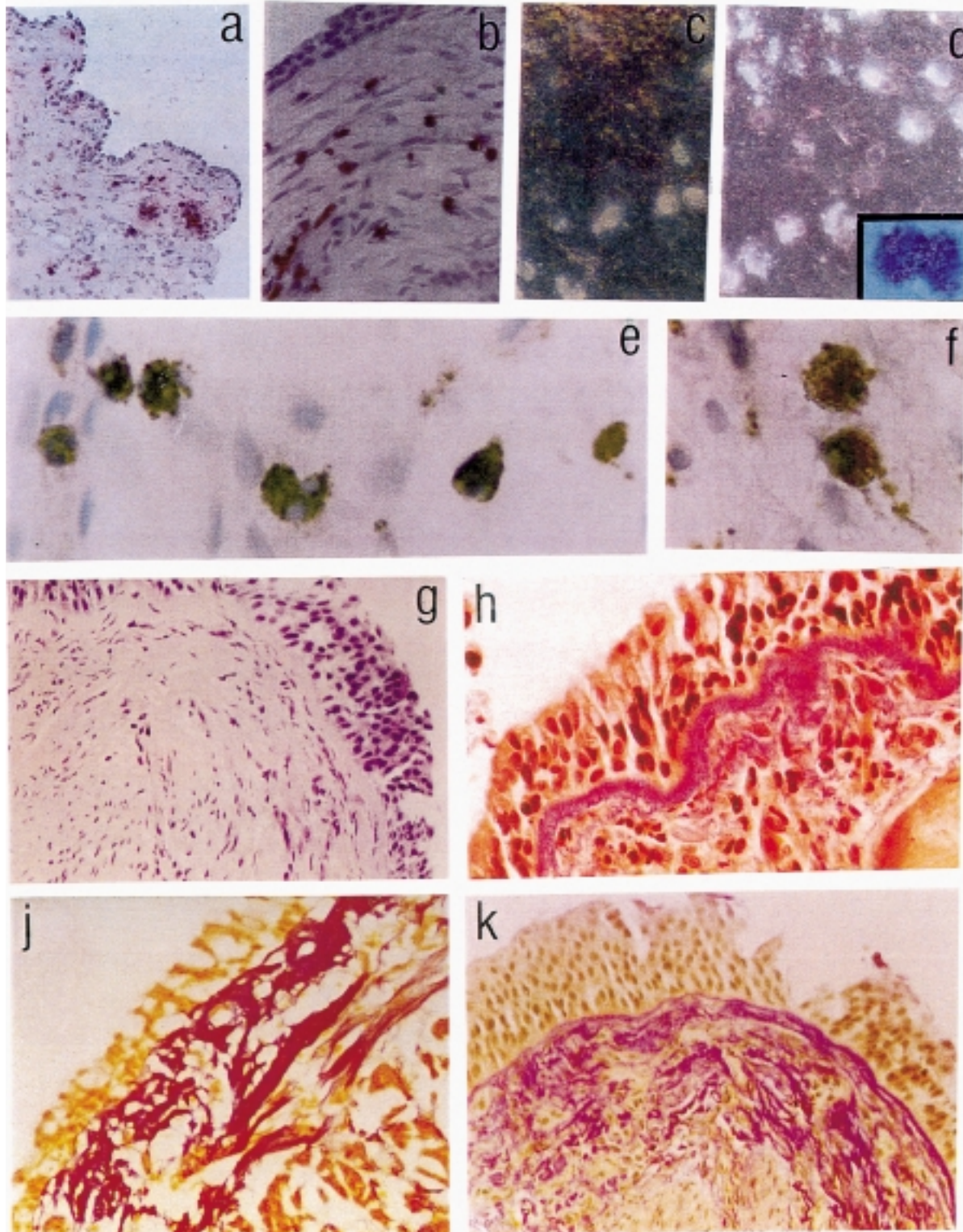


FIG 7. Expression of TGF- β 1 and its association with eosinophils in bronchial biopsy specimens. Immunostaining for EG2-positive cells in mild (a) and severe (b) asthmatic bronchial biopsy specimens and in situ hybridization detecting TGF- β 1 mRNA-positive cells in a moderate (c) and a severe (d) asthma. Inset in d, TGF- β 1 mRNA-positive cell, indicated by the silver grains, associated with EG2+ immunoreactivity (pink staining). Immunocytochemistry for TGF- β 1 immunoreactive protein in moderate asthma (e) and double immunostaining demonstrating presence of TGF- β 1 immunoreactivity in EG2+ eosinophils (f). Hematoxylin and eosin staining of bronchial biopsy specimen from a patient with severe asthmatic (g) and representative examples of subepithelial fibrosis as detected by van Gieson's staining in mild (h), moderate (j), and severe (k) asthma. (Reproduced with permission from Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF- β mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17:326-33.)

TABLE II. Mechanisms and cellular sources of profibrotic cytokines implicated in asthma

Cytokine	Actions	Cellular source
TGF- β	Stimulates fibroblast activation, chemotaxis, and proliferation; enhances collagen synthesis and smooth muscle proliferation	Eosinophils, fibroblasts, epithelial cells, macrophages
IL-11	Stimulates accumulation of myocytes, fibroblasts, and myofibroblasts; enhances production of collagens types I and III; stimulates TIMP-1	Eosinophils, fibroblasts, epithelial cells
PDGF	Stimulates fibroblast chemotaxis and proliferation, enhances smooth muscle proliferation	Eosinophils, epithelial cells
IGF	Stimulates fibroblast and smooth muscle proliferation, promotes collagen synthesis	Epithelial cells, smooth muscle
EGF	Stimulates smooth muscle proliferation	Epithelial cells, smooth muscle, mucus glands
GM-CSF	Stimulates TGF- β production	Smooth muscle, T cells, fibroblasts

TIMP, Tissue inhibitor of metalloproteinase; *PDGF*, platelet-derived growth factor; *IGF*, insulin-like growth factor; *EGF*, epidermal growth factor.

expressing α -actin and are thought to be the cell of origin of nascent smooth muscle cells. These potentially contractile cells are increased in numbers in biopsy samples from atopic asthmatic subjects and their numbers have been associated with the thickening of the reticular layer.¹⁰⁵ Increased numbers of myofibroblasts may result by differentiation of an existing population of fibroblasts, within the appropriate cytokine milieu or by dedifferentiation of smooth muscle cells in situ and their migration to a subepithelium site.¹⁰⁶

Recently the mechanisms underlying collagen homeostasis have come under investigation as a means of possibly inducing airway fibrosis. Matrix metalloproteinases (MMPs) are members of a family of proteolytic enzymes that degrade the extracellular matrix. These enzymes are inhibited by TIMPs, and any imbalance between these 2 enzyme families is thought to play a critical role in the synthesis or degradation of the extracellular matrix of the airway architecture. TIMP-1 levels within the BAL fluid are increased in asthma,¹⁰⁷ and these levels are observed to be far greater than the sum of MMP-1, MMP-2, MMP-3, and MMP-9 combined, suggesting an imbalance in favor of airway fibrosis. One of the major cell types implicated in the production of TIMP is the alveolar macrophage, which has been found to produce more TIMP-1 both at protein and mRNA levels than alveolar macrophages from either nonasthmatic or steroid-treated asthmatic subjects.¹⁰⁷ Indeed, steroids may reduce the profibrotic environment of the airways by either reducing TIMP-1 levels or by increasing the production of MMP-9. Serum ratios of MMP-9/TIMP-1 have been shown to predict the response of oral steroid therapy in asthma; patients with a low MMP-9/TIMP-1 ratio exhibit little or no improvement in FEV₁ with steroid administration.¹⁰⁸ MMPs have also been suggested as being able to induce chronic structural changes in asthma by their ability to degrade proteoglycans and thus their potential to release and activate latent matrix-bound growth factors. Whether these factors fine-tune the fibrotic response

given the overwhelming profibrotic milieu observed in asthma remains to be determined.

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