

The new millennium: The conquest of allergy

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Epithelial-mesenchymal interactions in the pathogenesis of asthma

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During lung development, repair, and inflammation, local production of cytokines (eg, transforming growth factor- β) and growth factors (eg, epidermal growth factor) by epithelial and mesenchymal cells mediate bidirectional growth control effectively creating an epithelial-mesenchymal trophic unit. In asthma the bronchial epithelium is highly abnormal, with structural changes involving separation of columnar cells from their basal attachments and functional changes including increased expression and release of proinflammatory cytokines, growth factors, and mediator-generating enzymes. Beneath this damaged structure there is an increase in the number of subepithelial myofibroblasts that deposit interstitial collagens causing thickening and increased density of the subepithelial basement membrane. Our recent studies suggest that the extent of epithelial damage in asthma may be the result of impaired epidermal growth factor receptor-mediated repair. In view of the close spatial relationship between the damaged epithelium and the underlying myofibroblasts, we propose that impaired epithelial repair cooperates with the T_H2 environment to shift the set point for communication within the trophic unit. This leads to myofibroblast activation, excessive matrix deposition, and production of mediators that propagate and amplify the remodeling responses throughout the airway wall. (J Allergy Clin Immunol 2000;105:193-204.)

Key words: Bronchial epithelium, myofibroblast, airway remodeling, stress, epidermal growth factor, transforming growth factor- β , morphogenesis, trophic unit, allergy, asthma

Evidence continues to grow that asthma and allergic disease are increasing on a worldwide scale, most likely because of changing factors in the environment.¹ The large intercountry differences in asthma prevalence, the observation that only 50% of the asthma phenotype can

Abbreviations used

a-FGF:	Acidic fibroblast growth factor
b-FGF:	Basic fibroblast growth factor
BHR:	Bronchial hyperresponsiveness
COPD:	Chronic obstructive pulmonary disease
ECM:	Extracellular matrix
EGF:	Epidermal growth factor
EGFR:	Epidermal growth factor receptor
ET-1:	Endothelin-1
HETE:	Hydroxyeicosatetraenoic acid
IGF-1:	Insulin-like growth factor-1
MCP:	Monocyte chemotactic protein
MMP:	Matrix metalloproteinase
NF- κ B:	Nuclear factor- κ B
PAR:	Protease-activated receptor
PCNA:	Proliferating cell nuclear antigen
PDGF:	Platelet-derived growth factor
SBM:	Subepithelial basement membrane
SMAD:	Sma- and Mad-related proteins
STAT:	Signal transduction and activator of transcription
TGF- β :	Transforming growth factor- β
T_H2 :	T-helper-2 lymphocyte
TIMP:	Tissue inhibitor of metalloproteinases

be accounted for by the coexistence of atopy, and the rising trends linked to a Western-type lifestyle point to important factors that underpin asthma pathogenesis in addition to allergen exposure.² On the basis of careful cohort longitudinal studies, susceptibility for asthma seems in large part to be determined during fetal development and the first 3 to 5 years of life.³ Genetic and environmental factors operate at a time of rapid lung growth and remodeling and set the course for airway structure and function later in life. Perturbations during this critical period predispose the airways to recurrent respiratory viral infection in early life, increase susceptibility to environmental pollutants such as cigarette smoke, and predispose the lower airways to sensitization with aeroallergens.⁴

In contrast to many animal models of T-helper-2 lymphocyte (T_H2) polarization in the lung that are self-limiting, asthma is a chronic disorder that is characterized by

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exacerbations and remissions and an underlying bronchial hyperresponsiveness (BHR) of the airways to a wide variety of environmental factors. In established disease a major part of the BHR can be separated from the inflammatory response and most likely represents structural changes in the airways with extensive epithelial damage, deposition of extracellular matrix (ECM) proteins throughout the airways and other features of "remodeling," which include epithelial goblet cell metaplasia, smooth muscle hypertrophy, and increases in nerves and blood vessels. Whether these features occur as a consequence of, or in parallel with, the inflammatory response is not known, but increasingly it is being appreciated that many of the airway's structural elements are themselves altered to produce cytokines, growth factors, and mediators that may contribute to sustaining the inflammatory response.

EPITHELIAL DAMAGE AND STRESS

The bronchial epithelium acts as the physical barrier separating the external environment and the internal milieu of the lung. Therefore, maintaining its integrity is an important component of airways defense. Impairment of this protective function in asthma was originally suggested by the extent of epithelial damage that was evident in asthmatic airways at autopsy.⁵ That epithelial shedding is an important feature of asthma is supported by the presence of increased numbers of epithelial cell clumps (Creola bodies) in sputum^{6,7} and the disruption of the bronchial epithelium that is frequently observed in mucosal biopsy specimens obtained from asthmatic airways by either rigid⁸ or fiberoptic⁹⁻¹¹ bronchoscopy. Although it has been suggested that much of the epithelial damage observed in fiberoptic bronchoscopy biopsy specimens is artifactual,¹² the increased expression of the adhesion molecule CD44¹³ in areas of damage provides evidence that this has occurred before the biopsy because in vitro CD44 is specifically expressed when epithelial cells lose cell-cell contact.¹⁴ Similarly, increased expression of the epidermal growth factor receptor (EGFR, c-erbB1),¹⁵ which typically occurs in response to injury, is also evident in areas of epithelial damage in asthmatic bronchial epithelium. Although the number of injured and repairing cells is increased in the asthmatic epithelium, there is no evidence of a gross structural defect. Ultrastructural analysis of undamaged areas has shown that cells are able to form normal cell-cell junctions.¹⁶ Thus, to explain the disordered morphologic features, the asthmatic epithelium is either more susceptible to or has an altered response to injury.

Under normal circumstances the damaged epithelium is able to repair itself with remarkable speed.¹⁷ This reflects selective pressure for rapid and effective repair of the airway epithelium, which will reduce individual morbidity and mortality caused by, for example, respiratory virus infection. In contrast to skin, where mechanical integrity is provided by numerous layers of keratinized cells, the airway epithelium is primarily a cellular barrier, consisting of a basal cell layer with a suprabasal layer of

ciliated and secretory columnar cells (Fig 1, A and B).¹⁸ The basal cells are more firmly attached to the underlying basal lamina than to the columnar cells, and when the epithelium is damaged it is columnar cells that are predominantly lost (Fig 1, C).¹⁹ This selective loss is clearly seen in asthmatic individuals where loss of columnar epithelial cells may be increased by 15% to 45% compared with that of nonasthmatic subjects.¹⁹ Removal of inhaled particulate material also relies on mucus production and ciliary clearance by the epithelial cells and may be compromised in the absence of differentiated epithelial cells. Only in severe asthma is significant loss of the basal layer seen and even then more than 85% of the basement membrane remains covered with cells of a basal phenotype.¹⁹ In full-thickness damage to the epithelium, repair is initiated by the laying down of a temporary fibrin-fibrinogen matrix and dedifferentiation of epithelial cells and their migration to cover denuded areas of basement membrane.²⁰ Once there is a layer of epithelial cells covering the basement membrane, the normal structure of the epithelium is then progressively restored by a process of cell proliferation and differentiation.

Apart from physical damage, the epithelium in asthma also exhibits features of stress with increased expression of heat shock proteins,²¹ activation of the transcription factors nuclear factor- κ B (NF- κ B),^{22,23} activator proteins (eg, activator protein-1),²⁴ and signal transduction and activator of transcription-1 (STAT-1)²⁵ and widespread increases in expression of the EGFR.¹⁵ This most likely reflects responses to tissue-damaging agents such as peroxynitrite, which is formed from the reaction between oxygen intermediates and nitric oxide, causing epithelial damage through the nitration of proteins, particularly on tyrosines and methionines.²⁶

THE EPITHELIUM AS A SOURCE OF PROINFLAMMATORY MEDIATORS

The airway epithelium is in a key position to coordinate responses to challenges from the environment, as signaled by damage to, or activation of, epithelial cells. In close proximity to the epithelium of the conducting airways are the underlying *lamina propria* and *lamina muscularis mucosa*. It is in these layers that the major structural remodeling of the airways associated with asthma occurs, including thickening of the subbasement membrane ECM and smooth muscle hypertrophy. The altered mediator production by the activated epithelial cells during stress and repair are most likely to affect these processes, reflecting the coordinating role of the epithelium.

In asthma the "stressed" epithelial phenotype is reflected in functional changes, with this structure becoming an important source of autacoid mediators, chemokines, and growth factors.^{27,28} These include oxidative arachidonic acid metabolites (PGE₂, 15-hydroxyeicosatetraenoic acid [HETE], 12,15-diHETE, and 8,15-diHETE), lipoxins nitric oxide, endothelin-1 (ET-1), fibronectin, α - and β -defensins, reactive oxygen (O⁻), IL-8, Gro- α /melanoma growth stimulating activity,

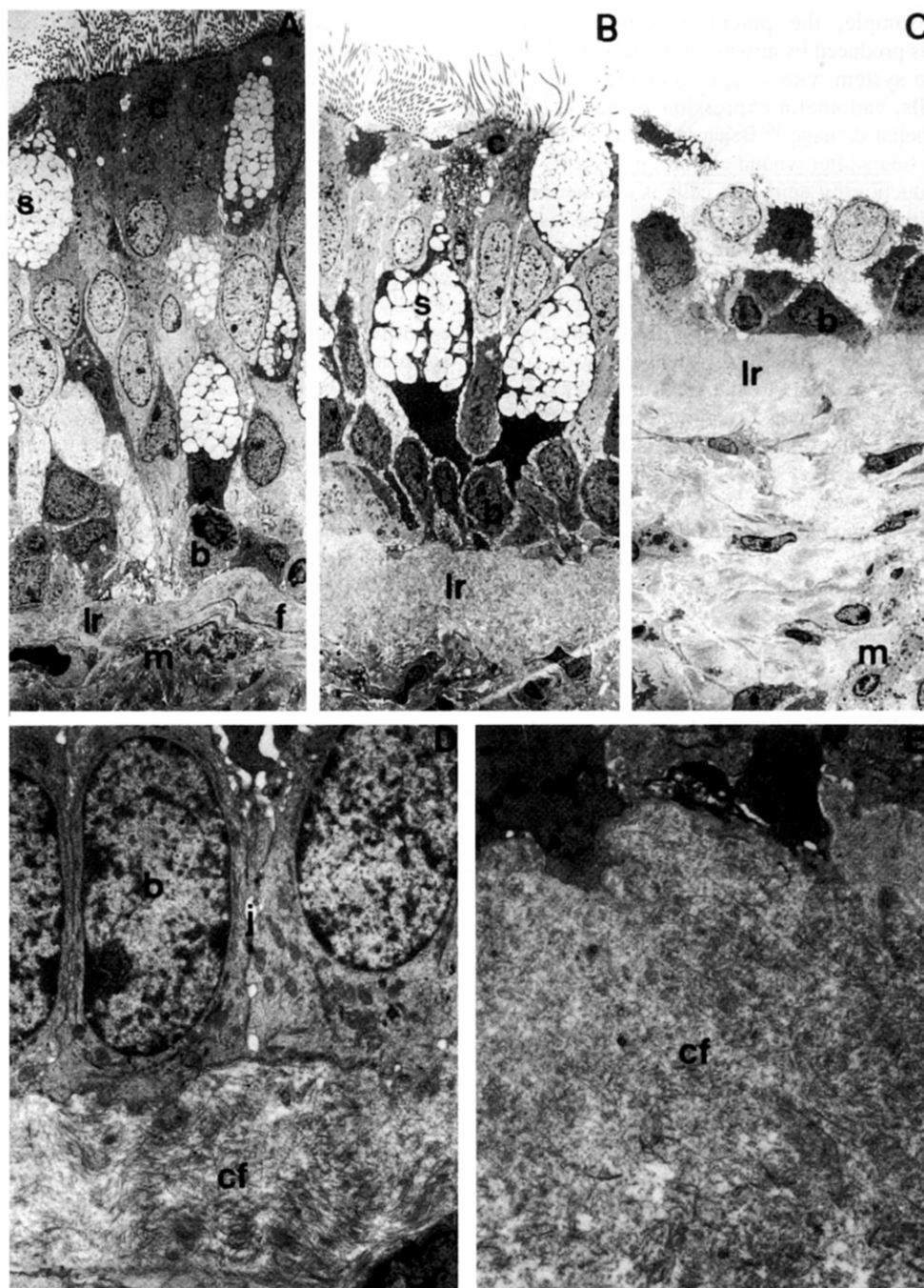


FIG 1. Ultrastructure of normal (**A**, **D**) and asthmatic (**B**, **C**, **E**) bronchial mucosa. **A** and **B**, Intact stratified bronchial epithelium composed of basal (*b*), secretory (goblet) (*s*), and ciliated (*c*) cells. Note hypertrophic appearance of goblet cells and increased thickness of lamina reticularis (*lr*) in asthmatic (**B**) bronchial mucosa. Mast cells (*m*) and fibroblast (*f*) can also be seen. **C**, Disrupted appearance of bronchial epithelium frequently observed in asthma. **D** and **E**, Difference in thickness of the lamina reticularis and density of the fibrils (*cf*) in healthy (**D**) and asthmatic (**E**) bronchial mucosa. (Original magnification $\times 1600$ [**A**] to [**C**] and $\times 8000$ [**D**] and [**E**].)

monocyte chemotactic protein (MCP-1), RANTES, eotaxin, TNF- α , IL-1 β , IL-6, oncostatin-M, IL-11, IL-10, IL-16, IL-18, GM-CSF, basic fibroblast growth factor (b-FGF), transforming growth factor- β_1 (TGF- β_1) and TGF- β_2 , insulin-like growth factor-I (IGF-I), platelet-derived growth factor (PDGF), stem cell factor, and,

most recently identified, the T_H2 cytokines IL-5²⁹ and IL-13.³⁰

Many endogenous and exogenous stimuli including the direct enzymic action of allergens,³¹ proteases,³² oxidants,³³ viruses,³⁴ and bacteria³⁵ and physical distortion³⁶ have all been shown to activate epithelial cells in

vitro. For example, the potent bronchoconstrictor endothelin³⁷ is produced by airway epithelial cells³⁸ and, in an in vitro system with scrape-wounded bronchial epithelial cells, endothelin expression is increased in areas of epithelial damage.³⁹ Because the increase persists up to 24 hours after wound closure, it suggests that mediator production by epithelial cells is elevated both during the initial response to damage and in the resolution that follows. Many of the stimuli that act on epithelial cells operate by specific receptor-mediated processes (eg, protease-activated receptors [PARs]⁴⁰) or by inducing stress responses with activation of NF- κ B and activator protein-1.^{41,42} This results in the generation and secretion of many of the cytokines and growth factors, suggesting that the widespread proinflammatory activity of the bronchial epithelium in asthma is the result of responses to environmental stress. However, primary cultures of asthmatic epithelial cells established from mucosal biopsy specimens also demonstrate higher basal production of IL-8, GM-CSF, and RANTES and increased sensitivity to diesel exhaust particles, as measured by their enhanced release of these cytokines compared with cultures from healthy individuals.^{43,44} Because these differences persist en passage, it is difficult to attribute them to the effects of the proinflammatory environment from which the cells were derived. It seems more likely that in asthmatic bronchial epithelial cells there exists an intrinsic difference in the set point for the expression of certain cytokines.

EGFR AS A PRIMARY REGULATOR OF EPITHELIAL REPAIR

Tissue repair is regulated by the activities of inducible genes whose expression is coordinated by intracellular biochemical networks that reflect extracellular signals provided by adhesion molecules and growth factors. These convey essential local spatial and environmental information to direct responses that are appropriate for a given phase of repair.

Among the growth factor receptors, the EGFR serves a central role as a primary regulator of epithelial function.⁴⁵ The pivotal role of the EGFR in controlling the behavior of the bronchial epithelium is emphasized by the finding that several other regulators of epithelial function exploit the EGFR to achieve a response. For example, induction of PGG/H synthase-2 expression by IFN- γ occurs by autocrine activation of the EGFR after up-regulation of EGFR ligand expression by IFN- γ .⁴⁶ EGFRs can also be transactivated independently of ligand, by G protein-coupled 7-transmembrane receptors including those activated by proteases (PARs), ET-1, and bradykinin,⁴⁷ as well as by cytokine receptors and by environmental stress by a mechanism involving reactive oxygen.⁴⁸ Our own studies have shown that the extent of EGFR involvement in bronchial epithelial cell behavior is extended by its ability to be transactivated by the thrombin receptor (PAR-1) or activated by oxidant stress.⁴⁹ This points to the EGFR as being a critical focus

and switch point responsible for coordinating many of the cellular responses elicited by exposure to a variety of external and internal stimuli pertinent to asthma.

The involvement of EGFRs in responses to acute lung injury is well documented.⁵⁰ For example, epidermal growth factor (EGF) enhances repair of sheep tracheal epithelium after cotton smoke exposure.⁵¹ In our recent studies of bronchial biopsy specimens from healthy airways, there is marked EGFR expression in areas of local epithelial damage, including the apical surface, which is usually devoid of EGFRs.⁵⁰ This serves to facilitate its exposure to ligands present in airway lining fluid⁵² and so enhance repair. We have shown that closure of scrape-wounded monolayers of human bronchial epithelial cells in vitro is enhanced by EGF or heparin-binding EGF-like growth factor but not by keratinocyte growth factor.^{15,53} Direct activation of the EGFR occurs even in the absence of exogenous ligand and involves release of autocrine ligands because both spontaneous and EGF-stimulated wound closure can be inhibited with an EGFR-selective tyrosine kinase inhibitor, tyrphostin AG1478.^{15,53} Consistent with enhanced EGFR expression in repairing bronchial epithelium, we have found that mechanical damage¹⁵ and oxidant stress⁴⁹ enhance surface EGFR levels in bronchial epithelial cells and that in vivo exposure to ozone causes a marked increase in immunoreactive EGFR in nasal epithelium.⁵⁴

BRONCHIAL EPITHELIAL REPAIR AND ITS IMPAIRMENT IN ASTHMA

Unlike the normal bronchial epithelium, where elevated EGFR expression was only observed in areas of structural damage,⁵⁰ in asthma we have found a striking disease-related overexpression of the EGFR both in damaged and morphologically intact epithelium, the extent of which correlates with subbasement membrane (SBM) collagen thickness.¹⁵ Further contrasting with normal epithelium, EGFR immunostaining in asthma occurs *throughout the epithelial layer*, which is indicative of widespread functional changes. This confirms a recent Japanese study with asthmatic bronchial tissue post mortem or from surgical resections.⁵⁵ Because our in vitro findings indicate that EGFR expression occurs as an early response to damage, it is possible that a high level of EGFR expression is a "reporter" of damage and that the extent of epithelial injury in asthma is more widespread than previously appreciated. Alternatively, there may be a failure to down-regulate EGFR expression after restitution, with the consequence that the epithelial cells are inappropriately "held" in a repair phenotype.

Overexpression of the EGFR in psoriasis⁵⁶ and in carcinomas⁵⁷ is accompanied by hyperplasia driven by an EGFR autocrine loop. However, in asthma, at least under basal conditions, the epithelium is not hyperproliferative. Proliferating cell nuclear antigen (PCNA) is expressed at a low level by basal cells in asthmatic bronchial epithelium, although extensive epithelial shedding was observed in the asthmatic biopsy specimens studied.⁵⁸ However,

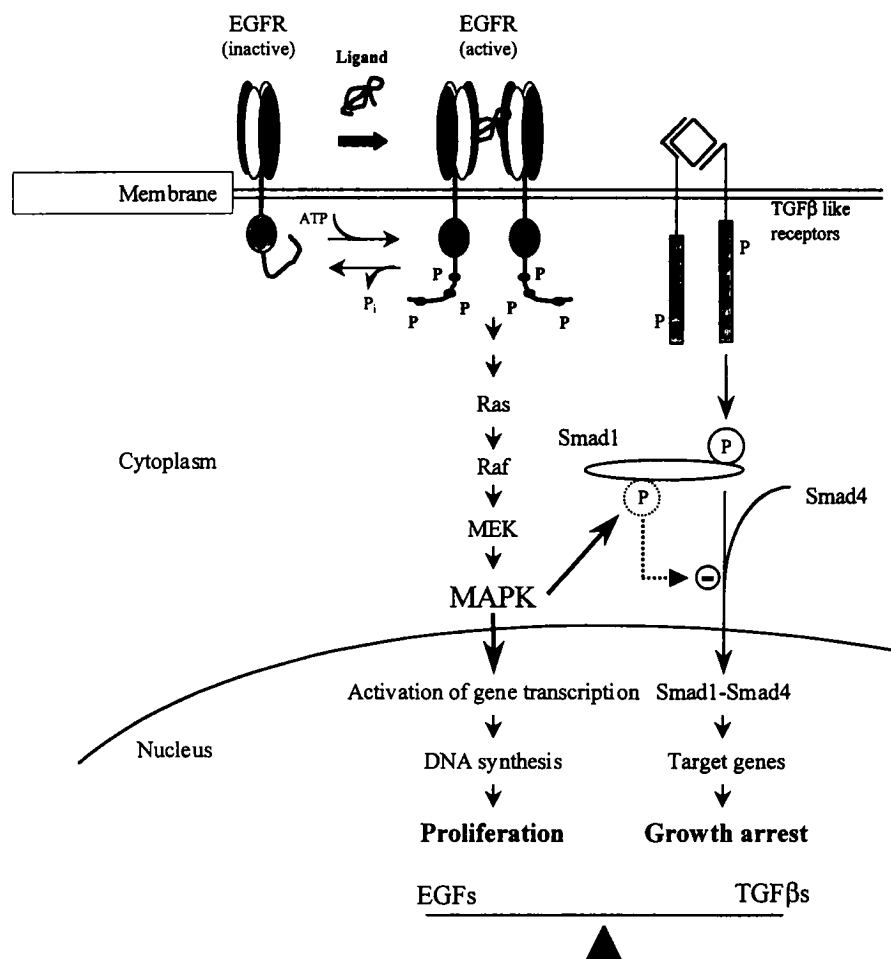


FIG 2. Interaction between EGF and TGF- β signal transduction pathways. EGF-like growth factors interact with the EGFR to cause dimerization and activation of ras/mitogen-activated protein kinase pathway, leading to cell proliferation. In contrast, TGF- β -like growth factors activate SMAD proteins, which translocate to nucleus to initiate transcription of genes that cause growth arrest. These 2 pathways interact at the level of MAPK, which can phosphorylate SMAD1, preventing its nuclear translocation. Thus cellular outcome (proliferation or growth arrest) will depend on relative amount of signal transduced through each pathway.

on exposure to allergen, sufficient stimulus is created to initiate epithelial proliferation as measured by the increased expression of the proliferation marker Ki-67.⁵⁹ A key candidate responsible for suppression of proliferative signals in the epithelium is TGF- β which acts in opposition to EGF to inhibit epithelial proliferation.⁶⁰ As EGF and TGF- β signaling pathways converge on the Sma- and Mad-related proteins (SMAD) proteins,⁶¹ it follows that the overall proliferative potential of the epithelium will be determined by the balance of mitogenic and antimitogenic signals (Fig 2). Although in asthma we have not found any increase in epithelial expression of EGFR ligands,⁶² bronchoalveolar lavage fluid levels of TGF- β are elevated.⁶³ Thus this imbalance may be a plausible explanation for the apparent failure to mount an adequate proliferative response in the bronchial epithelium in asthma. The antagonistic effect of TGF- β on EGFR-mediated bronchial epithelial proliferation is reminiscent of similar interactions that occur during

embryonic lung development to regulate branching morphogenesis (see section on attenuated fibroblast sheath and epithelial-mesenchymal trophic unit below).

Although PCNA expression is markedly increased in the asthmatic epithelium on corticosteroid treatment,⁶⁴ this treatment has no measurable effect on epithelial repair in vitro.¹⁵ Therefore it seems likely that corticosteroids influence epithelial repair favorably by reducing inflammatory tissue damage. Given that in corticosteroid-treated asthmatic patients with persistent symptoms and high EGFR levels persist in morphologically intact bronchial epithelium,¹⁵ failure to down-regulate EGFR expression after resolution is suggestive of a steroid-insensitive component and a key abnormality of this disease.

EPITHELIAL SBM COLLAGEN DEPOSITION

A retrospective analysis of a bronchial biopsy study conducted in 27 children aged 1 to 11 years has shown

that in those in whom asthma subsequently developed, airway eosinophilia and increased epithelial SBM collagen thickening (both cardinal features of asthma) were present up to 4 years before asthma was clinically expressed.⁶⁵ In a separate study, these abnormalities have been observed in early-onset asthma in children as young as 5 months old.⁶⁶ Airway eosinophilia is a reflection of coordinate up-regulation of a cluster of cytokines encoded on chromosome 5q31-33 (IL-3, IL-4, IL-5, IL-13, and GM-CSF) by T_H2 polarized T lymphocytes, mast cells, and basophils.²⁷ The deposition of interstitial (repair) collagens in the epithelial SBM region (lamina reticularis) (Fig 1) is a pathologic feature of asthma irrespective of etiology and is not seen in any other inflammatory airway disease.⁶⁷ Some⁶⁸⁻⁷⁰ but not all⁷¹ studies have reported that the thickness of the SBM collagen increases in proportion to disease severity. SBM thickening has been described as a feature of nonatopic occupational asthma,⁷² intrinsic asthma,⁷³ and asthma with eosinophilia superimposed on chronic obstructive pulmonary disease (COPD)⁷⁴ but not in COPD alone.⁷⁵ In hyperresponsive atopic nonasthmatic subjects SBM thickening is intermediate between asthmatics and nonasthmatics.⁷⁶ Probably of more significance than the thickness of this collagen layer is its increased density, which is best observed by electron microscopy (Fig 1, *D* vs *E*) across the age spread of asthma. Mathematic modeling indicates that an increase in thickness and density of the lamina reticularis would increase folding of the epithelium when the airway smooth muscle shortens, thereby disproportionately reducing the caliber of the airway lumen.⁷⁷

THE ATTENUATED FIBROBLAST SHEATH AND THE EPITHELIAL-MESENCHYMAL TROPHIC UNIT

In 1990 we described a layer of subepithelial mesenchymal cells that immunostain with the mAb PR2D3 with features of myofibroblasts whose number was increased in asthma in proportion to the thickness of the SBM-collagen layer.⁷⁸ These correspond to the attenuated fibroblast sheath described by Evans et al⁷⁹ lying adjacent to the lamina reticularis and forming a network similar to stellate cells in fibrotic liver⁸⁰ and juxta-parenchymal cells in the intestine, kidney, alveoli, synovium, granuloma, breast, and endometrium.^{81,82} When it is analyzed as a 3-dimensional model, the large surface area of the attenuated (myo)fibroblast sheath close to the epithelium forms an integrated unit that is capable of responding to exogenous and endogenous stimuli.⁸¹ The close spatial relationship seen between the epithelium and the cells of the attenuated fibroblast sheath indicates not only bidirectional communication via soluble mediators but also through release of neuropeptides,⁸³ interactions with the surrounding matrix, and cell-cell contact through gap junctions as shown for the pericryptal fibroblasts in the large intestine.⁸⁴ Additional evidence for a sophisticated level of organization between the

epithelium and underlying basement membrane is the existence of discrete channels of communication between the overlying epithelium and the submucosa.⁸⁵ These channels provide conduits during immune and inflammatory cell migration into the epithelium.

The epithelial-mesenchymal trophic unit is so named because of its pivotal function in fetal lung development where the release of soluble mediators by epithelial cells (eg, transforming growth factor- α , IGF-1, b-FGF, nerve growth factor, TGF- β s, ET-1) and (myo)fibroblasts (eg, keratinocyte growth factor, acidic fibroblast growth factor [a-FGF], amphiregulin, PDGF, TGF- β s) is crucial at different times of airway growth and branching.⁸⁶⁻⁸⁹ Central to the regulation of branching is the ECM, which accumulates and restricts epithelial growth at clefts whereas adjacent unrestricted epithelium continues growing to create branches.⁹⁰ These complex processes of branching and matrix remodeling are orchestrated by the combined activities of many growth factors, but prominent among these are the EGF-like growth factors and the TGF- β s. EGFs stimulate epithelial proliferation and production of matrix metalloproteinases (MMPs) that degrade the ECM, whereas the TGF- β s enhance ECM synthesis and inhibit both EGF-induced proliferation and MMP synthesis.⁹⁰ In this way, cleft regions are characterized by low proteolytic activity (or an abundance of tissue inhibitors of MMPs [TIMPs]), whereas branch formation is characterized by proliferation and rapid matrix degradation.

REACTIVATION OF THE EPITHELIAL-MESENCHYMAL TROPHIC UNIT IN ASTHMA

The extensive deposition of interstitial collagens and other matrix proteins in the lamina reticularis in asthma, but notably not in other airway diseases, is strong evidence that the epithelial-mesenchymal trophic unit is "reactivated" in this disease. This results in an alteration(s) in the set point for communication between the 2 compartments, leading to a subsequent increase in mesenchymal volume. Such a hypothesis is consistent with the increased amount of TGF- β detected in asthmatic airways,⁶³ the low degree of epithelial proliferation in areas of epithelial damage,⁵⁸ and the concomitant decrease in the MMP/TIMP ratio in asthmatic airways especially in severe disease⁹¹ in which enhanced matrix accumulation is likely to be most prominent. However, in a separate study corticosteroid treatment has been reported to decrease MMP-9 expression and to up-regulate TIMP-1; paradoxically this was shown to correlate with decreased collagen deposition.⁹² Because inflammatory cells are also a source of MMP-9, it is clear that more detailed study of the individual MMPs and their role in matrix turnover is required.

We have recently demonstrated the influence of local proteinase activity on the interaction between the epithelium and the underlying mesenchymal cells by showing that active MMP-2, derived from epithelial cells and activated by the membrane-associated protease MT1-MMP

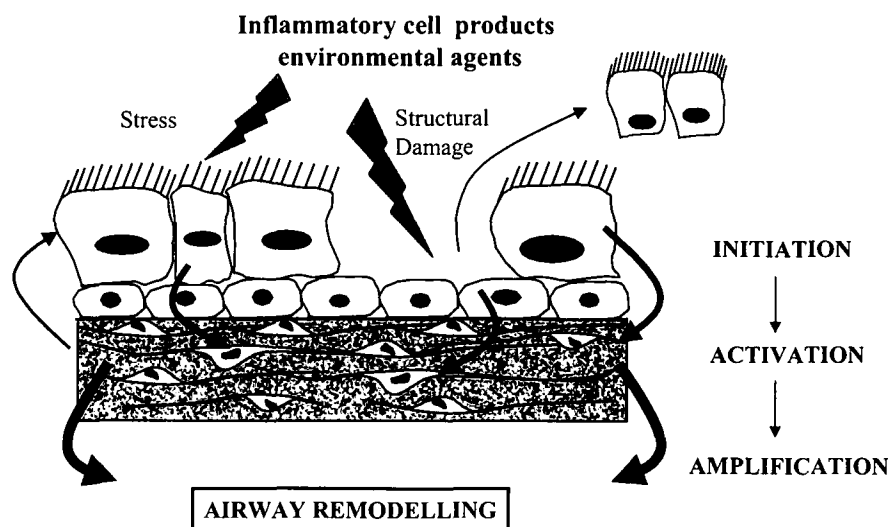


FIG 3. Schematic representation of cascade of events initiated by epithelial damage, leading to myofibroblast activation and propagation of structural changes throughout airway wall.

(MMP-14), stimulates subepithelial fibroblasts to proliferate.⁹³ Together with factors that regulate matrix biosynthesis, the level of activity of MMPs in the airways and the overall protease/antiprotease balance will determine the extent of matrix turnover, the availability of matrix-associated cytokines, and instructive signaling between matrix and epithelium as has been shown in lung development in the developing fetus.⁹⁰

To study the interactions between epithelial cells and (myo)fibroblasts, monolayers of bronchial epithelial cells have been grown on collagen gels seeded with human myofibroblasts. This *in vitro* coculture model has shown that chemical (with use of polyarginine as a surrogate for eosinophil basic proteins) or mechanical damage to confluent monolayers of epithelial cells leads to enhanced proliferation and increased collagen gene expression because of the combined effects of b-FGF (FGF-2), IGF-1, PDGF-BB, TGF- β , and ET-1.⁹⁴ Because each growth factor demonstrates a different temporal pattern of release, this is likely to reflect phenotypic changes occurring within the epithelial cells during the process of repair. These data provide direct evidence that injured and repairing bronchial epithelial cells are important regulators, not only of inflammation (see section on the epithelium as a source of proinflammatory mediators above) but also of remodeling through increased production of proliferative and profibrogenic growth factors (Fig 3). In an extension of these studies, we have investigated the effect of blocking EGFR-mediated bronchial epithelial repair on epithelial-mesenchymal signaling and found augmented release of profibrogenic growth factors,¹⁵ which enhanced collagen gene expression when applied to cultures of bronchial myofibroblasts. These observations provide evidence for a reciprocal relationship between the rate of epithelial repair and the production of profibrogenic growth factors and suggest functional mechanisms that

may underlie the remodeling responses that are characteristic of chronic asthma.

On activation, fibroblasts and myofibroblasts not only produce matrix proteins such as collagens I, III, and V⁹⁵ and fibronectin and tenascin,⁹⁶ but they also elaborate the production of growth factors that act as autocrine factors, as well as stimulating proliferation of fibroblasts, nerve cells, smooth muscle cells, and vessels.⁹⁷⁻⁹⁹ In this way we postulate that signals from the reactivated epithelial-mesenchymal trophic unit are propagated and amplified throughout the airway wall, causing the structural changes that characterize this disease (Fig 4).

INTERACTION OF THE EPITHELIAL-MESENCHYMAL TROPHIC UNIT WITH T_H2 CYTOKINES

Of the T_H2 cytokines, IL-4 and IL-13 are well recognized for their effects on immune cells and have been studied extensively in relation to allergy and asthma.¹⁰⁰⁻¹⁰² IL-13 is a pleiotropic immune regulatory cytokine that shares many structural and functional characteristics with IL-4.¹⁰³ Both cytokines are predominantly produced by activated T_H2 cells, mast cells, and basophils and have many similar effects on target cells except on T cells. This overlapping effector profile is partly the result of the shared use of receptor components in the multimeric IL-4 and IL-13 receptor complexes.¹⁰⁴ However, the sphere of influence of IL-4 can be extended by interaction of the IL-4 receptor with the common γ chain of the IL-2 receptor.¹⁰⁴ Both cytokines inhibit the production of inflammatory cytokines, up-regulate expression of MHC class II and CD23 on monocytes, induce CD40-dependent IgE class switch, and cause B cells to produce IgE and IgG4.¹⁰³ However, IL-13 differs from IL-4 in not being able to support T_H2 differentiation and prolong cell survival.¹⁰³

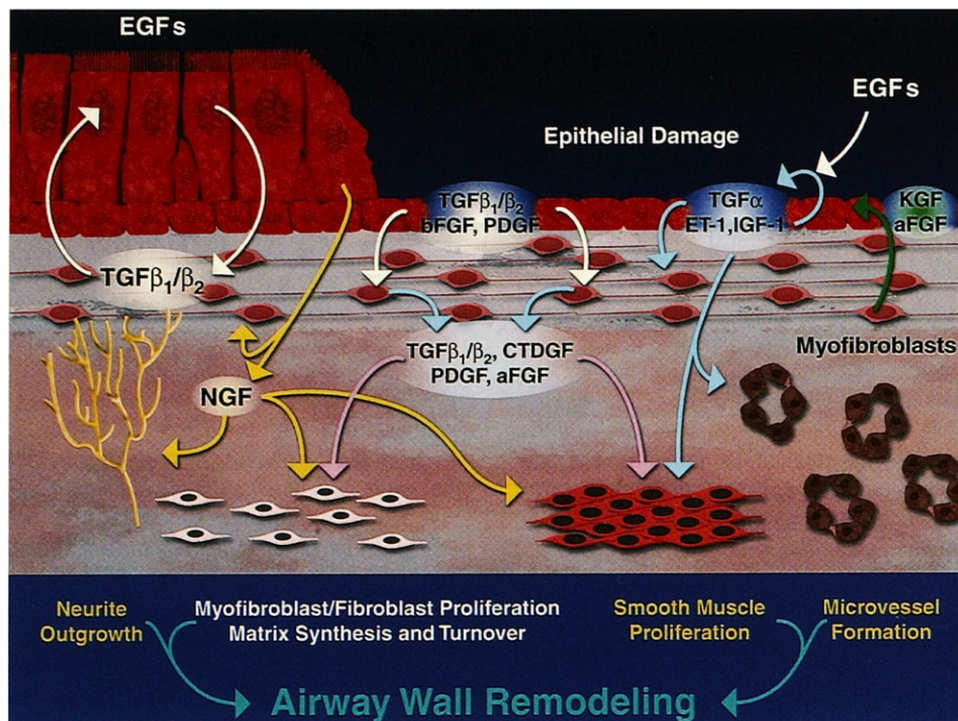


FIG 4. Epithelial-mesenchymal trophic unit in asthma. Epithelial damage alters set point for communication between bronchial epithelium and underlying mesenchymal cells, leading to myofibroblast activation, increase in mesenchymal volume, and induction of structural changes throughout airway wall.

In addition to their effects on immune cells, studies with animal models have revealed important effects of IL-4 and IL-13 on the structural cells of the airways, which may account for some of the remodeling in asthma. For example, goblet-cell metaplasia and mucus hypersecretion, which are characteristic features of asthma, can be induced by IL-4¹⁰⁵ or IL-13¹⁰⁶ as well as by EGFR ligands.¹⁰⁷ Both IL-4 and IL-13 signal through transcription factor STAT-6.¹⁰⁴ When this gene is deleted in mice, both antigen-induced airway hyperresponsiveness and the goblet-cell hyperplasia are ablated as well as the expected inhibition of T_H2 functions and IgE synthesis.¹⁰⁸

In addition to the common effects of IL-4 and IL-13 in these mouse models, overexpression of IL-13 has several important additional effects, including subepithelial fibrosis and smooth muscle proliferation associated with airway hyperresponsiveness.¹⁰⁶ This suggests that IL-13 may play a key role in sustaining the asthmatic phenotype.

IL-4 and IL-13 are also involved in the regulation of expression of epithelial inducible nitric oxide synthase,^{109,110} eotaxin,¹¹¹ and IgA secretory component,¹¹² which itself is a powerful activator of eosinophils.¹¹³ In fibroblasts, IL-4 or IL-13 stimulation causes RANTES,¹¹⁴ eotaxin,^{114,115} IL-6, MCP-1, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and $\beta 1$ -integrin expression,¹¹⁶⁻¹¹⁸ and IL-4 also promotes the differentiation of myofibroblast from fibroblasts.¹¹⁹ On the basis of these nonimmunologic properties, IL-4 and IL-13 may influence properties of the epithelial-mesenchymal trophic unit that are relevant to the asthmatic phenotype.

Because polymorphisms of the IL-4 receptor are associated with enhanced activation of STAT6,^{120,121} dysregulation of the T_H2 component has the potential to impinge on immune, epithelial, and mesenchymal cell function, providing a common link between the airways structural and inflammatory cells, which might cooperate to produce the asthmatic phenotype (Fig 5).

AN INTEGRATED MODEL OF ASTHMA

Atopy is the strongest known risk factor for the development of asthma, but alone it is insufficient to cause chronic disease. Martinez⁴ has hypothesized that an abnormality in lung maturation may itself create the local microenvironment for polarization of T cells to the T_H2 phenotype; this may have significant implications and consequences with respect to the activation state of the epithelial-mesenchymal trophic unit.

Dendritic cells form a complex interconnecting network of antigen recognition and processing cells in the epithelium and submucosa and are the sentinel cells mediating primary allergen sensitization.¹²² Polarization of T cells to the T_H2 phenotype requires both cognate interactions between T cells and dendritic cells involving the presentation of antigen peptide in the cleft of the MHC class II to the T-cell receptor. One or more second (costimulatory) signals are required for T-cell activation and cytokine production involving CD28 on T cells interacting with either CD80 or CD86 on dendritic cells.¹²³ Another factor of crucial importance in shaping the T-cell

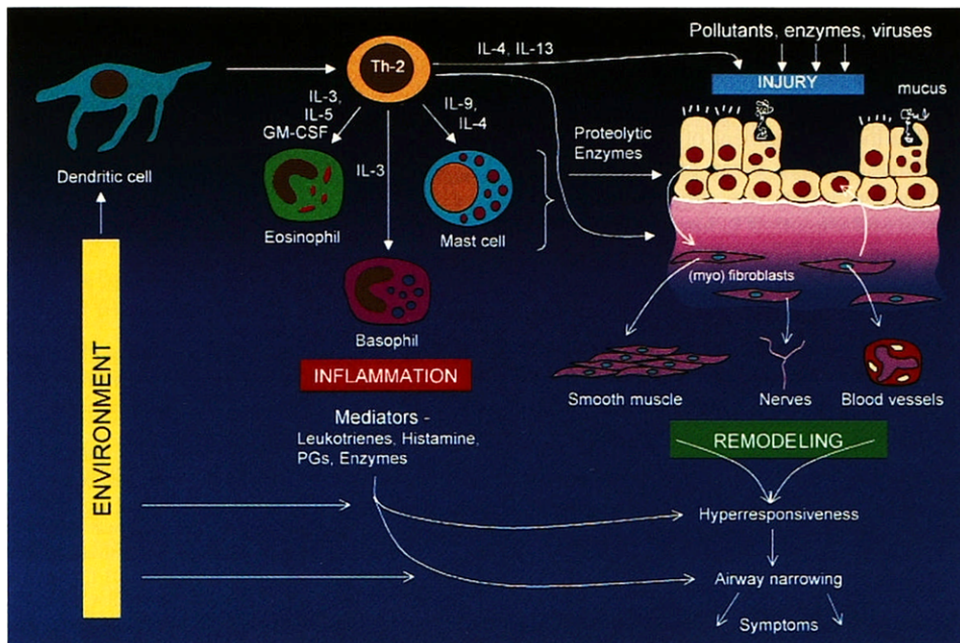


FIG 5. Integrated model of asthma. Diagram shows interrelationship between T_H2 cytokines and formed elements of airways in evolution of asthma. According to this scheme, clinical expression of asthma is the result of an intrinsic susceptibility of the airways epithelium to T_H2 -mediated inflammation and environmental stimuli. This reactivates morphogenetic mechanisms, skewing the set point for regulation of epithelial-mesenchymal signaling, resulting in an increase in mesenchymal volume and structural changes in the airways. These processes determine the chronicity and severity of the disease.

response is the secretion of cytokines by dendritic cells, especially IL-12, which deviates the pathway of differentiation toward IFN- γ , producing T_H1 cells and down-regulation of T_H2 responses.¹²⁴ The enhanced production of PGE_2 by epithelial cells and myofibroblasts has been suggested as one mechanism for reducing dendritic cell IL-12 production and, as a consequence, a T_H2 -biased immune response.¹²⁵ Bronchial epithelial cells are also an important source of IL-18,¹²⁶ which exerts effects on T cells similar to those of IL-12.¹²⁷ IL-6, IL-11, and oncostatin-M, as well as IL-15 from the epithelium, potentiate T-cell responses,¹²⁸ whereas IL-16, also produced by the asthmatic epithelium, selectively recruits T cells.¹²⁹ The chemokines RANTES and MCP-1, which are both up-regulated in the bronchial epithelium in asthma,¹²⁸ provide additional signals for T-cell recruitment, whereas these chemokines and GM-CSF from both epithelial cells and myofibroblasts are crucial for directing antigen-primed dendritic cells to migrate to local lymphoid collections where antigen presentation takes place.¹²⁴ Thus in abnormal functioning in asthma, the epithelial-mesenchymal unit provides ample opportunity for directing the mucosal immune response.

A logical extension of this concept is that the epithelial-mesenchymal tropic unit is abnormally sensitive to environmental factors impinging on the airways during early life because this has either a genetic or prenatal environmental basis. Population-based genetic studies have been able to separate heritable factors controlling atopy from those driving lower airways responsiveness in

asthma.¹³⁰ The impaired ability of the epithelium in asthma to respond to injury and stress with enhanced mediator production and evidence for continued activation of epithelial-mesenchymal signaling support the concept of a primary end-organ determinant underlying the clinical expression of asthma.⁴ Furthermore, the persistence of asthma in lungs transplanted from asthmatic donors into nonasthmatic recipients, but not the converse, is strong evidence for the involvement of intrinsic, local determinants of disease expression.¹³¹ The bronchial epithelium is a rich source of genes that are sensitive to environmental signals. Thus, in searching for known or novel candidate genes involved in controlling epithelial-mesenchymal signaling, the observation that asthmatic epithelial cells in culture exhibit enhanced basal and stimulus-induced production of cytokines such as RANTES and GM-CSF that persists through passage is particularly significant.^{43,44}

Although relatively little is known about the natural history of asthma, at a population level basal pulmonary function decays more rapidly compared with those without asthma irrespective of treatment. Thus, although inhaled corticosteroids are highly effective at reducing dendritic cell numbers, inhibiting T_H2 cytokine release, accelerating eosinophil apoptosis, and suppressing inducible genes in the epithelium (eg, cyclooxygenase-2 and inducible nitric oxide synthase), their effects on the enhanced epithelial expression of TGF- β , PDGF, or EGFR are either absent or only slightly reduced.^{15,132} Thus, in patients with chronic severe asthma receiving

high doses of corticosteroids, persistence of symptoms and decline in lung function may reflect the inadequacy of the drugs to suppress remodeling events linked to activation of the epithelial-mesenchymal trophic unit.

Recognizing that the development of the lung and its immune competence both in utero and during early childhood occur in parallel and are subjected to a wide range of environmental stimuli, it is important to understand how these two components interact to create the full asthma phenotype.¹³³ On the basis of this interrelationship, a challenge for the next century will be to determine how different environments interact with candidate genes expressed locally in the airways, which could hold the key for the discovery of novel targets for the prevention and cure of asthma as a disease of civilization.

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