

Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling

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A new paradigm for asthma pathogenesis is presented in which exaggerated inflammation and remodeling in the airways are a consequence of abnormal injury and repair responses arising from a subject's susceptibility to components of the inhaled environment. An epithelial-mesenchymal trophic unit becomes activated to drive pathologic remodeling and smooth muscle proliferation through complex cytokine interactions. Histamine, prostanoids, and cysteinyl leukotrienes (CysLTs) are potent contractile agonists of airway smooth muscle (ASM). The CysLTs appear to play a central role in regulating human ASM motor tone and phenotypic alterations, manifested as hypertrophy and hyperplasia in chronic severe asthma. The CysLTs augment growth factor-induced ASM mitogenesis through activation of CysLT receptors. Although they mediate their contractile effects by increasing phosphoinositide turnover and inducing increased cytosolic calcium, new data suggest that part of the contractile effect may be independent of calcium mobilization. Prostaglandin E₂, the predominant eicosanoid product of the airway epithelium, is a potent inhibitor of mitogenesis, collagen synthesis, and mesenchymal cell chemotaxis and therefore can suppress inflammation and fibroblast activation. The capacity of the epithelium for CysLT synthesis is inversely related to its ability to make PGE₂. The ASM is capable of expressing both leukotriene-synthesizing enzymes and CysLT receptors, and cytokines upregulate the receptor expression. This may be an explanation for the CysLTs promoting airway hyperresponsiveness in asthma. The CysLTs play an important role in the airway remodeling seen in persistent asthma that includes increases of airway goblet cells, mucus, blood vessels, smooth muscle, myofibroblasts, and airway fibrosis. Evidence from a mouse model of asthma demonstrated that CysLT₁ receptor antagonists inhibit the airway remodeling processes, including eosinophil trafficking to the lungs, eosinophil degranulation, T_H2 cytokine release, mucus gland hyperplasia, mucus hypersecretion, smooth muscle cell hyperplasia, collagen deposition, and lung fibrosis. (J Allergy Clin Immunol 2003;111:S18-36.)

Key words: Epithelial mesenchymal trophic unit, asthma pathophysiology, airway inflammation, airway remodeling, cysteinyl leukotrienes

Increasingly, it is being recognized that chronic asthma is accompanied by striking changes in the structure and function of the formed elements of the lower airways, including epithelial metaplasia, to one enriched in mucus-secreting goblet cells, increased matrix deposited in the submucosa and adventitia, increased smooth muscle (both hyperplasia and hypertrophy), and proliferation of airway microvessels and nerves. Conventional wisdom has led to the proposed formulation of a linear model for the development of chronic asthma that progresses from aeroallergen sensitization to T_H2-mediated inflammation that then becomes persistent, providing the microenvironment for airway wall remodeling.¹ A variable interaction between mediators released from inflamed airways such as cysteinyl leukotrienes (CysLTs), prostanoids, proteolytic enzymes, and kinins and the remodeled airway leads to hyperresponsiveness, variable airflow obstruction, and asthma symptoms.

Evidence suggests that CysLTs are important molecules that promote airway inflammation and modulate airway smooth muscle (ASM) cell function. Airway smooth muscle plays a central role in regulating bronchomotor tone and also undergoes phenotypic alterations manifested by hypertrophy and hyperplasia in chronic severe asthma. By secreting chemokines and cytokines, or by expressing cell adhesion molecules or altering extracellular matrix, ASM may also perpetuate and orchestrate airway inflammation.

The role of CysLTs in both airway inflammation and remodeling has been studied in a mouse model of asthma that shows chronic lung inflammation and fibrosis. It is characterized by eosinophil trafficking to the lungs, eosinophil degranulation, mucus gland hyperplasia, mucus hypersecretion, smooth muscle cell hyperplasia, and collagen deposition beneath the epithelial layer and in the lung interstitium at sites of leukocyte infiltration. An important role for the CysLTs in these events has been suggested and tested.

This review will explore the link between inflammation and remodeling by considering an alternate paradigm for their relation. In addition, it will examine recent evidence implicating CysLTs as important effectors of ASM function and important elements of both inflammation and remodeling, which, if blocked, would appear to be therapeutically beneficial.

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Abbreviations used

AA:	Arachidonic acid
AHR:	Airway hyperresponsiveness
ASM:	Airway smooth muscle
BHR:	Bronchial hyperresponsiveness
CAMP:	Child Asthma Management Program
COX:	Cyclooxygenase
CysLTs:	Cysteinyl leukotrienes
EGFR:	Epidermal growth factor receptor
EMTU:	Epithelial-mesenchymal trophic unit
ET:	Endothelin
FGF:	Fibroblast growth factor
5-LO:	5-lipoxygenase
FLAP:	5-LO-activating protein
GM-CSF:	Granulocyte macrophage colony-stimulating factor
ICS:	Inhaled corticosteroid
IGF:	Insulin-like growth factor
IL:	Interleukin
IFN:	Interferon
LTRAs:	Leukotriene receptor antagonists
MMP:	Metalloproteinase
OVA:	Ovalbumin
PDGF:	Platelet-derived growth factor
PG:	Prostaglandin
STAT:	Signal transducer and activation of transcription
TGF:	Transforming growth factor
TIMP:	Tissue inhibitor of metalloproteinase

EPITHELIAL MESENCHYMAL COMMUNICATION IN ASTHMA AND THE ROLE OF LEUKOTRIENES

Difficulties in linking atopy and asthma in a linear model for asthma

Atopy is one of the strongest risk factors associated with asthma, but it is difficult to explain asthma purely in terms of lower respiratory allergy. Although up to 50% of a population has atopy, less than 10% actually have persistent asthma, and the level of exposure in early life is not associated with the development of asthma in those who become sensitized to aeroallergens.² In countries that have not adopted a Western lifestyle (eg, Albania), the prevalence of atopy is similar to that in the United Kingdom, but the prevalence of asthma is much lower.

Evidence is accumulating that regarding asthma only as a T_H2-mediated inflammatory disorder underplays other important factors.³ For example, the T_H1-like cytokine tumor necrosis factor- α greatly enhances the tissue effects of interleukin (IL)-4 and IL-13. Even the presumed pivotal role for eosinophils in the inflammatory response of asthma has been challenged. Both allergen challenge⁴ and clinical⁵ studies with an anti-IL-5–blocking monoclonal antibody failed to reveal efficacy despite markedly reducing circulating and airway eosinophils.

Thus, although associated with asthma, airway eosinophilia driven by IL-5 would not seem to be a critical requirement for disease expression, although persistence of eosinophils in the lung supported by other cytokines (eg, granulocyte-macrophage colony-stimulat-

ing factor [GM-CSF]) is a possibility. Genetic studies have also demonstrated that atopy and bronchial hyperresponsiveness (BHR) have different patterns of inheritance.⁶ Taken together, these findings imply that locally operating factors play an important role in predisposing individuals to asthma and provide an explanation for epidemiologic evidence that identifies pollutant exposure,⁷ diet,⁸ and respiratory virus infection⁹ as other important disease risk factors.

Inhaled corticosteroids (ICS) are the mainstay controller therapy for asthma. In a significant proportion of patients, however, the clinical response is incomplete, requiring supplementary drugs such as inhaled long acting β_2 -agonists or leukotriene receptor antagonists (LTRAs). In chronic asthma, noninvasive imaging has revealed that the thickening of asthmatic airways accounts for at least a component of BHR and the excessive airway narrowing seen in established disease. The majority of BHR, then, would appear to be associated with abnormalities in smooth muscle both in volume and contractile functions.¹⁰

This “remodeling” response involving multiple structural elements has also been linked to the progressive decline of pulmonary function observed in asthma.¹¹ Although remodeling has been considered to be secondary to longstanding inflammation, a recent biopsy study has revealed that all the structural changes in adult disease are present as early and consistent components of childhood asthma, including fibroblast proliferation, collagen deposition in the subepithelial lamina reticularis, and smooth muscle hyperplasia.^{12,13} Early life development of corticosteroid-insensitive airway remodeling would also explain the outcome of the recent Childhood Asthma Management Program Research Group (CAMP) study in children 5 to 11 years of age.¹⁴ This study showed that the initial beneficial effect of an ICS on the postbronchodilator improvement in airway function, observed during the first year of treatment, was lost over the following 3 years. These studies strongly suggest that “remodeling” processes begin early in the course of asthma (“premodeling”) and most likely occur in parallel with, or may be obligatory for, the establishment of persistent inflammation. Our recent finding that variable airflow obstruction and BHR is critically dependent on the presence of increased mast cells in smooth muscle indicates that abnormal asthmatic smooth muscle itself is able to support an increased mast cell population.¹⁵

A new paradigm for asthma pathogenesis

Even though asthma has a strong genetic basis, a dramatic increase in its prevalence over the last 3 decades has occurred in too short a time for new genetic changes to be responsible. This leads to the proposal that environmental changes have uncovered a preexisting susceptibility within the population. Environmental agents affect the bronchial epithelium, the protective layer of cells that lines the airways; any difference in the ability of the asthmatic epithelium to withstand environmental insults would provide a plausible, local gene-environment interface.

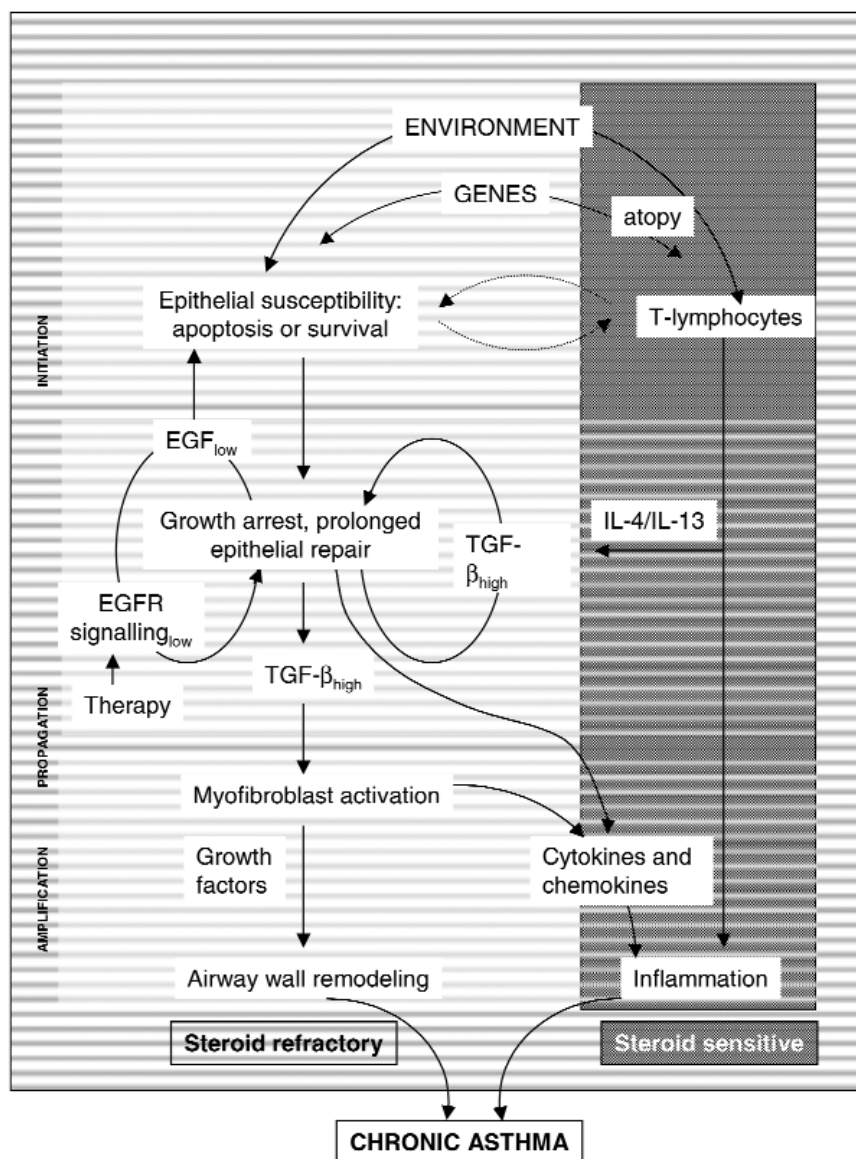


FIG 1. Cell-cell communication in the EMTU. In this parallel model for asthma pathogenesis, an inherited or acquired epithelial susceptibility to environmental agents leads to the induction of stress/injury and repair responses. Growth arrest and prolonged repair enhances cell-cell communication within the EMTU, leading to myofibroblast activation and the propagation of remodeling responses into the submucosa. At each level, T_H2 cytokines are able to interact with the EMTU to enhance or amplify these responses. Fundamental to the new hypothesis of "parallel pathways" is the concept that altered epithelial-mesenchymal signaling provides a microenvironment in the airways for proliferating responses of smooth muscle, fibroblasts, microvessels, and nerves as well as a cytokine and chemokine milieu facilitating the persistence of inflammation. *EGF*, Epidermal growth factor; *EGFR*, epidermal growth factor receptor; *TGF*, transforming growth factor.

We propose a new paradigm for asthma pathogenesis, the "epithelial-mesenchymal trophic unit" (EMTU) in which exaggerated inflammation and remodeling in the airways are a consequence of abnormal injury and repair responses arising from the bronchial epithelium's susceptibility to components of the inhaled environment (Fig 1). This modifies the function of the epithelium and its ability to communicate with the underlying mesenchymal cells to provide the appropriate microenviron-

ment for promoting tissue remodeling and for sustaining persistent inflammatory responses characteristic of chronic asthma. Positioning atopy or T_H2 -mediated inflammation in parallel with the altered tissue response rather than sequential to it explains why asthma does not develop in a significant proportion of atopic individuals and why some individuals have asthma that is susceptible to steroid therapy (inflammation dominating) or more resistant to it (remodeling dominating).

INCREASED EPITHELIAL INJURY AND DISORDERED REPAIR IN ASTHMA

The normal differentiated bronchial epithelium is a stratified structure consisting of a columnar layer comprising ciliated and secretory cells that are supported by basal cells. This polarized structure is the physical barrier protecting the internal milieu of the lungs from inhaled pollutants, infectious agents, and other particulate matter. Under normal conditions, the bronchial epithelium is engaged actively in defense of the airways by secreting mucus and many specific and nonspecific cytoprotective molecules that trap and inactivate inhaled components. The epithelium also responds to environmental stimuli by signaling to, and interacting with, cells of the innate and adaptive immune systems through secretion of cytokines and chemokines and expression of adhesion molecules such as ICAM-1 and CD40.¹⁶ This enables the epithelium to work in conjunction with the immune system to provide a mechanism whereby, when the natural epithelial barrier is compromised, the immune and inflammatory cells can promote tissue repair by removing cell debris and providing a transient supply of locally acting growth factors.

In asthma, the epithelium shows evidence of stress as evidenced by widespread activation of the transcription factors for nuclear factor- κ B, activator proteins (eg, AP-1), signal transducer and activation of transcription-1 (STAT-1), and the increased expression of both heat shock proteins and the cyclin-dependent kinase inhibitor p21^{waf}. Consistent with an injured phenotype, the asthmatic epithelium is also an important source of autacoid mediators, chemokines, and growth factors that create a local microenvironment to sustain ongoing inflammation. It has been proposed that epithelial damage is artifactual¹⁷; however, our findings of enhanced expression of epidermal growth factor receptor (EGFR), (HER1, c-erbB1),¹⁸ and the epithelial isoform of CD44¹⁷ indicate that injury has occurred *in vivo*. We have found that EGFR expression in asthma increases with disease severity and is evident throughout the epithelium (ie, involves columnar and basal cells), suggesting that stress and damage is widespread.¹⁸ Although the extent of injury may reflect damage by T_H2-mediated inflammatory cell products, the fact that EGFR expression persists in the face of corticosteroid treatment¹⁸ might indicate that environmental stimuli make a primary contribution to epithelial stress and injury in asthma rather than a secondary effect through corticosteroid-sensitive T_H2-driven pathways.

When cells enter into apoptosis, activation of caspase-3 by either caspase-8 or caspase-9 results in early change of intracellular proteins pivotal to cell survival. One of these is 5-poly-ADP-ribose polymerase (PARP), whose cleavage can be shown after oxidant-induced apoptosis *in vitro* and increased asthma-related immunostaining *in vivo*.¹⁹

Because they are preserved through several generations *in vitro* (imprinting), these differences are unlikely to be secondary to the microenvironment created by airway inflammation. Considering that epidemiologic stud-

ies have identified multiple interacting risk factors for asthma, we propose that the effect of environmental stimuli on such a susceptible epithelium provides a plausible triggering mechanism for the induction of epithelial activation and damage in asthma. Once initiated, the resulting inflammatory cell influx will cause secondary damage through production of endogenous reactive oxygen linked to inflammation, tissue injury, and remodeling. We propose that as a result of activation by environmental triggers, the susceptible asthmatic epithelium creates a microenvironment that supports repeated chronic cycles of injury and inflammation.

The epithelial mesenchymal trophic unit and airway remodeling

Under normal conditions, the epithelium releases primarily factors that suppress mesenchymal cells, such as prostaglandin (PG)E₂ and 15-HETE. With epithelial injury or damage, production of PGE₂ and 15-HETE is diminished, and the ensuing repair responses could promote airway remodeling by activating the fibroblasts/myofibroblasts that lie directly under the epithelial layer in the lamina reticularis (Fig 2).²⁰ This signaling between the epithelium and fibroblasts involves the provision of growth factors that support the growth and survival of mesenchymal cells likely to contribute to the component of asthma unresponsive to corticosteroids. *In vitro* studies have shown that injury to epithelial monolayers results in increased release of fibroproliferative and profibrogenic growth factors including fibroblast growth factor (FGF-2), insulin growth factor (IGF-1), platelet-derived growth factor (PDGF), endothelin (ET-1), and transforming growth factor (TGF)- β .²¹

In asthma, the epithelium has increased susceptibility to oxidant injury through the activation of the caspase-3/apoptosis pathway, a feature that carries over into cultured asthmatic epithelial cells *in vitro*.¹⁹ Impaired epithelial repair is also a feature of asthma and is linked to the increased production of profibrotic growth factors such as TGF- β /or FGF-2, as evidenced by low expression of cell markers of proliferation (eg, proliferating cell nuclear antigen [PCNA]). This can be modeled *in vitro* by slowing epithelial repair in the presence of a selective tyrosine kinase inhibitor of EGFR, tyrosine kinase. This markedly augments the release of TGF- β ,¹⁸ which may play a key role in converting fibroblasts into myofibroblasts.

Overexpression of EGFR (cerb B1) in asthmatic bronchial epithelium *in vivo*, or *in vitro* after injury, is insensitive to the action of corticosteroids and increases in proportion to disease severity and chronicity. By immunostaining asthmatic and normal epithelium, the related cerbB2, B3, and B4 receptors, as well as their ligands EGF, heparin-binding EGF-like growth factor (HB-EGF), TGF α , and amphiregulin appear unaltered. In moderate-to-severe asthma, ICS reduced airway inflammation and levels of IGF-1 but provided minimal improvement in BHR and had little or no effect on either latent or active TGF- β levels.²² As corticosteroid treatment reduces inflammation, persistently high TGF- β

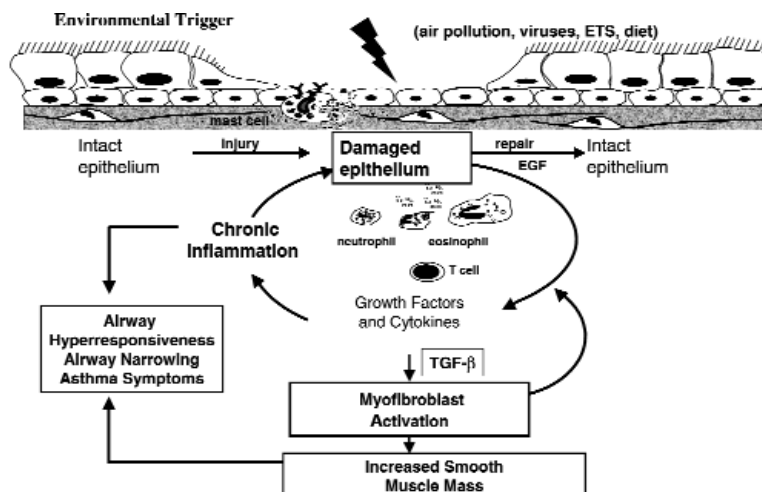


FIG 2. Model for the interaction between environmental agents and a susceptible epithelium: a trigger for persistent airway inflammation and remodeling in asthma. Susceptibility to environmental oxidants causes epithelial damage that triggers a normal injury-repair response involving release of mediators that promote inflammation and tissue repair (which involves transient remodeling responses). The release of endogenous oxidants by inflammatory cells causes further injury to the susceptible epithelium, however, resulting in a chronic state of tissue damage, which maintains the appropriate environment for persistent inflammation and tissue remodeling. *TGF-β*, Transforming growth factor; *EGF*, epidermal growth factor; *ETS*, environmental tobacco smoke.

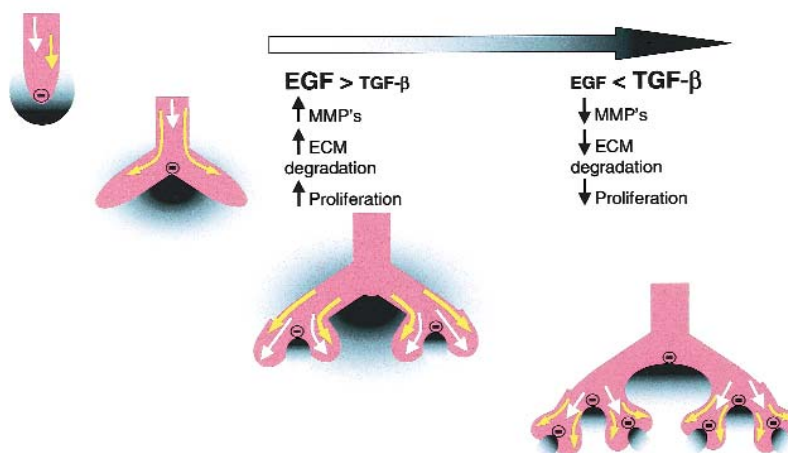


FIG 3. Mechanisms of branching morphogenesis involving reciprocal signaling between EGF and $TGF-\beta$ involving the epithelium and underlying attenuated fibroblast sheet. *EGF*, Epidermal growth factor group of cytokines; *TGF-β*, transforming growth factor-β family; *MMP*, metalloproteinase; *ECM*, extracellular matrix.

most likely derives from the injured and repairing epithelium and associated matrix turnover rather than from infiltrating leukocytes such as the eosinophils. Because both epithelial EGFR expression and $TGF-\beta$ production are refractory to corticosteroids, we propose that the combined effects of the remodeling of these signaling pathways explain the incomplete resolution of pulmonary function with ICS seen in chronic asthma.

Although myofibroblasts are recognized as key effector cells in tissue fibrosis because of their enhanced ability to synthesize interstitial collagens, those from asth-

matic bronchial biopsy specimens also release greater amounts of ET-1 and vascular endothelial growth factor,²³ which are mitogens for smooth muscle and vascular endothelial cells, respectively. Using a panel of cell markers, including α -smooth muscle actin, heavy chain of myosin, and an early smooth muscle differentiation marker SM-22, mesenchymal transformation in the presence of $TGF-\beta$ creates a cell indistinguishable from a smooth muscle cell. Moreover, when these cells are cultured from asthmatic airways and compared with normal airways, their proliferative response in the absence of

exogenous growth factors is enhanced as the result of the autocrine production of proliferative factors. In this way, remodeling responses initiated at the cell surface are propagated and amplified through the submucosa by way of the subepithelial myofibroblasts to smooth muscle.

Communication between the epithelium and the subepithelial fibroblast sheath is reminiscent of the processes that drive physiologic remodeling of the airways during embryogenesis, when the epithelium and mesenchyme act as a “trophic unit” to regulate airway growth and branching (Fig 3).²⁴ Consequently, we propose that the EMTU becomes activated or is reactivated in asthma to drive pathologic remodeling and smooth muscle proliferation (Fig 1).²⁵ In subjects with asymptomatic BHR, longitudinal studies have shown that those who progress to asthma show parallel changes in inflammation and remodeling.²⁶

Thickening of the lamina reticularis in bronchial biopsy specimens from young children is also present several years before asthma becomes clinically manifest.¹³ This feature is pathognomonic of asthma and, although it reflects activation of the EMTU, it should not be regarded as remodeling per se. The downstream consequences of EMTU activation more directly relates to the remodeling events of altered airway structure and function. During fetal lung development, epithelial and mesenchymal growth is regulated in part by the balance of EGF, FGF, and TGF- β BMP signaling.²⁴ In those individuals who are destined to progress to asthma, we propose that in early life, environmental factors interact with the EMTU to initiate structural changes in the airways (pre-modeling). This may explain the decrease in pulmonary function observed in young children who are susceptible to early wheezing²⁷ and the loss of corticosteroid responsiveness on baseline pulmonary function observed in the CAMP study. Thus, for asthma to fully develop, bronchial epithelial susceptibility precedes or occurs in parallel with factors predisposing to T_H2-mediated inflammation and is an absolute requirement to establish the microenvironment for inflammation to become persistent in the airways and for remodeling to occur.

Interaction between IL-4 and IL-13 and the epithelial-mesenchymal trophic unit

Irrespective of atopy, T_H2-type inflammation of the airways is a characteristic feature of asthma. In transgenic mice, the expression of IL-13 or IL-4 transgenes in the bronchial epithelium led to many of the inflammatory responses characteristic of asthma, but submucosal remodeling was only evident in the case of IL-13.²⁸ Using fibroblasts derived from asthmatic mucosal biopsy specimens, we have shown that IL-13 was able to induce myofibroblast transformation but, although it is equipotent with IL-4, it is 2 orders of magnitude less potent than TGF- β in this effect.²³ Because IL-13 causes a corticosteroid-insensitive increase in the release of TGF- β ₂ from bronchial epithelial cells, IL-13-mediated submucosal remodeling is initiated largely through the bronchial epithelium. In human epithelial cells, however, IL-4 is as

effective as IL-13 in promoting TGF- β release, raising the possibility of an important species difference in epithelial functions of these 2 related T_H2 cytokines.

Although the remodeling effects of IL-4 and IL-13 can be attributed to epithelial activation, these cytokines also have direct proinflammatory effects on both epithelial cells and fibroblasts. Cultures of bronchial epithelial cells respond to IL-4 and IL-13 with increased STAT-6 phosphorylation accompanied by enhanced GM-CSF, IL-8, and TGF- α production, which is further augmented by enzymatically active extracts of house dust mite, *Der-matophagoides pteronyssinus*. Asthmatic epithelial cells differ from normal in their greatly enhanced production of TGF- α in response to IL-4, IL-13, and the direct effects of allergen. This may be important because TGF- α is a powerful agonist of the EGFR linked to “frustrated” repair and differentiation in favor of a mucus-secreting phenotype. T_H2 cytokines may also influence mesenchymal cell functions,^{29,30} including the enhanced release of eotaxin from asthmatic “fibroblasts” and may help explain the accumulation of eosinophils beneath the lamina reticularis in asthma. Thus, by interacting with the EMTU, IL-4 and IL-13 have the potential to augment both ongoing inflammation and remodeling responses.

The role of inflammatory cells and their mediators in remodeling

Autacoid mediators such as histamine, prostanoids, and CysLTs are potent contractile agonists of ASM. In addition, their effects in augmenting smooth muscle proliferation has attracted recent attention. Activation of their G-protein-coupled, 7-transmembrane receptors initiated activation of membrane bound metalloproteinases (MMPs) such as MMP-2, which in turn either reduced proliferative growth factor binding factors (eg, IGF-2 binding factor) or released soluble forms of the growth factors from their membrane-bound precursors (eg, amphiregulin or EGF), with the net result of enhanced proliferation of smooth muscle progenitor cells.^{31,32} In asthma, the increased infiltration of smooth muscle by mast cells also may play a role in maintaining smooth muscle hyperplasia,¹⁵ because the MC_{TC} (connective tissue) mast cells are major sources of LTC₄, PGD₂, histamine, and tryptase, all of which engage G-protein-linked receptors.

Human ASM cells also express leukotriene-synthesizing enzymes in addition to CysLT₁ receptors, some of which are further upregulated by TGF- β and, in the case of LTA₄ hydrolase, by histamine.³³ Most recently, we have shown that primary airway epithelial cells contain a full set of CysLT synthetic enzymes, and when activated, they are capable of releasing appreciable amounts of LTC₄. Epithelial cells do not express the CysLT₁ receptor.³⁴ Thus, the generation of CysLTs can occur in the absence of “inflammation,” and their actions on precursor mesenchymal cells may be important in augmenting airway wall remodeling in asthma. The capacity of the epithelium for CysLT synthesis is inversely related to its ability to make PGE₂, as discussed below. If these events occur early in life, in children at risk of asthma, this

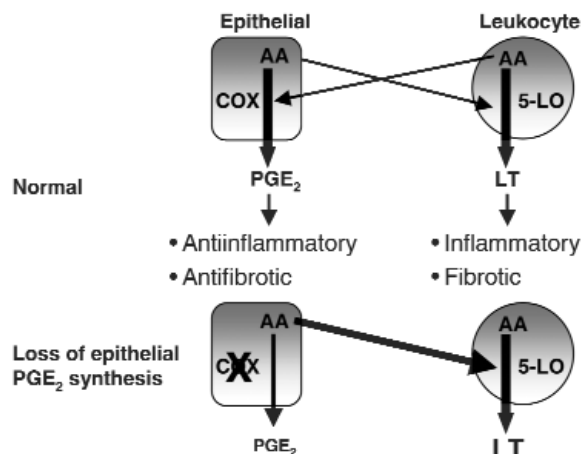


FIG 4. The influence of epithelial cell PGE₂ synthesis on leukocyte leukotriene generation through transcellular interactions. Pulmonary epithelial cells metabolize AA primarily through cyclooxygenase (COX) to PGE₂, whereas leukocytes metabolize AA primarily through the 5-lipoxygenase (5-LO) pathway to leukotrienes. When these 2 cell types are cocultured, however, AA from either cell type can also be used by the metabolic pathway of the other. Impairment of epithelial cell capacity for PGE₂ synthesis, as may exist in asthma, increases the extent to which epithelial cell-derived AA is available for leukotriene synthesis by leukocytes.

could form the basis of an intervention study with LTRAs, in an attempt to prevent the consolidation and progression of asthma in childhood.

CYSTEINYL LEUKOTRIENES, FIBROBLASTS, REMODELING, AND PULMONARY FIBROSIS

Airway remodeling refers to the structural changes that can occur in asthma that may be associated with irreversible airflow obstruction. These alterations include hyperplasia of smooth muscle cells and fibroblasts as well as deposition of matrix proteins such as collagen in the airway wall. First, TGF- β , implicated in the differentiation of fibroblasts to myofibroblasts, also increases CysLT synthesis by macrophages.^{35,36} Second, CysLTs increase the proliferative response of lung fibroblasts to known mitogens³⁷ as well as the capacity of these cells to synthesize collagen.³⁸ Thus, CysLTs may contribute to remodeling not only through their diverse roles in inflammation but by direct effects on mesenchymal cells. Finally, it is important to recognize that the key biological features that characterize airway remodeling—epithelial injury, a T_H2 phenotype, accumulation and activation of inflammatory cells, mesenchymal cell hyperplasia, and matrix protein deposition—are common to remodeling responses at other anatomic sites, including the lung parenchyma.³⁹ There is evidence supporting a role for CysLTs (and other 5-lipoxygenase [5-LO] metabolites) in the pathogenesis of pulmonary fibrosis. Leukotriene levels have been shown to be elevated in the lungs of patients with idiopathic pulmonary fibrosis.^{40,41} Moreover, bleomycin-induced pulmonary fibrosis in mice is characterized by overproduction of CysLTs and is significantly ameliorated in 5-LO knockout

mice.⁴² A role for leukotrienes in remodeling responses in other organs, including the skin⁴³ and the liver,⁴⁴ has also been suggested.

EPITHELIAL-DERIVED PROSTAGLANDIN E₂: EFFECTS ON INFLAMMATION/REMODELING AND INFLUENCE ON LEUKOTRIENE BIOSYNTHESIS

The epithelial cell–fibroblast dyad has recently been highlighted as being of central importance in the development of both pulmonary fibrosis³⁹ and airway remodeling.⁴⁵ The current paradigm posits that under normal conditions, epithelial cells suppress fibroblast proliferation and collagen synthesis and that fibrosis is favored by conditions that result in a loss of this usual epithelial suppression or a state of epithelial activation of fibroblast function. The predominant eicosanoid product of pulmonary epithelial cells, PGE₂,^{46,47} is a potent inhibitor of mesenchymal cell chemotaxis,⁴⁸ mitogenesis,^{49,50} and collagen synthesis.⁵¹ Furthermore, it has a variety of other downregulatory effects on such processes as leukocyte recruitment and generation of cytokines, growth factors, reactive oxygen intermediates, endothelin, and leukotrienes.^{52–57} PGE₂ is therefore an excellent candidate for mediating the capacity of epithelial cells to suppress inflammation as well as fibroblast activation. Interestingly, a diminished capacity for PGE₂ synthesis by respiratory epithelium has been reported in an equine model of asthma⁴⁶ as well as in patients with aspirin-sensitive asthma.⁵⁸

Arachidonic acid (AA) metabolism, occurring in vivo at the epithelial surface of the airways or the alveoli, cannot be understood adequately by studying isolated cells. Such studies exclude a form of cell–cell interaction known as transcellular metabolism in which AA (or another intermediate in the eicosanoid biosynthetic pathway) released from one cell type can be utilized for eicosanoid synthesis by another. These interactions occur when alveolar epithelial cells, whose major eicosanoid product is PGE₂, were cocultured with alveolar macrophages (whose major eicosanoid products are leukotrienes).⁵⁹ Thus, epithelial cell–derived AA could be used instead by macrophages to generate leukotrienes, and macrophage–derived AA could be used instead by epithelial cells to produce PGE₂. Importantly, the cell's capacity to serve as a donor of AA for transcellular metabolism was dramatically increased when its own eicosanoid synthetic pathway was pharmacologically inhibited. The addition of a 5-LO inhibitor to the coculture resulted in an increase in conversion of macrophage–derived AA to PGE₂ (modeling the increased PGE₂ synthesis observed in the lungs of 5-LO knockout mice),⁴² whereas the addition of a cyclooxygenase (COX) inhibitor resulted in a large increase in the conversion of epithelial–derived AA to leukotrienes⁵⁹ (again modeling what has been observed in the lungs of COX-deficient mice).⁶⁰ Because transcellular interactions between epithelial cells and leukocytes are likely to be important in vivo, the prostanoid synthetic capacity of epithelial

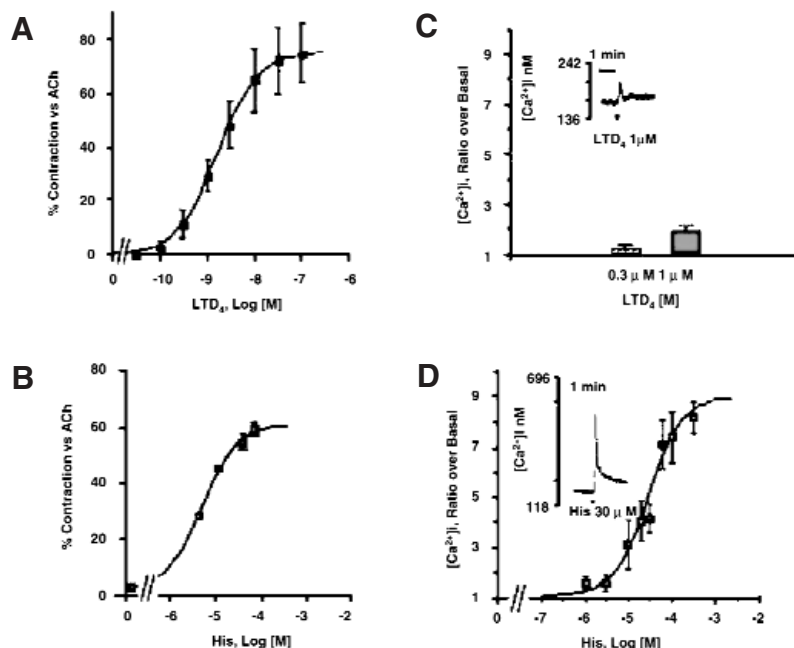


FIG 5. Concentration-response curves of agonist-induced contraction (**A** and **B**) of isolated human bronchi and [Ca²⁺] increase (**C** and **D**) in human ASM cells. **A** and **C**, LTD₄. **B** and **D**, Histamine. Insets in **C** and **D** are representative tracings of the individual effect of agonist concentrations in inducing [Ca²⁺] increases. Curves in **A** and **B** were constructed with strips from 7 different lung specimens. Curves in **C** and **D** were obtained from 4 to 7 experiments on cells obtained from 3 to 5 different lung specimens, respectively. From Accomazzo et al. *Am J Respir Crit Care Med* 2001;163:266-72.

cells may be a key determinant of the profile of eicosanoids produced in the lung. For this reason, a loss of PGE₂ synthetic capacity associated with epithelial cell injury or dysfunction would not only promote inflammation and fibrosis/remodeling in its own right but also by driving transcellular metabolic interactions toward leukotriene synthesis (Fig 4).

Airway smooth muscle and cysteinyl leukotriene bronchoconstriction

The CysLTs are potent bronchoconstrictors: LTC₄ and LTD₄ are 1000-fold more potent than histamine in contracting human ASM.⁶¹ The effects of the CysLTs are mediated by activation of two G-protein-coupled receptor subtypes, CysLT₁ and CysLT₂.⁶²⁻⁶⁵ Most CysLT receptor antagonists, including montelukast, pranlukast, zafirlukast, and pobelukast, are potent CysLT₁ receptor antagonists.^{32,66,67} The cloning of the human CysLT₁⁶⁶ and the CysLT₂⁶⁸ has greatly advanced our understanding of CysLT effects on human ASM cells.

Leukotriene D₄ markedly induces shortening of smooth muscle in a variety of tissues. This constrictor response results from the activation of a receptor-mediated pathway that increases phosphoinositide turnover and cytosolic calcium (Ca²⁺). In human ASM cells, LTD₄ potently and effectively increases Ca²⁺; however, there appears to be a discrepancy between force generation by the muscle and LTD₄-induced increases in Ca²⁺, although LTD₄ contracts human bronchial smooth muscle strips in vitro to a level comparable with histamine.

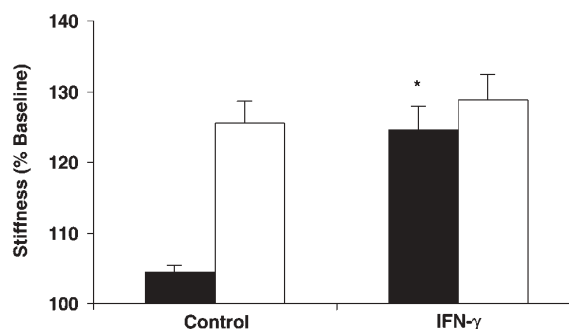


FIG 6. Cell stiffness responses to LTD₄ (10⁻⁷ mol/L) (black bars) and bradykinin (10⁻⁶ mol/L) (white bars) of human ASM cells treated with IFN-γ (1000 U/mL for 24 hours) and of untreated (control) cells were measured. Results are expressed as percent of baseline cell stiffness. Data are mean ± SEM of 8 wells in each group and were obtained from 2 different donors on 2 experimental days. **P* < .001 compared with control. From Amrani et al. *Am J Respir Crit Care Med* 2001;164:2098-101.

The maximal response is comparable, albeit at 1/10,000th the dose of histamine. LTD₄ is far less effective than histamine in maximally mobilizing cytosolic Ca²⁺ (Fig 5).⁶⁷ Evidence suggests that LTD₄-induced contraction of human ASM is in part independent of Ca²⁺ increases. Such calcium-independent contraction may involve the activation of a protein kinase C isoform, PKC-ε.⁶⁷ Further studies are necessary to address the precise mechanisms by which LTD₄ may promote ASM

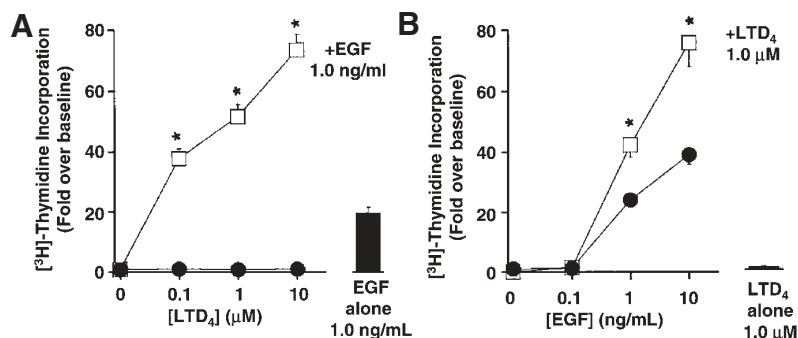


FIG 7. A, Effects of LTD₄ alone (filled circles) and the influence of increasing concentrations of LTD₄ on EGF-induced (1.0 ng/mL) human ASM cell proliferation (open squares). **B**, Influence of 1.0 μmol/L LTD₄ (open squares) on EGF-induced human ASM cell proliferation (filled circles). Effects of 1.0 μg/mL EGF (**A**) and 1.0 μmol/L LTD₄ (**B**) alone are shown in the histograms. The data are expressed as [3H]-thymidine incorporation and are the mean ± SEM of 6 experiments; each condition requires 6 replicates. *Significant compared with EGF alone, $P < .05$. From Panettieri et al. *Am J Respir Cell Mol Biol* 1998;19:453-61.

cell contraction and induce airway hyperresponsiveness (AHR) in asthma.

LEUKOTRIENE RECEPTOR EXPRESSION ON AIRWAY SMOOTH MUSCLE CELLS

Evidence suggests that CysLTs, especially LTD₄, play an important role in the asthmatic bronchoconstriction. For example, leukotriene modifier drugs that block the action of LTD₄, either by inhibiting its synthesis or by abrogating its ability to bind to the CysLT receptor, are efficacious in the treatment of asthmatic patients.⁶⁹ Despite the characterization of the structure and the sequencing of the CysLT receptors, little is known concerning the cellular and molecular mechanisms that alter CysLT receptor expression.

CYTOKINE INFLUENCE

According to a recent study, interferon (IFN)-γ enhances the expression of the CysLT₁ receptor and increases contractile responses to LTD₄ in human ASM cells.⁷⁰ When measured by flow cytometry, the upregulation of CysLT₁ was associated with an increase in steady-state mRNA levels of the CysLT₁ and CysLT₂ genes. An upregulation of the CysLT₁ receptor and an enhanced sensitivity to LTD₄ was also observed in response to IL-5 in HL-60 cells.⁴⁹ Furthermore, in untreated eosinophils, the CysLT₁ mRNA levels decreased in a time-dependent manner, whereas mRNA levels were maintained in the presence of IL-5. These studies suggest that increased gene expression may account for cytokine-induced increases in CysLT₁ receptor in some cells.

VIRAL INFECTIONS

The ability of IFN-γ to modulate CysLT₁ receptor expression and function in human ASM cells has important implications for airway diseases such as asthma. First, both CysLT₁ receptor protein and mRNA are

expressed in vivo in ASM.⁷¹ Second, IFN-γ levels within the airways are dramatically increased in asthmatic persons^{72,73} and after viral infections.⁶⁹ The primary components of the IFN-γ signaling system, STAT-1, and STAT-1-dependent genes, have been activated in the airway epithelium of asthmatic patients.⁷⁴ Finally, IFN-γ has been implicated in virus-induced and allergen-induced AHR.^{75,76}

Viral infections are a common trigger for asthma exacerbations⁶⁹ and, even in nonasthmatic persons, they may induce AHR and cough that can persist for weeks or months. The precise mechanisms by which viruses alter ASM function remain unknown. Typically, viral syndromes are characterized by intense inflammatory responses in the airways with marked leukocyte trafficking and production of leukotrienes and T_H1 cytokines such as IFN-γ.⁷⁷ In young children with acute episodes of virus-induced wheezing, levels of IFN-γ and CysLTs were significantly increased in sputum.⁷⁷ The role of CysLTs is further supported by the fact that the administration of montelukast, a CysLT₁ receptor antagonist, resulted in a significant clinical improvement in children with persistent wheezing associated with viral infection.⁷⁸ These data suggest that IFN-γ may promote the development of virus-induced AHR by enhancing ASM contractile responses to LTD₄. More clinical studies are needed to determine whether leukotriene antagonists may be useful in treating virus-induced AHR and cough.

SMOOTH MUSCLE AND CELL STIFFNESS

Interferon-γ has been shown to enhance LTD₄-induced changes in human ASM cell stiffness. Measured by magnetic twisting cytometry, cell stiffness can be used as a proxy for force generation in ASM cells.^{79,80} It is likely that the observed changes in cell stiffness are caused by increases in CysLT₁ expression with IFN-γ treatment (Fig 6).⁷⁰ Because the measured stiffness relates to the stiffness of all cytoskeleton elements including actin and myosin, it is plausible that IFN-γ effects on cell stiffness

responses may result from the effects of the cytokine on cytoskeleton proteins, as has been described in lung carcinoma cells⁸¹; however, this is unlikely because bradykinin caused similar cell stiffness responses with or without IFN- γ present.⁷⁰ The lack of effect of IFN- γ on responses to bradykinin suggests that other mechanisms, such as an increase in G-protein-coupled signaling events,⁸² are also unlikely explanations for the effect of IFN- γ on LTD₄-induced force generation.

MODULATORS OF ASM CELL PROLIFERATION

Although the leukotrienes mimic many asthma features, little is known about the influence of the leukotrienes on ASM proliferation. Both LTB₄ and LTD₄ augmented ASM proliferation in vitro when combined with EGF or IGF,⁸³ and growth factors play a crucial role in regulating cell proliferation and survival.^{84,85} In a model of asthma, repeated challenge with ovalbumin (OVA) in OVA-sensitized rats produced increased ASM mass in airways larger than 2 mm in diameter, which was partially inhibited by the CysLT receptor antagonist MK-571, suggesting that CysLTs play a role in this phenomenon.⁸⁶ Furthermore, LTD₄ potentiated the IGF-1-induced proliferation of rabbit tracheal smooth muscle cells.⁸⁷ In human ASM cells, LTD₄ induced the release of matrix MMP-1, which may be an IGF-binding protease.³¹ These preliminary data support an important role for CysLTs in lung remodeling. Although LTD₄ alone has no effect on the proliferation of human ASM cells, LTD₄ markedly potentiated mitogenesis elicited by growth factors (eg, EGF and thrombin) (Fig 7).³²

The CysLT receptor antagonists pranlukast and pobilukast inhibited LTD₄-induced proliferation, indicating a receptor-mediated phenomenon. Furthermore, the lack of influence of LTD₄ on expression of collagen or extracellular matrix protein mRNAs suggests that the LTD₄-mediated effect on human ASM proliferation is specific and does not represent a generalized activation of these cells.

Although in human bronchus, pranlukast, zafirlukast, and pobilukast inhibited LTD₄-induced contractions with similar potencies, their ability to inhibit the LTD₄ potentiation of EGF-induced human ASM cell growth were markedly different.³² The augmented proliferation elicited by LTD₄ was abolished by pranlukast (1 μ mol/L) and pobilukast (30 μ mol/L), whereas zafirlukast, at a concentration of 1 μ mol/L, was without effect; a higher concentration of zafirlukast (10 μ mol/L) caused detachment of the human ASM cells. The differences in the relative potencies to inhibit LTD₄-induced contraction and the potential of EGF-elicited DNA synthesis provide preliminary evidence to suggest that different CysLT receptors may mediate these activities.

REMODELING AND AIRFLOW OBSTRUCTION

Persistent allergic airway inflammation in asthma is accompanied by airway remodeling changes, including

TABLE I. Airway remodeling in asthma

Airway wall thickening
Subepithelial fibrosis
Hyperplasia of mucus glands
Myofibroblasts
Smooth muscle
Vasculature

TABLE II. Airway wall thickening in asthma

Deposition beneath basement membrane of collagen
Fibronectin
Tenascin
Laminin

hyperplasia of airway mucus glands, myofibroblasts, smooth muscle and vasculature, and the thickening of the airway wall with subepithelial fibrosis (Table I).^{88,89} Airway thickening beneath the basement membrane occurs with collagen deposition and other extracellular matrix proteins, including fibronectin and tenascin in the connective tissue layer surrounding the blood vessels, and alveolar interstitium (Table II). In situ hybridization studies demonstrated type I collagen gene expression in connective tissue fibroblasts.⁹⁰ This airway wall thickening correlated with clinically severe asthma⁹¹ and was a prominent feature of lung tissue from patients dying with fatal asthma. Degradation of the extracellular matrix may also decrease secondary to an imbalance between MMPs and their tissue inhibitors of metalloproteinases (TIMPs).⁹² Myofibroblasts are important for the remodeling process because they secrete extracellular matrix proteins, whereas epithelial cells and infiltrating inflammatory cells release profibrotic cytokines (eg, TGF- β 1, IGF, and PDGF). The result is increased collagen expression in the airways.^{21,22,93,94} Of these cytokines, TGF- β 1 may play a key role in the development of airway fibrosis in asthma and is implicated in the differentiation of fibroblasts to myofibroblasts and increased CysLT synthesis by macrophages.^{82,83}

VARIABLE ASTHMA PHENOTYPES

Asthmatic patients have shown significant variability in cellular inflammation and structural changes, suggesting that distinct immunologic/pathologic phenotypes may exist.⁹⁵ Patients with mild persistent asthma have shown features of airway remodeling in bronchial biopsy specimens, including thickening of the subepithelial lamina reticularis.⁹⁶ Most patients with mild persistent asthma, however, do not have progressive airflow limitation characteristic of severe asthma. A 15-year longitudinal study of the decline in FEV₁ in adults with self-reported asthma (400 asthmatic persons vs 17,506 nonasthmatic persons) found a greater decline in pulmonary function in patients with asthma.¹¹ Cigarette smoking significantly added to this decline in both asthmatic and nonasthmatic persons.¹¹ In a 10-year follow-up of 92 lifelong nonsmoking asthmatic patients, 23% had nonreversible

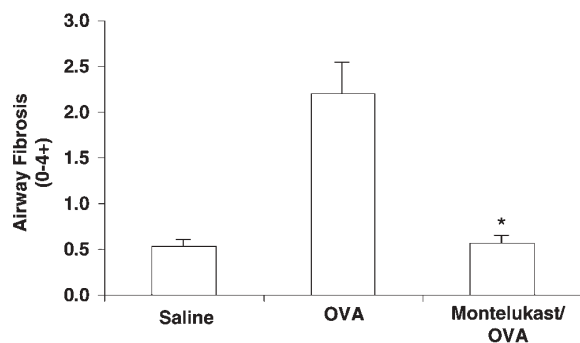


FIG 8. Inhibition of airway fibrosis by CysLT₁ receptor blockade. Lung tissue was obtained from saline-treated mice (*Saline*) and OVA-sensitized/challenged mice in the absence (*OVA*) or presence of treatment with montelukast (*Montelukast/OVA*). Airway collagen deposition/fibrosis was assessed by Masson trichrome staining on a 0 to 4+ scale by morphometric analysis. Montelukast significantly reduced the airway fibrosis seen in OVA-treated mice. From Henderson et al. *Am J Respir Crit Care Med* 2002;165:108-16.

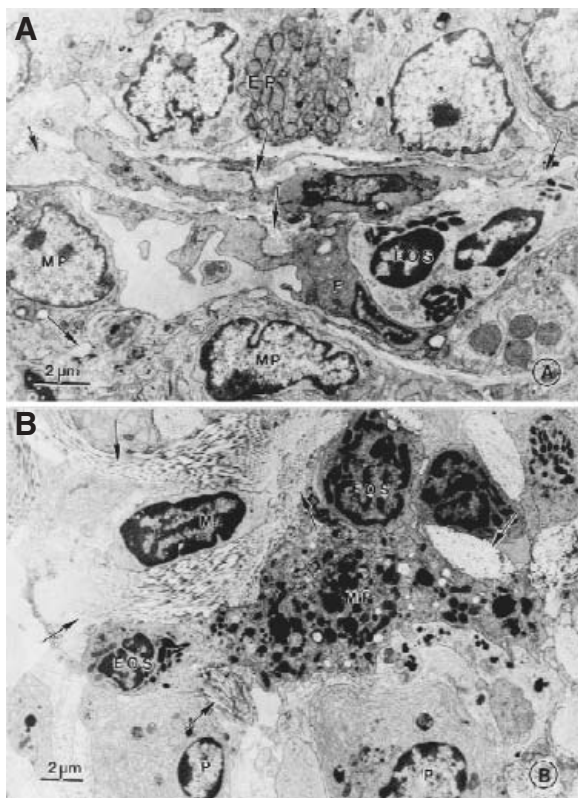


FIG 9. Reduction in airway collagen deposition by CysLT₁ receptor blockade. Deposition of collagen (arrows) in the airways of OVA-sensitized/challenged mice in the presence (A) or absence (B) of montelukast was assessed by electron microscopy. Dense collagen bundles seen in the lung interstitium of OVA-treated mice (A) were markedly decreased by montelukast treatment. From Henderson et al. *Am J Respir Crit Care Med* 2002;165:108-16.

obstruction.⁹⁷ It is not known why some patients show a decline of airway function that may be progressive and unresponsive to therapy.

The FEV₁ of persons with severe asthma after therapy with bronchodilators and prednisone is lower than predicted (suggesting irreversible airflow obstruction) and correlates inversely with age, duration, and asthma severity. Asthmatic patients in respiratory failure have shown evidence of airway remodeling with basal lamina thickening.^{98,99} Patients with severe asthma may have a different airway pathologic process than that seen in asthmatic patients with less severe disease.¹⁰⁰ In 34 patients with severe corticosteroid-dependent asthma, 2 inflammatory subtypes were identified: a group lacking eosinophils on bronchial biopsy specimens and a group with eosinophils. The eosinophil (+) group had greater thickening of the subbasement membrane and more intubations than the eosinophil (–) group.⁹⁹

Leukotrienes and extracellular matrix secretion

Smooth muscle cells express a wide variety of extracellular matrix macromolecules in vitro.¹⁰¹ Little is known about human ASM cell expression of extracellular matrix or collagen, however. Evidence suggests that cultured human ASM cells express types I and IV collagen, fibronectin, elastin, small dermatan/chondroitin sulfate, and proteoglycans (biglycan and decorin). The matrix macromolecules, collagen types III and IV, constitute the bulk of the connective tissue in the arteries and airways of the lungs. It is well known that the extracellular matrix influences the migration and proliferation of many cell types. The expression of genes that encode the collagen and noncollagen matrix proteins are regulated by several cytokines, including TGF- β . A study in human ASM cells has shown that TGF- β but not LTD₄ (unlike fibroblasts) upregulates expression of several of the extracellular matrix genes. Fibroblasts upregulate collagen, in particular type I and IV collagen.³² TGF- β also increased elastin, fibronectin, and biglycan mRNAs severalfold. In contrast, TGF- β markedly reduced decorin mRNA levels in human ASM cells, whereas decorin mRNA and protein levels were unaltered in lung fibroblasts.¹⁰² Although LTD₄, alone or in conjunction with TGF- β , does not appear to modulate the production of extracellular matrix molecules by human ASM cells directly, the LTD₄ potentiation of EGF-induced human ASM cell proliferation may yield a net increase in these molecules at the tissue level.

Leukotrienes in airway inflammation and remodeling in a mouse asthma model

Important insights into the mechanisms of chronic inflammation in asthma have come from studies of allergen-induced airway inflammation in animal models. Mice, sensitized to OVA by intraperitoneal and intranasal routes and challenged with airway administration of the allergen, had pathologic and immunologic features characteristic of human asthma. The OVA challenge elicited eosinophil infiltration into the lungs and widespread mucus occlusion of the airways and increased the expression of T_H2 cytokines (IL-4, IL-5, IL-10, and IL-13) in

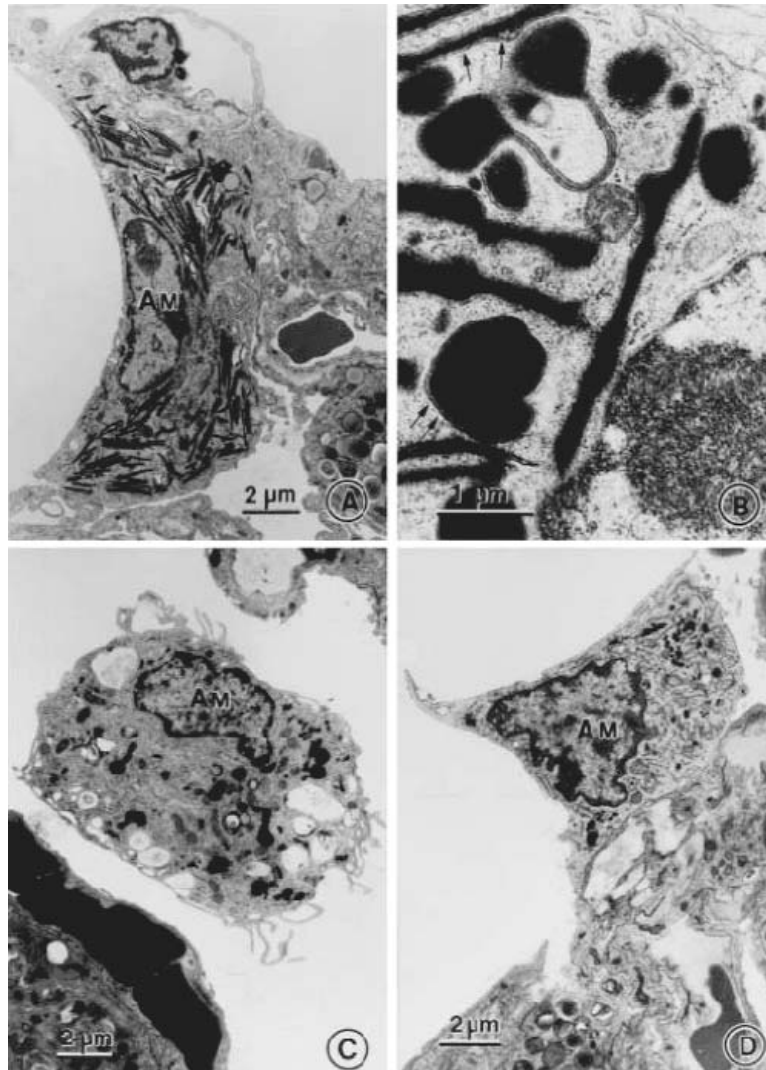


FIG 10. Inhibition of Charcot-Leyden-like crystal release by CysLT₁ receptor blockade. Charcot-Leyden-like crystal protein was noted by electron microscopy in alveolar macrophages in OVA-treated mice (**A**, **B**) but not in OVA-sensitized/challenged mice treated with montelukast (**C**) and saline controls (**D**). From Henderson et al. *Am J Respir Crit Care Med* 2002;165:108-16.

lung and bronchial lymph node tissue and of AHR to methacholine.¹⁰³⁻¹⁰⁵

The role of leukotrienes in mediating airway eosinophil infiltration, mucus release, and AHR to methacholine has been examined. In OVA-sensitized/challenged mice, 5-LO and 5-LO-activating protein (FLAP) gene expression were found in leukocyte infiltrates around airways and pulmonary blood vessels.¹⁰⁶ 5-LO, FLAP mRNA, and protein expression were also induced in pulmonary blood vessel endothelial cells in OVA-treated mice.¹⁰⁶ Specific inhibitors of 5-LO and FLAP that prevent leukotriene synthesis block airway mucus release and infiltration by eosinophils, indicating an important role for leukotrienes in these features of allergic airway inflammation.¹⁰³ Leukotriene blockade, however, did not affect AHR in OVA-sensitized mice as measured by

methacholine, indicating that AHR in these mice can occur without airway eosinophilia.¹⁰³

The role of leukotrienes was examined in a mouse model of allergen-induced chronic lung inflammation in which fibrosis and other features of airway remodeling developed (Figs 8 and 9).¹⁰⁷ After intraperitoneal OVA sensitization on days 0 and 14, BALB/c mice received intranasal OVA periodically from days 14 through 75. The OVA-treated mice had an extensive eosinophil and mononuclear cell inflammatory response, goblet cell hyperplasia with mucus occlusion of the airways, and increased thickness of the ASM layer compared with saline-treated control mice. A striking feature of this inflammatory response was the widespread deposition of collagen beneath the airway epithelial cell layer and also in the lung interstitium that was not observed in the control mice.

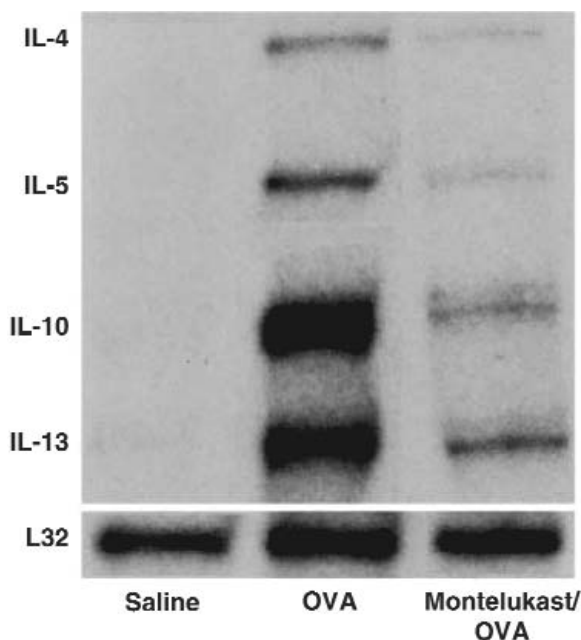


FIG 11. Inhibition of T_H2 cytokine expression by CysLT₁ receptor blockade. The increased lung mRNA expression of T_H2 cytokines (IL-4, IL-5, IL-10, and IL-13) seen in OVA-treated mice (compared with saline controls) was markedly reduced by montelukast. From Henderson et al. *Am J Respir Crit Care Med* 2002;165:108-16.

Cysteinyl leukotriene blockade by the CysLT₁ receptor antagonist montelukast significantly inhibited the airway eosinophil infiltration, hyperplasia of smooth muscle cells and goblet cells, mucus plugging, and subepithelial fibrosis in the OVA-sensitized/challenged mice. Eosinophil degranulation and release of Charcot-Leyden-like crystals in airway tissue in the OVA-treated mice were inhibited by CysLT₁ receptor blockade (Fig 10). Montelukast administration also reduced the increased T_H2 cytokine mRNA expression (Fig 11) in lung tissue and protein in bronchoalveolar lavage fluid found in OVA-sensitized/challenged mice. These results suggest that CysLTs are important in the pathogenesis of chronic allergic airway injury and fibrosis.

EFFECTS OF LEUKOTRIENE MODIFIERS ON INFLAMMATION AND REMODELING

A number of leukotriene modifiers have been introduced as alternative or additional anti-inflammatory agents (Table III). The 5-LO inhibitor (zileuton) and CysLT₁ receptor antagonists (montelukast, zafirlukast, and pranlukast) have been effective in a considerable percentage of asthmatic individuals, both adults and children.^{108,109} Leukotriene modifiers improved pulmonary function, reduced the need for rescue medication with β_2 -agonists, relieved asthma symptoms, decreased the frequency of exacerbations, and reduced the dose of steroids required to control asthma.¹⁰⁹⁻¹¹¹ In patients with mild persistent asthma, montelukast reduced the

TABLE III. Anti-inflammatory and antiremodeling effects of LTRAs

CysLT ₁ receptor antagonists block key features of allergic airway inflammation and remodeling including
Eosinophil trafficking to the lungs and eosinophil degranulation
Pulmonary T_H2 cytokine release
Airway goblet cell hyperplasia and mucus hypersecretion
Airway smooth muscle hyperplasia
Collagen deposition and fibrosis in the airways

concentration of leukotrienes in the respiratory tract,¹¹² and both zafirlukast and montelukast attenuated the early- and late-phase responses to inhaled allergen¹¹³ and reduced antigen-induced AHR to histamine.¹¹⁴

Eosinophil trafficking and degranulation

Cysteinyl leukotrienes are key mediators of the trafficking of eosinophils and other leukocytes to airway sites of allergic inflammation. The CysLTs are potent eosinophil chemoattractants, and CysLT₁ receptors are expressed on eosinophils, monocytes, macrophages, basophils, and B lymphocytes.⁷¹ The CysLT₁ receptor expression was also found on CD34⁺ pregranulocytic cells,⁷¹ suggesting that CysLTs may modulate the differentiation pathways of these cells to eosinophils and other leukocytes. Furthermore, leukotrienes promoted eosinophil survival in vitro, and CysLT₁ receptor antagonists reversed the prolonged survival of eosinophils observed in patients with asthma.¹¹⁵ Thus, CysLT₁ receptor antagonists may block eosinopoiesis and eosinophil chemotaxis and promote eosinophil apoptosis, leading to the decreased accumulation of eosinophils in allergic airways. Inhibition of eosinophil degranulation and Charcot-Leyden crystal protein release were additional anti-inflammatory effects of the CysLT₁ receptor blockade observed in the mouse asthma model.¹⁰⁷ Zileuton inhibited leukocyte synthesis by ~70% to 90%,¹¹⁶ and montelukast reduced the recruitment of eosinophils and IL-5-expressing cells into the airways of mice and rats after allergen challenge.¹¹⁷⁻¹¹⁹ In patients with mild asthma, montelukast has been effective in reducing blood eosinophilia and asthma symptoms and improving pulmonary function.^{108-111,114}

T_H2 Cytokine release

Inhibition of T_H2 cytokine release by CysLT₁ receptor blockade probably is an important anti-inflammatory action of this treatment. Allergen-induced production of T_H2 cytokines, including IL-4, IL-5, and GM-CSF by peripheral blood mononuclear cells from patients with bronchial asthma after allergen stimulation, was inhibited by the CysLT₁ receptor antagonist pranlukast.¹²⁰ In a rat model of asthma, airway eosinophilia and IL-5-expressing cells were inhibited by montelukast.¹¹⁸ Thus, the inhibition of T_H2 cytokine (eg, IL-5) production may be the mechanism by which leukotriene inhibitors attenuate mucus gland hyperplasia and mucus hypersecretion.¹¹⁸ T_H2 cytokines (IL-4 and IL-13) and CysLTs induced

mucus hypersecretion^{121,122}; IL-13 was a profibrotic cytokine.²⁸ Inhibition by montelukast of mucus gland hyperplasia and mucus occlusion of airways in the mouse asthma model may be through interference with T_H2 cytokine production and through blocking direct actions of CysLTs on the airway goblet cells.¹⁰⁷

Airway smooth muscle hyperplasia

Previous studies have shown that the increase in ASM observed in allergen-treated Brown-Norway rats^{86,123,124} was reduced by CysLT₁ receptor antagonism, and LTD₄ increased growth factor-induced human ASM proliferation.³² These data, together with findings that ASM hyperplasia was inhibited by montelukast in a mouse model of asthma with airway remodeling,¹⁰⁷ suggest that CysLTs have important effects on smooth muscle proliferation in allergic airways.

Collagen deposition

The excessive release of leukotrienes may be important for the development of interstitial lung fibrosis in patients with excessive deposition of extracellular matrix proteins.⁴¹ Mouse alveolar macrophage release of FGF was stimulated by CysLTs.¹²⁵ Fibroblast proliferation in cell cultures was also increased by the LTs³⁷: LTC₄ and LTE₄ stimulated mouse bone collagen synthesis in vitro,¹²⁶ and LTC₄ bound to rat lung fibroblasts and stimulated collagen production in vitro.³⁸ In human ASM cells, LTD₄ was capable of releasing MMP-1.³¹ LTC₄ upregulated collagenase expression and synthesis in human lung fibroblasts,¹²⁷ suggesting that CysLTs may modulate extracellular matrix remodeling during pulmonary inflammation. Decreased parenchymal collagen deposition in bleomycin-treated mice, whose 5-LO gene was disrupted, additionally supports a role for CysLTs in promoting fibrosis.⁴² The dramatic reduction in airway collagen deposition by montelukast in the chronic mouse asthma model indicates a potent antifibrotic action of CysLT₁ receptor blockade.¹⁰⁷

Thus, evidence from animal and human studies of asthma indicate that antileukotriene therapy may help prevent such structural changes of airway remodeling as the goblet cell and smooth muscle cell hyperplasia and the fibrosis accompanying allergen-induced inflammatory responses. It will be important to test this possibility directly in appropriately designed clinical trials.

CONCLUSIONS

A new paradigm for asthma pathogenesis was presented in which exaggerated inflammation and remodeling in the airways are a consequence of abnormal injury and repair responses arising from the susceptibility of the bronchial epithelium to components of the inhaled environment. At this environment-gene interface, an EMTU becomes activated to drive pathologic remodeling and smooth muscle proliferation through complex cytokine interactions. This paradigm also suggests that remodeling and inflammation may be parallel processes for which the structural changes in adult disease are present

as early and consistent components of childhood asthma. Each is subsequently influenced in parallel rather than in a sequence from inflammation to remodeling.

Although histamine, prostanoids, and CysLTs are potent contractile agonists of ASM, the CysLTs appear to play a central role in regulating human ASM motor tone and phenotypic alterations, manifested as hypertrophy and hyperplasia in chronic severe asthma. The CysLTs augment growth factor-induced ASM mitogenesis through the activation of CysLT receptors. Although these receptors mediate their contractile effects by increasing phosphoinositide turnover and inducing increased cytosolic calcium, data suggest that part of the contractile effect may be independent of calcium mobilization. Prostaglandin E₂, the predominant eicosanoids of the airway epithelium, is a potent inhibitor of mitogenesis, collagen synthesis, and mesenchymal cell chemotaxis and therefore can suppress inflammation and fibroblast activation. The capacity of the epithelium for CysLT synthesis is inversely related to its ability to make PGE₂. This capacity may explain why the CysLTs promote airway hyperresponsiveness in asthma.

The CysLTs play an important role in the airway remodeling seen in persistent asthma, which includes increases of airway goblet cells, mucus, blood vessels, smooth muscle, myofibroblasts, and airway fibrosis. Evidence from a mouse model of asthma demonstrated that CysLT₁ receptor antagonists inhibit the airway remodeling processes, including eosinophil trafficking to the lungs, eosinophil degranulation, T_H2 cytokine release, mucus gland hyperplasia, mucus hypersecretion, smooth muscle cell hyperplasia, collagen deposition, and lung fibrosis.

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QUESTIONS AND DISCUSSION

Hans Bisgaard: Stephen, I think what you are suggesting is fascinating. You are taking allergy out of the equation. You are suggesting that asthma develops as an interaction between genes and environment and that allergy does not really cause the process of asthma. I think it's important for our appreciation of the early development of asthma that we should be looking for the critical event much earlier.

Stephen Holgate: Yes. I think that the German Multicenter Asthma Study (MAS) has shown that children at the age of 7 seem to reflect an allergen sensitization over time but not the development of asthma over time. And there are some very nice studies in Albania showing that ATP was equivalent between children from Albania and

northwest of London. About 35% of the children showed the same size wheal to the same antigens. But asthma and bronchial hyperresponsiveness in the children from Albania were only 1%, whereas they were 17% in the children from northwest London. There seems to be something causing the asthma that is separate from allergen. Furthermore, in a study from John Warner's group, they tried to reduce the allergens in homes to very low levels by removing carpets and putting plastic covers on bedding. Rather than decreasing the occurrence of asthma, the asthma increased by a highly significant amount, which was the opposite of what they expected.

Hans Bisgaard: That changes the strategy for early intervention. Where does that leave the models based on allergen exposure or allergen challenge?

Stephen Holgate: If there is an interaction between the cytokine mediators from inflammation on the susceptible airway, then an inflammatory response can reveal a phenotype. But the mediator alone is insufficient to support the phenotype. I think that really is the principle we're trying to move toward. It changes the way you think about therapeutics and you may want to protect the airway against environmental injury from an early age, such that these other things just cannot take root.

Anthony Sampson: I was relieved to see the anti-IL-5 data because I had believed that the eosinophil was dead. That data was very much misinterpreted. If you are developing a drug inhibitor, you want to see at least a 1 or 2 log inhibition, but the data only showed a 75% reduction, and that is not enough to say that you've removed the eosinophils from the equation. The Leachy paper showed an effect on the late effect that was poorly analyzed with multiple comparisons. It seemed to me that the exacerbation rates showed a clear dose-dependent trend for exacerbations to be reduced, but it was not significant because it was underpowered (6 exacerbations in 20 weeks), and the FEV₁ data were not corrected for baseline. It looked to me that there is very good evidence that eosinophils are involved in asthma, not the reverse.

Stephen Holgate: I would have expected a more powerful effect on the basis of steroid effects on eosinophils or an anti-IgE effect on eosinophils. It is surprising that with such major changes in those two compartments that there was not something more powerful that's coming through. It doesn't mean that there is none, but it doesn't give you confidence that many patients would want this treatment if they were offered it.

Reynold Panettieri: In your wound model, you showed important findings within 9 hours. That's a rather short period of time for cell proliferation to repopulate the lesion. Did you see cell proliferation or migration? I suspect it was migration.

Stephen Holgate: You could well be right. If one actually stains up that particular series of blocks with PCNA or PC67, for example, as a proliferative marker, you can see proliferation taking place, but I would strongly suggest that the majority of the closure is due to cell migration. The principle we're trying to present is that there is an enhanced generation of a variety of other profiles with

the fibrogenic growth factors that may be relevant to events that happen subsequently, if you stop the epithelium from restituting properly.

Stephen Holgate: Marc, what is the evidence that PGE₂ is deficient in asthmatic epithelial cells or airways?

Marc Peters-Golden: In the heaves model of asthma in horses, the time from when the horse went into the barn until it developed clinical disease inversely correlated with epithelial PGE₂ synthesis. The time to develop disease could be shortened by giving indomethacin.¹ These data fit with the idea that when there was already a deficiency of PGE₂, if you further reduced PGE₂ levels, you could precipitate disease sooner. Data from Caesar Picado also showed that in patients with aspirin-sensitive asthma, the COX-2 mRNA expression was less.² We have shown that fibroblasts from idiopathic pulmonary fibrosis also have reduced PGE₂ synthetic capacity, and one of the mechanisms that we established was that those fibroblasts had an inability to induce COX-2. Therefore, COX-2 is protective largely under chronic disease conditions. The reason you see COX-2 at inflammation sites is because it represents a counterregulatory attempt to reduce inflammation and remodeling, and in disease states where COX-2 cannot be induced, as in pulmonary fibrosis and aspirin-sensitive asthma, you cannot develop that counterregulation, and the diseased phenotype or remodeling response predominates.

Qutayba Hamid: Is there evidence to suggest that PGE₂ increases in asthma?

Marc Peters-Golden: I think it was Peter Barnes and Ian Roger who stained for COX-2 in airway biopsies, and they reported higher levels of COX-2 in asthma, so that might suggest a difference from our data.

Stephen Holgate: It is surprising that the selective inhibitors of COX-2 do not have more adverse reactions, such as augmenting inflammation and tissue remodeling responses. And in aspirin-intolerant asthma, it's the opposite.

Marc Peters-Golden: My theoretical explanation is that the reason that COX-2 inhibitors might be well tolerated in aspirin-sensitive asthma is that based on Picado's data, aspirin-sensitive asthmatics already are lacking COX-2, so that COX-1 is the sole source of PGE₂. Therefore, a COX-2 inhibitor would not have any effect on them.

Anthony Sampson: In aspirin-induced asthmatic persons, the COX is the problem, and much of the PGE₂ break is coming from COX-1, not COX-2. This is why Picado's data do not fit the prevailing view in aspirin-induced asthma. It has nothing to do with COX-2 at all; the PGE₂ break is from COX-1. It does not depend on shunting; rather, it depends on a receptor-dependent process.

Marc Peters-Golden: I agree completely, and the reason is that they are already lacking COX-2. If you inhibit COX-2 in someone who does not have a challenge to their lung, you are not going to see adverse effects. For example, COX-2 knockout mice do not have an abnormal phenotype when unchallenged. But when you challenge them with antigen or with bleomycin, you will produce exaggerated respiratory responses. The other part of the answer to your

question is that the role of PGs in the joint is very different from their role in the lung. Most of the current data with COX-2 in the lung is suggesting that PGE₂ is a protective molecule. I don't think that the clinical experience in arthritis has been able to appropriately test that.

Anthony Sampson: Right. The COX-2 knockouts have no problem, but the COX-1 knockouts have something very like aspirin-induced asthma: reduced PGE₂, very, very high LTC₄, lots of eosinophilia, and it seems that these fit with the idea that COX-2 inhibition, removal of that break, is what triggers these longer-term effects in aspirin asthma as well as the short-term exacerbations.

Marc Peters-Golden: Yes, and what they've also shown is that the relative importance of COX-1 or COX-2 depends on the kind of challenge presented. In allergen challenge, both COX-1 and COX-2 gave more inflammation, but the inflammation was worse and the hyper-reactivity was worse in the COX-1 knockouts. But another challenge, using lipopolysaccharide, produced the opposite result. Only COX-2 led to worse responses, so there's a lot of stimulus-specificity in these.

Bill Henderson: Rey, does the CysLT₂ receptor play a role in growth or proliferation? How would you go about answering that question?

Reynold Panettieri: I do not know what its role is. Our approach will be to use the individual receptor clones and overexpress them in ASM. We'll see if we get augmented growth. I think that's the only way to do it. We have a human CysLT₁ receptor overexpressing mouse from which we will grow the ASM that will have the selective increase of that receptor. We will also be looking at AHR in that mouse.

Mark Liu: Do you have any evidence that β_2 -agonist alone inhibits smooth muscle growth?

Reynold Panettieri: Yes, absolutely. Formoterol is the best, salmeterol is second best, and albuterol does the least at inhibiting human ASM cell growth.

Mark Liu: Have you tested cytokines besides IFN- γ ?

Reynold Panettieri: What was reported recently^{3,4} was that in an eosinophil cell line, IL-4 and IL-5 induced increased expression of CysLT₁ receptor. Based on that finding, we did the same experiment in human ASM, and there was no effect. When we looked at the effects of IL-13 and IL-4, they have disparate effects separately, and when you put them together, they have a different effect. So, the signaling here is really quite intriguing. We want to get the promoter segments of this gene to answer the questions. When you carefully examine the papers and look at expression, what they showed in the presence of IL-4 and IL-5 was not increased expression but rather maintained expression (while the control cells lose expression). The authors' interpretation was that expression was induced, but my interpretation is that expression is sustained without a loss of expression.

Stephen Holgate: There was quite a lot of evidence suggesting that LTD₄ induces certain cell membrane-expressed metalloproteinase enzymes. The CysLT may work mostly on MMP-2, but I think there are other ones as well. Have you done anything on what causes MMP-2 to clip progrowth factors?

Reynold Panettieri: There's a very intimate relationship between MMP expression and the consequences of growth because migration is an important harbinger for proliferation. That is, often cell proliferation requires cell migration. These two processes are intimately related and may depend on MMP expression.

Stephen Peters: Bill, what are the implications from your animal model for the design of human asthma clinical trials of the antiremodeling effects of CysLT₁ receptor antagonism?

Bill Henderson: The striking inhibition by CysLT₁ receptor antagonist therapy of airway wall collagen deposition, mucus production, and smooth muscle hyperplasia in the mouse asthma model suggests that this pharmacologic approach may ameliorate structural changes occurring in the airways of patients with asthma. Clinical trials of long-term treatment with CysLT₁ receptor antagonists in patients with either new-onset or persistent asthma would be of great interest to determine whether airway remodeling changes in asthma can be prevented, or if established, be reversed by such therapy. These studies would likely entail sequential airway biopsies before and after initiation of CysLT₁ receptor treatment. This research would help address whether CysLT₁ receptor therapy is disease modifying in asthma.

Anthony Sampson: In your model, do corticosteroids block airway remodeling fibrosis?

Bill Henderson: We have recently found a marked increase in laminin expression in the lungs of OVA-treated mice compared with saline controls by Western blot analysis (Christie et al, unpublished data). By immunocytochemistry, laminin staining was intense in the pulmonary blood vessel endothelial cell layer and subepithelial lung interstitium. Laminin deposition was reduced in the OVA-treated mice by dexamethasone to indicate an antifibrotic effect of corticosteroid treatment in this model.

Reynold Panettieri: Would you see differences in remodeling changes in different mouse models, and what is the effect of LTD₄ in mice?

Bill Henderson: Marc Peters-Golden and colleagues have recently found that bleomycin induced less lung fibrosis and fewer lung inflammatory cells in 5-LO-knockout mice (129-Alox5^{tm1Fun}) than in strain-matched wild-type mice. In our study of allergen-induced airway fibrosis, BALB/c mice were used. Thus, leukotrienes appear important for development of lung fibrosis in different mouse models of fibrosis. In BALB/c mice, instillation of LTD₄ into the airways induces mucus hypersecretion.⁵

Marc Peters-Golden: Did you measure IL-6 and IFN- γ in the lungs of the OVA-treated mice?

Bill Henderson: In the OVA-sensitized/challenged mice by day 28, there was increased expression of T_H2 cytokines (eg, IL-4, IL-5, IL-13) and decreased expression of T_H1 cytokines (eg, IL-2, IFN- γ) in bronchial lymph node tissue as determined by PCR.⁶ Montelukast inhibits T_H2 cytokine expression in the OVA-treated mice,⁷ but its effects on T_H1 cytokine expressed have not been examined.

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