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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

Activity Objectives

- (a) To understand the structural changes that occur in asthmatic airways.
- (b) To appreciate the importance of gene-environment interactions and the early life origins of asthma.
- (c) To recognize potential mechanisms of airway wall remodeling.

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Airway remodeling in asthma: New insights

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Asthma is increasing in prevalence worldwide as a result of factors associated with a Western lifestyle. The prevalence and chronic nature of the disease represent significant economic burdens. Despite advances in understanding the inflammatory and immunologic components of asthma, there is relatively little understanding of the cellular and molecular mechanisms underlying the structural changes seen in the asthmatic lung (airway remodeling). These changes include hypertrophy of bronchial smooth muscle, transformation of fibroblasts to myofibroblasts, and deposition of subepithelial collagen. Airway remodeling is linked to bronchial hyperresponsiveness to diverse triggers and a steeper trajectory of long-term decrease in lung function in asthmatic patients. Until recently, these

remodeling changes have been considered to be secondary phenomena, developing late in the disease process as a consequence of persistent inflammation. We discuss an alternative view of asthma pathogenesis by emphasizing the importance of the airway microenvironment (the epithelial mesenchymal trophic unit) in the origins of the disease. Our proposals are supported by the recent identification of *ADAM33* as an asthma susceptibility gene, the expression of which is abundant in airway fibroblasts and smooth muscle but absent from T lymphocytes or inflammatory cells that infiltrate the airway wall in patients with asthma. (*J Allergy Clin Immunol* 2003;111:215-25.)

Key words: Asthma, remodeling, ADAM33, airway epithelium, airway mesenchyme

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HISTORICAL PERSPECTIVE

In 1859, Henry Hide Salter¹ was the first to describe asthma as a disease of reversible airway obstruction; in 1960, the definition was similar but with the inclusion of bronchial hyperresponsiveness (BHR). By 1997, the National Heart, Lung, and Blood Institute defined asthma as "a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells,

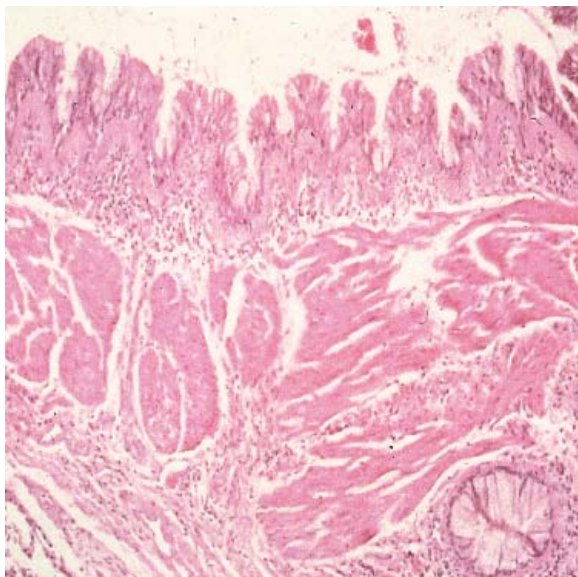


FIG 1. Histologic section through an asthmatic airway. The plate shows that, in addition to the presence of large numbers of inflammatory cells, there are characteristic structural changes, including epithelial goblet cell hyperplasia and metaplasia, thickening of the lamina reticularis, and increased amounts of smooth muscle.

Abbreviations used

BHR:	Bronchial hyperresponsiveness
ECM:	Extracellular matrix
EGF:	Epidermal growth factor
EGFR:	Epidermal growth factor receptor
EMTU:	Epithelial-mesenchymal trophic unit
α SMA:	α Smooth muscle actin
SRF:	Serum response factor
TGF:	Transforming growth factor

eosinophils, and T lymphocytes. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough particularly at night and/or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partially reversible, either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli.”²

Over the last 30 years, this expansion of the definition of asthma illustrates the rapid progress that has been made in understanding this complex disease. This increase is due in part to the progress made in the techniques used to investigate the disease. Originally, investigations into asthma were restricted to the measurement of airways obstruction and inflammatory cell numbers in the peripheral blood, whereas much of the early pathologic investigation relied on the use of postmortem samples. It was not until the advent of the fiberoptic bronchoscope that the detailed pathology of asthma was advanced in a systematic manner. Fiberoptic bronchoscopy is now a commonly used technique in the field of respiratory disease research,^{3,4} allowing collection

of biopsy specimens, bronchial brushing samples, and bronchoalveolar lavage fluid for immunochemical or molecular analysis and, more recently, for establishment of in vitro models, enabling functional studies aimed at defining underlying disease mechanisms.

ASTHMA: A DISEASE OF INFLAMMATION AND REMODELING

Immunohistochemical analysis of bronchial biopsy specimens has enabled detailed description of the inflammatory cells present in asthmatic airways. These infiltrates are characterized by the presence of mast cells, basophils, eosinophils, monocytes, and T lymphocytes of a T_H2 inflammatory profile.⁵ Consequently, treatments have been developed to suppress this inflammatory response for therapeutic benefit.⁶ Some success has been achieved with the use of corticosteroids, leukotriene receptor antagonists, and mast cell stabilizers.⁷ However, there are many asthmatic patients with chronic symptoms for whom anti-inflammatory therapy is inadequate and who account for up to 50% of the health costs of this disease.⁸

Although inflammation undoubtedly plays a central role in asthma, inflammation alone does not explain many of the features characteristic of this chronic and relapsing disease.⁹ Eosinophils have been assumed to play a central role in disease pathogenesis; however, studies with recombinant human IL-12¹⁰ or an anti-IL-5 blocking mAb^{11,12} have failed to reveal efficacy, despite markedly reducing circulating (by approximately 90%), sputum (by approximately 60%-80%), and tissue (by approximately 55%) eosinophil numbers in the case of anti-IL-5. Thus despite being associated with asthma, atopy and airway eosinophilia would not seem to be critical requirements for disease expression. Genetic studies have also demonstrated that atopy and BHR have different patterns of inheritance.¹³ These findings imply that locally operating factors play an important role in predisposing individuals to asthma¹⁴ and provide an explanation for the epidemiologic evidence that identifies environmental factors, such as pollutant exposure,^{15,16} diet,¹⁷ and respiratory virus infection,¹⁸ which all increase oxidant stress in the airways, as important disease risk factors.

The traditional viewpoint that inflammation is the cause of asthma has meant that airway remodeling, the other major histologic feature of the disease, has received considerably less attention, and its relevance to disease pathogenesis is still controversial. Yet changes involving epithelial goblet cell hyperplasia and metaplasia, collagen deposition and thickening of the lamina reticularis, smooth muscle hyperplasia, and proliferation of airway blood vessels and nerves are consistently observed in ongoing disease, as well as in postmortem asthmatic airways (Fig 1).¹⁹⁻²² Morphometry has revealed that mucosal and adventitial thickening of asthmatic airways is theoretically able to account for a large component of BHR and excessive airway narrowing observed in established disease.^{23,24} At best, these structural changes in established asthma are poorly responsive to corticosteroids.

Although with prolonged treatment some improvement in BHR is evident,²⁵ it frequently remains abnormal, especially in patients with moderate-to-severe disease. Airway remodeling also provides an explanation for corticosteroid-resistant BHR²⁵ and the accelerated decline in lung function observed over time in adult asthma.²⁶ In 5- to 11-year-old children the recent Childhood Asthma Management Program study has shown that the initial beneficial effect of an inhaled corticosteroid on the postbronchodilator improvement in airway function observed during the first year of treatment was lost over the following 3 years.²⁷ This is best explained by airway remodeling that is insensitive to corticosteroids. The importance of tissue remodeling as an early and consistent component of childhood asthma has been emphasized in a recent biopsy study that describes collagen deposition in the *lamina reticularis* and underlying fibroblast proliferation, rather than eosinophil infiltration, as diagnostic features of this disease.²⁸ Although remodeling has been considered to be caused by longstanding inflammation, airway biopsy studies in young children have shown tissue restructuring up to 4 years before the onset of symptoms,²⁹ indicating that this process begins early in the development of asthma and might occur in parallel with inflammation or even be required for the establishment of persistent inflammation.

To understand how and why airway remodeling occurs in asthma, we have likened the tissue responses to those of a chronic wound in which failure to resolve the normal processes of inflammation and remodeling that accompany wound repair leads to establishment of a tissue microenvironment that sustains both airway inflammation and remodeling.^{30,31}

THE EPITHELIAL MESENCHYMAL TROPIC UNIT IN ASTHMA

Although asthma is a heritable trait, the increasing prevalence of this disorder over the last 30 years is too large and widespread to be caused by genetic change. This has led to the proposal that the increasing trends in asthma are due to changes in the environment acting on a susceptible genotype both in respect to disease induction and worsening of established disease.¹⁴ This proposal is supported by epidemiologic studies identifying multiple interacting environmental risk factors for asthma induction and exacerbations.³²⁻³⁴ As the barrier to the external environment, the bronchial epithelium is in a key position to translate gene-environment interactions. Evidence is now accumulating that the epithelium of a patient with asthma is structurally and functionally abnormal. We have shown that epithelial cells from patients with asthma are more susceptible to oxidant-induced apoptosis,³⁵ whereas others have reported that bronchial epithelial cells from atopic patients with mild asthma have increased permeability after exposure to ozone or nitrogen dioxide³⁶ and release more IL-8 and GM-CSF than those from nonasthmatic individuals when stimulated with diesel exhaust particles.³⁷ The effects of

oxidants and particles on epithelial function are consistent with the increasing trends in asthma because the presence of these agents in the environment would be expected to trigger epithelial activation and damage at a lower threshold in susceptible individuals. Sensitivity to oxidants might explain why a diet low in antioxidants is linked to the development of asthma.¹⁷ Assuming that a proportion of the population is more susceptible to environmental pollutants, these concepts have important implications for environmental health and safety.

As a reflection of its increased susceptibility, epithelial shedding in asthma,^{38,39} although somewhat controversial,⁴⁰ is a characteristic feature that is not observed in other inflammatory diseases, such as chronic obstructive pulmonary disease, in which the epithelium becomes multilayered because of squamous metaplasia. Desquamation appears to be due to apoptotic cell loss³⁵ and is accompanied by increased expression of the epidermal growth factor receptor (EGFR) in areas of damage, which is consistent with damage occurring *in vivo*.⁴¹ However, the increase in epithelial EGFR is paradoxical because, unlike chronic obstructive pulmonary disease, there is little evidence of increased proliferation.^{42,43} This might be due to high expression of the cyclin/cyclin-dependent kinase inhibitor p21^{waf}, particularly at the severe end of the disease spectrum.⁴³ This negative regulator of G1 cyclins can be induced by the antiproliferative transforming growth factor (TGF) β_1 and β_2 , the levels of which are increased in patients with asthma.^{44,45} As a consequence of p21^{waf} expression, the proinflammatory phenotype of the epithelium can be maintained by prolonging the period of epithelial repair. In addition, the EGFR might contribute directly to these proinflammatory responses because epidermal growth factor is known to elicit IL-8 expression and release from bronchial epithelial cells, a response that is not fully suppressed by corticosteroids. Because the EGFR is overexpressed in the morphologically intact epithelium of patients with severe asthma and strongly correlates with epithelial IL-8 expression,⁴⁶ this might contribute to airway neutrophilia in patients with severe asthma.

In vivo animal studies⁴⁷ and *in vitro* studies with cocultures of bronchial epithelial cells and fibroblasts⁴⁸ have shown that, in addition to proinflammatory mediator release, injury to the epithelium causes the release of fibroproliferative and profibrogenic growth factors. These include TGF- β_2 ⁴⁸ and other members of the TGF- β superfamily, including fibroblast growth factor, insulin growth factor, platelet-derived growth factor, endothelin 1, and bone morphogenetic proteins 2, 4, and 6,⁴⁷ which promote the proliferation of the underlying fibroblasts. Among these growth factors, TGF- β is particularly important because it promotes differentiation of fibroblasts into myofibroblasts that secrete interstitial collagens, as well as growth factors, such as endothelin 1 and vascular endothelial growth factor, which are mitogens for smooth muscle and endothelial cells.⁴⁹ Thus through release of TGF- β and its effects on the underlying fibroblasts and myofibroblasts, remodeling signals initiated in

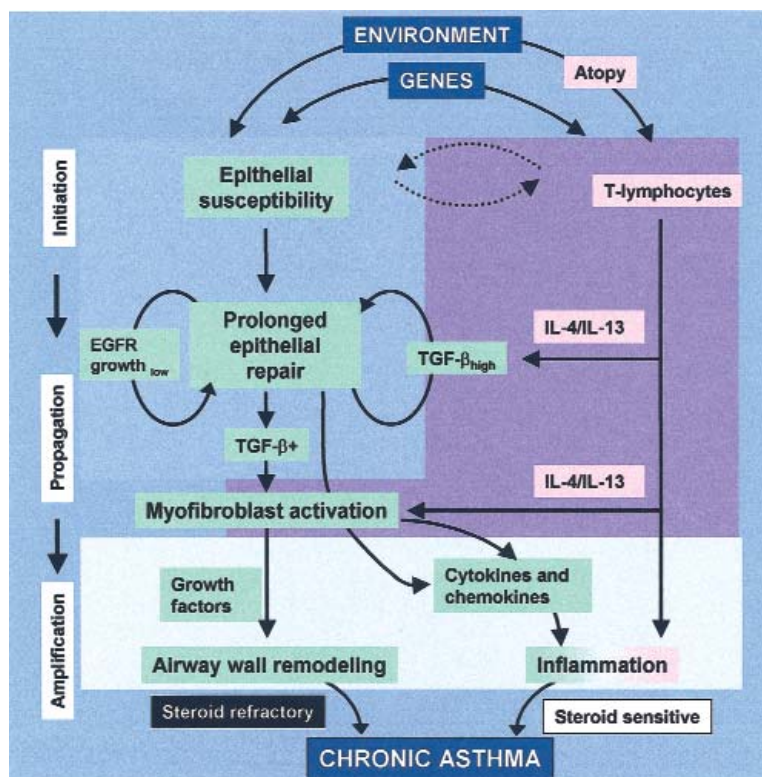


FIG 2. The interaction between T_H2 inflammation and the EMTU in asthma pathogenesis. A parallel model for asthma pathogenesis in which an inherited or acquired epithelial susceptibility to environmental agents leads to induction of stress-injury and repair responses is shown. Growth arrest and prolonged repair enhances cell-cell communication within the EMTU, leading to myofibroblast activation and 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. Adapted with permission from Holgate and Davies (Hogrefe & Huber Publishers, Seattle, Wash).⁵¹

the repairing epithelium are propagated and amplified into the deeper layer of the submucosa. This communication between the epithelium and mesenchymal cells is reminiscent of the modeling of the airways during embryonic development⁵⁰ and has led us to propose that the epithelial-mesenchymal trophic unit (EMTU) remains activated after birth or becomes reactivated in asthma and drives the remodeling of the asthmatic airways.^{30,51} According to this model, airway inflammation and remodeling occur as a consequence of increased susceptibility to injury, impaired wound healing (healing by secondary intention), or both.

T_H2 INFLAMMATION AND THE EMTU: A NEW PARADIGM FOR ASTHMA PATHOGENESIS

T_H2 -type inflammation of the airways is a characteristic feature of asthma, irrespective of atopy. Although studies in transgenic mice suggest that IL-13 is able to cause remodeling changes when expressed as a transgene in the bronchial epithelium, we have found that IL-13 is only poorly able to induce myofibroblast transformation in comparison with TGF- β .⁴⁹ Because IL-13 causes a corticosteroid-insensitive increase in the release of TGF- β_2 from bronchial epithelial cells, it seems likely that IL-13-mediated

submucosal remodeling is initiated through the bronchial epithelium.⁴⁹ Although the remodeling effects of IL-4 and IL-13 can be attributed to epithelial activation, these cytokines also have direct effects on both epithelial cells and fibroblasts. Cultures of bronchial epithelial cells respond to IL-4 and IL-13 with increased signal transducer and activator of transcription 6 phosphorylation accompanied by enhanced GM-CSF and IL-8 production, which is further augmented by enzymatically active extracts of house dust mite (*Dermatophagoides pteronyssinus*).^{52,53} These cytokines also enhance release of TGF- α ,⁵³ a ligand for the EGFR that has been linked to enhanced mucin gene expression.⁵⁴ IL-4 and IL-13 also enhance release of eotaxin from asthmatic fibroblasts,⁴⁹ which might help explain the accumulation of eosinophils beneath the *lamina reticularis* in patients with asthma. By interacting with cells within the EMTU, IL-4 and IL-13 can augment chronic inflammation and airway remodeling. Therefore we postulate that epithelial damage and T_H2 cytokines can act in concert to cause a functional disturbance of the EMTU, which leads to myofibroblast activation and induction of inflammatory and remodeling responses characteristic of chronic asthma (Fig 2).⁵¹ This model differs from the traditional sequential view of asthma pathogenesis by setting the structural abnormalities in parallel with T_H2 inflamma-

tion. On the one hand, the expression of asthma is dependent on inheritance of local susceptibility genes, which have the potential to interact with the atopic predisposition, whereas on the other hand, inheritance of atopy can occur in the absence of asthma (Fig 3).

EARLY LIFE ORIGINS OF ASTHMA

Thickening of the *lamina reticularis* in bronchial biopsy specimens from young children is present several years before asthma becomes clinically manifest.²⁹ During lung development, epithelial and mesenchymal growth is also regulated by the balance of epidermal growth factor (EGF) and TGF- β signaling, as we suggest occurs in chronic asthma (Fig 4). Thus in susceptible individuals we suggest that environmental factors are able to interact with the EMTU in early life to initiate structural changes in the airways (Fig 5) and might, as a consequence, contribute to the decrease in lung function observed in young children who are susceptible to early wheezing⁵⁵ and to the loss of corticosteroid responsiveness on baseline lung function observed in the Childhood Asthma Management Program study.²⁷ This is supported by very recent studies in nonhuman primates in which intermittent exposure to ozone in the presence of allergen creates a phenotype resembling chronic asthma,⁵⁶ and prenatal exposure to nicotine causes collagen deposition around the large airways.⁵⁷ We hypothesize that bronchial epithelial susceptibility either 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.

PHENOTYPIC PLASTICITY OF AIRWAY MYOFIBROBLASTS AND SMOOTH MUSCLE

Evidence suggests that myofibroblasts play a central role in the EMTU by propagating and amplifying signals from the bronchial epithelium into the deeper layers of the submucosa through release of soluble mediators.⁴⁹ Consistent with this, immunohistochemistry and electron microscopy clearly show that airway myofibroblasts and the smooth muscle bundles lie in close physical proximity in asthma.²¹ There is also an increase in the number of myofibroblast-like cells in the *lamina reticularis* of asthmatic subjects after allergen challenge.⁵⁸ This suggests that exposure of the epithelium to allergen causes myofibroblasts to migrate toward the smooth muscle or that smooth muscle cells migrate from the muscle bundles toward the *lamina reticularis* in response to myofibroblast-derived chemoattractants. Because myofibroblasts have a phenotype that is essentially an intermediate between that of the fibroblast and the smooth muscle cell, this raises the possibility that myofibroblasts might contribute directly to increased smooth muscle mass because of their phenotypic plasticity.

Microscopic analysis shows that myofibroblasts are elongated cells containing intracellular filaments, abundant rough endoplasmic reticulum, and irregularly

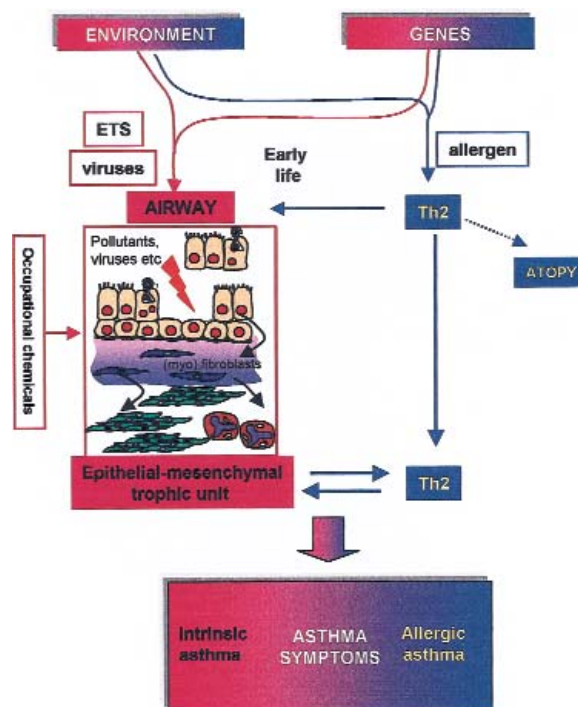


FIG 3. Gene-environment interactions in the development of asthma and atopy. By separating local tissue susceptibility factors from those that predispose to the development of atopy, this scheme shows how atopy can develop in the absence of asthma and how the allergic predisposition can interact with the airway susceptibility factors to promote the asthmatic phenotype. ETS, Environmental tobacco smoke.

shaped nuclei.⁵⁸ Although there are no known specific markers for fibroblasts, myofibroblasts are classically characterized by the presence of filaments of α smooth muscle actin (α SMA). However, the definition of myofibroblast is more complex than this. There are several situations in vivo in which myofibroblasts do not express α SMA.^{59,60} They do, however, express stress fibers containing nonmuscle actins and myosins. Therefore, 2 subclasses of myofibroblast phenotype have been proposed: protomyofibroblasts (ie, expression of stress fibers containing cytoplasmic actins) and differentiated myofibroblasts (ie, expression of stress fibers containing α SMA).⁶⁰ In addition, myofibroblasts can express other smooth muscle proteins, such as heavy-chain myosin and calponin. Smooth muscle cells express additional muscle-associated proteins, such as smooth muscle γ -actin,⁶¹ muscarinic M3 receptor,⁶² and desmin.⁶³

The origin of the myofibroblast in vivo is not fully understood. In granulation tissue the most likely progenitor cells are the local fibroblasts because myofibroblasts are seen to revert back to a fibroblast phenotype after wound closure.⁶⁴ Other in vitro studies report smooth muscle cells acquiring morphologic and functional characteristics of the myofibroblast,⁶⁵ including collagen gene expression. Work on obstructed bladders in rabbits has shown that myofibroblasts can, under certain condi-

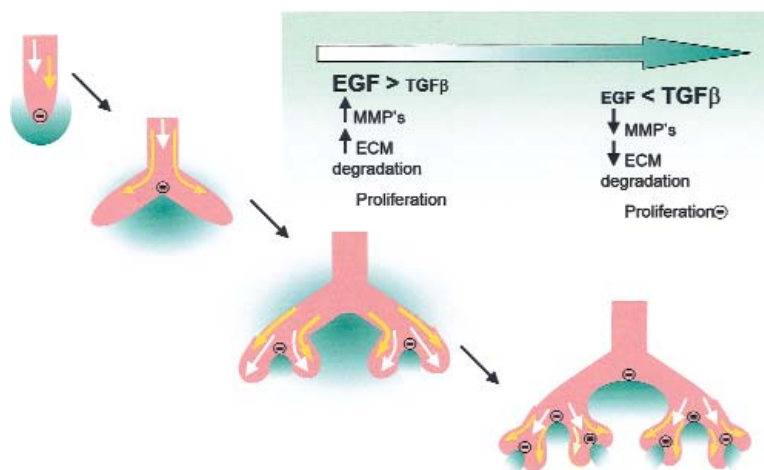


FIG 4. The involvement of EGF and TGF- β in branching morphogenesis of the airways. EGF and TGF- β act in opposing fashion to promote or inhibit growth and elongation of the airways. Where EGF dominates, the airways extend because of the combined effects of EGF on growth and matrix degradation. Where TGF- β dominates, growth and matrix degradation are inhibited, and branching occurs. *MMP*, Matrix metalloproteinase.

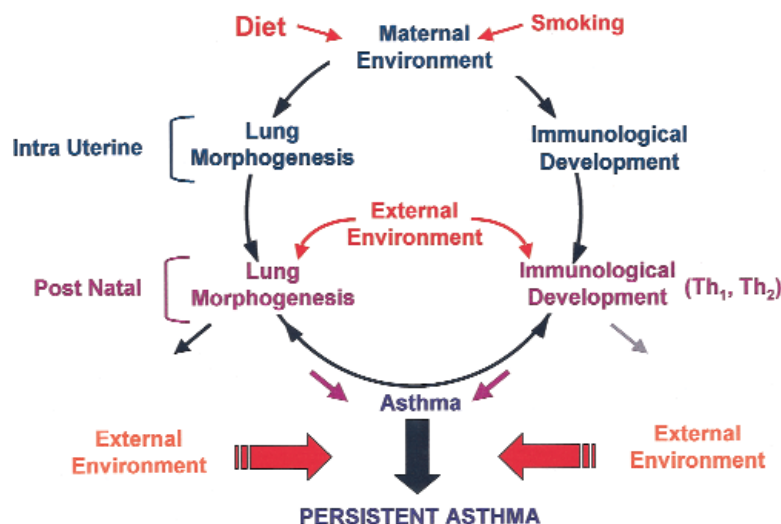


FIG 5. Gene-environment interactions and the early life origins of asthma. It is proposed that the early life origins of asthma involve developmentally regulated genes that control branching morphogenesis and the structure of the airways and their interactions with the maternal and external environment. Reproduced with permission from Holgate and Davies (Hogrefe & Huber Publishers, Seattle, Wash).⁵¹ *MMP*, Matrix metalloproteinase.

tions, express α SMA, heavy-chain myosin, and desmin, indicating that it is possible for myofibroblasts to differentiate to a bona fide smooth muscle phenotype.⁶⁶ These examples illustrate the plastic nature of the myofibroblast phenotype, which appears to shift toward a fibroblastic or smooth muscular phenotype according to the microenvironment. Several studies have identified factors that affect fibroblast phenotype and can be used reliably to manipulate the fibroblast phenotype in vitro. TGF- β family members are the most potent mediators of fibroblast-myofibroblast transformation, and TGF- β is reported to prolong the longevity of myofibroblasts by blocking IL-1 β -induced apoptosis.⁶⁷ Other studies have suggested that IL-4 can induce α SMA expression in fibroblasts,

although in our own studies⁴⁹ these cytokines were poor differentiation factors by comparison with TGF- β , the levels of which are known to be increased in asthmatic bronchoalveolar lavage fluid.⁴⁵ Mast cell histamine and tryptase also upregulate α SMA expression in human foreskin fibroblasts.

Mechanical influences also seem important in the regulation of the myofibroblast phenotype. For example, it has been demonstrated that myofibroblast features appear earlier in splinted wounds, in which the fibroblast population is under mechanical tension, than in control wounds.⁵⁹ This observation has been confirmed in vitro with free-floating and tethered collagen lattices.⁶⁸ In similar work Yang et al⁶⁹ grew undifferentiated mes-

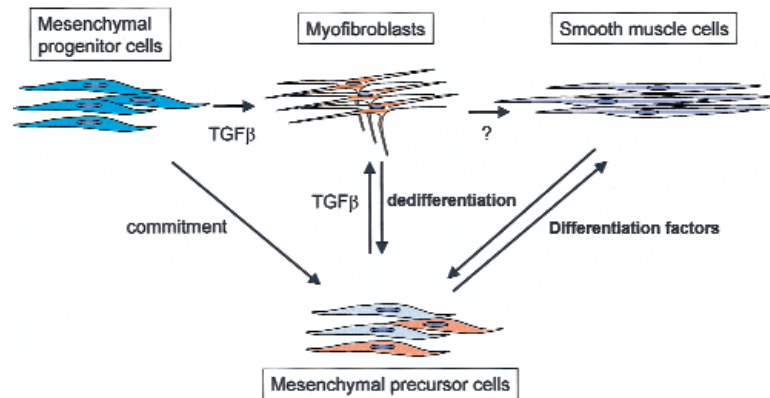


FIG 6. The relationship between myofibroblasts and smooth muscle cells. In this scheme we propose that airway myofibroblasts and smooth muscle cells are derived from a common mesenchymal progenitor cell (ie, stem cells). This might involve a sequential pathway, as shown at the top, or might involve an intermediate progenitor that becomes committed to become either a myofibroblast or a smooth muscle cell. It is likely that commitment and subsequent differentiation involves instructive signals from the ECM and growth factors, such as TGF- β .

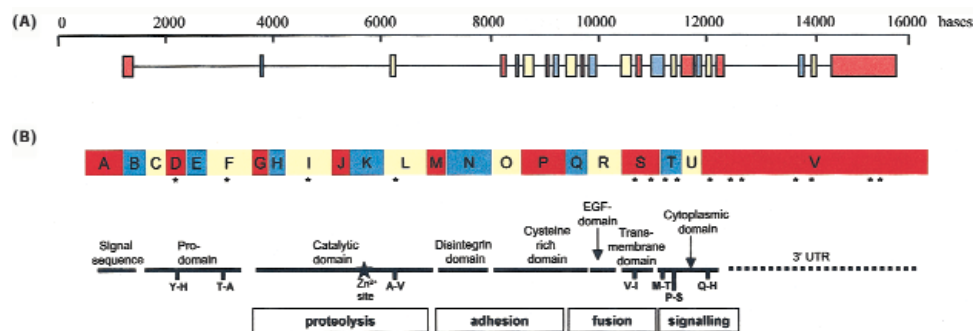


FIG 7. The domain organization of ADAM33 and its potential functions in asthmatic mesenchymal cells. A schematic diagram showing the exon-intron structure of ADAM33 (A) and the exon structure and domain organization of full-length ADAM33 (B) is shown. The location of disease-related SNPs in the coding region and 3' untranslated region are indicated with asterisks; those SNPs leading to amino acid changes are indicated. Likely functions associated with each domain are also indicated.

enchymal cells on a collagen-coated silicon membrane, which was stretched once the cells had reached the desired confluency. Sustained stretching induced the expression of smooth muscle proteins and accelerated smooth muscle myogenesis. The cause of this stretch-induced myogenesis was shown to be due to alternative splicing of serum response factor (SRF). SRF is a transcription factor known to be critical for the induction of muscle-specific gene expression. An alternatively spliced variant, SRF Δ 5, was found to downregulate bronchial myogenesis, whereas the full transcript was a promoter of myogenesis. When undifferentiated smooth muscle cells are starved of serum for 7 days, approximately 15% of the cells return to a contractile phenotype, and this has been attributed to differences in SRF cellular location, where responses were seen only when SRF was located in the nucleus.⁷⁰ Thus the continuous expansion and contraction of the airways has the potential to influence fibroblast phenotype, and this might be of particular importance with respect to BHR and asthma.^{71,72}

There is also considerable information regarding the smooth muscle phenotype and the importance of the extracellular matrix (ECM) in mesenchymal cell survival⁷³ and differentiation. Collagen I and IV, fibronectin, vitronectin, and, in particular, laminin have been shown to maintain the contractile phenotype of the smooth muscle cell in vitro.^{74,75} An independent investigation by Beqaj et al⁷⁶ suggested a mechanism by which laminin 2 could regulate smooth muscle differentiation. They observed that before smooth muscle differentiation, RhoA, a small GTPase-signaling protein, inhibits smooth muscle gene expression, although after differentiation, RhoA maintained the smooth muscle phenotype. RhoA levels were seen to decrease rapidly when cells were grown on laminin 2, and thus smooth muscle differentiation was more prevalent on cultures grown on laminin. ECM components are also of importance to the differentiating myofibroblast phenotype. For example, the ECM component vitronectin and vitronectin integrins have already been shown to downregulate myofibroblast transformation.⁷⁷

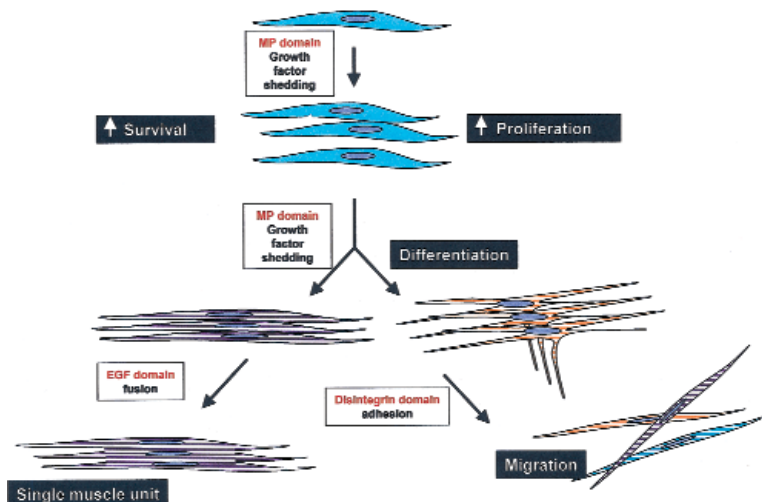


FIG 8. Possible functions of *ADAM33* and the domains involved in these functions on the basis of knowledge of other *ADAM* family members. It is conceivable that *ADAM33* might have multiple roles in mesenchymal cell function, depending on genotype or on interactions with environmental or endogenous factors.

PHENOTYPIC PLASTICITY AND MESENCHYMAL CELL ABNORMALITIES IN ASTHMA

Like the bronchial epithelium, the mesenchymal cells of the asthmatic EMTU also exhibit functional abnormalities. In our own studies we have found that fibroblasts from asthmatic patients, but not those from healthy patients, proliferate in vitro in the absence of exogenous growth factors.⁷⁸ Johnson et al⁷⁹ have reported a similar phenotype in asthmatic smooth muscle cells, although in this case growth was still supported by the presence of serum factors. This shared hyperproliferative property might be due to the cells arising from a common mesenchymal progenitor cell population (ie, stem cells) that carries an acquired or inherited defect. Such an abnormality might underlie the increased number of myofibroblasts in the *lamina reticularis* and the increase in smooth muscle mass, both of which are characteristic features of the remodeled airways in asthma. However, at present, we do not know whether these progenitor cells follow a linear pathway of differentiation, becoming myofibroblasts and then smooth muscle cells, or whether committed progenitor cells (transit amplifying cells) are derived to provide a precursor pool for either cell type (Fig 6). It is likely that commitment and subsequent differentiation involves instructive signals from growth factors, as occurs during vessel development.⁸⁰

WHERE NEXT?

As implied in the National Heart, Lung, and Blood Institute definition, BHR is fundamental to disordered function in asthma. A significant advance in defining the genetic origin of BHR has come from a genome-wide screen that has led to the identification of *ADAM33*, a member of the *ADAM* (a disintegrin and metalloprotease)

family, as a novel asthma susceptibility gene encoded on chromosome 20p13.⁸¹ In the linkage case-control association and transmission disequilibrium tests and haplotype analyses that led to the identification of *ADAM33*, statistical significance was strengthened when BHR was included in the asthma phenotype but weakened when asthma was conditioned for serum total IgE or allergen-specific IgE levels, indicating the presence of a gene more closely linked to altered airway function than to inflammation per se. The significance of *ADAM33* for asthma is reinforced by the existence of a syntenic region on mouse chromosome 2 at 73.9 cM, which has been linked to BHR overlying an orthologue of *ADAM33* that exhibits approximately 70% homology with its human counterpart.⁸²

ADAM33 is the most recently reported member of the *ADAM* gene family of Zn⁺⁺-dependent metalloproteases (metzincins). *ADAMs* have a complex organization, involving signal sequence, pro-, catalytic, disintegrin, cysteine-rich, and EGF domains, followed by a transmembrane and a cytoplasmic domain with signaling-specific sequences (Fig 7). *ADAM33* belongs to the *ADAM12*, *ADAM13*, and *ADAM19* subfamily, all of which possess proteolytic activities.^{83,84} *ADAM33* is preferentially expressed in smooth muscle, myofibroblasts, and fibroblasts but not in epithelial cells, T cells, or inflammatory leukocytes. The selective expression of *ADAM33* in mesenchymal cells strongly suggests that alterations in its activity might underlie abnormalities in the function of airway smooth muscle cells and fibroblasts linked to BHR in asthma. *ADAM* proteins have diverse functions that reflect the complex domain structure of these molecules.⁸³ They are anchored in the trans-Golgi network or plasma membrane, but in some cases secreted splice variants have been identified. Although the function or functions of *ADAM33* are still unknown, potential roles for a molecule with both cell-adhesion and protease activity are numerous. For instance, it has been shown that other members of the *ADAM* family are involved in the release of

proliferative growth factors,^{84,85} and it would be logical to postulate that ADAM33 could cleave proliferative or profibrogenic growth factors that modify responses in the EMTU. However, other domains of the ADAM proteins also play important roles in cell adhesion,⁸⁶ migration,⁸⁷ and membrane fusion.⁸⁸ As shown in Fig 8, it is possible that ADAM33 has multiple roles in mesenchymal cell function and that dysregulation of one or several of these functions contributes to the development of BHR. Several splice variants of the *ADAM33* gene have already been identified, suggesting that alternative splicing might regulate the ability of ADAM33 to participate in a given function. Given that several disease-related single nucleotide polymorphisms (SNPs) lie in introns that might affect alternative splicing,⁸¹ this is one way in which polymorphic variation might lead to abnormalities in asthma. Alternatively, there are other disease-related SNPs that cause amino acid changes in the coding sequence of *ADAM33*, which might affect the regulation, trafficking, or both of ADAM33. In the case of ADAM12, an evolutionarily close relative of ADAM33, proteolytic shedding of HB-EGF by ADAM12 has been linked to cardiac hypertrophy,⁸⁹ whereas ectopic expression in rhabdomyosarcoma cells of a minigene encoding a secreted form of ADAM12S that lacks the metalloprotease domain results in growth of tumor xenografts, which are infiltrated with large numbers of host-derived smooth muscle cells.⁹⁰ Clearly, understanding the function or functions of ADAM33 and its role in airway hyperresponsiveness is a well-defined goal that offers many exciting and novel prospects for new asthma treatments.

More detailed analysis of ADAM33 activity and its contribution to asthma chronicity and severity will give rise to diagnostic tests and novel therapeutic targets close to the disease origins. It is a rarity to have such a novel molecule about which so little is known as a target for research. We believe there to be considerable potential for novel findings within this field. We are truly entering a new era of understanding in regard to airway remodeling.

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