

Current reviews of allergy and clinical immunology

Series editor: Harold S. Nelson, MD

Airway remodeling contributes to the progressive loss of lung function in asthma: An overview

Rodolfo M. Pascual, MD, and Stephen P. Peters, MD, PhD *Winston-Salem, NC*

This activity is available for CME credit. See page 34A for important information.

Airway inflammation, airflow obstruction, and bronchial hyperresponsiveness are characteristic phenotypic features of asthma. Clinically, airflow obstruction in asthma often is not fully reversible, and many asthmatic subjects experience an accelerated and progressive loss of lung function over time. Histopathologic studies of the asthmatic airway have demonstrated stereotypic changes that might explain the loss of lung function that many patients with asthma experience. The notion of airway remodeling in asthma postulates that the alteration of the structure and function of key airway constituents, including airway smooth muscle, epithelium, blood vessels, and mucus glands, might explain, at least in part, the progressive loss of lung function that is observed clinically. Inflammation driven by CD4⁺ lymphocytes and mediated by effector cells, particularly the eosinophil, appears to modulate the function of mesenchymal cells, including fibroblasts and myofibroblasts, changing the composition of the airway wall matrix. Changes in the airway epithelium might alter the function of the underlying smooth muscle and the composition of the matrix and could drive inflammation. Alterations in

the structure and function of airway smooth muscle change the mechanical properties of the airway wall and might also affect the function of other airway constituents. A variety of experimental models have identified candidate mechanisms and mediators for these observed changes, which are thus potential therapeutic targets. However, clinical studies to date have been disappointing, and it remains to be seen whether targeted therapies will prevent the progressive loss of lung function seen in asthma. (*J Allergy Clin Immunol* 2005;116:477-86.)

Key words: Asthma, airway, remodeling, inflammation

Asthma is a disease characterized by episodic airflow obstruction that is at least partially reversible; lung inflammation, particularly in the airways; and bronchial (airways) hyperresponsiveness (AHR). Detailed histologic and physiologic analyses of asthmatic subjects have demonstrated that both the structure and function of the airways are altered in asthma. Conceptually, airway remodeling in asthma includes not only structural changes but also fundamental changes in the relationships between and among various airway constituents. Cell-cell interactions, the regulation of cells by extracellular matrix, and the modulation of the composition of matrix by cells have all been demonstrated to be altered.

The presence of AHR implies a fundamental change in the function of airway smooth muscle (ASM) that is partially due to changes in ASM structure and its relationships with the surrounding airway wall structures. In addition, longitudinal studies have shown that asthmatic subjects demonstrate greater losses of lung function over time than nonasthmatic subjects,¹⁻³ and a consensus of expert opinion now agrees that irreversible airflow obstruction (in some ways similar to chronic obstructive pulmonary disease) might occur in asthma, a concept proposed many years ago as part of the so-called Dutch hypothesis.⁴ Therefore the concept of airway remodeling in asthma can entail the histologic and structural changes associated with asthma, their physiologic consequences, and the progressive loss of lung function and permanent, irreversible airway obstruction that is observed in some patients with asthma. In this review we will focus on changes observed in ASM, inflammatory cellular

From the Center for Human Genomics and the Department of Internal Medicine, Section on Pulmonary, Critical Care, Allergy and Immunologic Diseases, Wake Forest University School of Medicine.

Supported in part by grants HL067663 and HL074225 to SPP.

Disclosure of potential conflict of interest: R. Pascual—none disclosed. S. Peters—As a member of the Wake Forest University Clinical Trials Group, Dr Peters has been involved in clinical trials supported by Abaris, AstraZeneca, Altana, Boehringer Ingelheim, Centocor, Genentech, Glaxo-SmithKline, Novartis, Pfizer, and Wyeth. He has also performed consulting duties under the auspices of the National Institutes of Health, Adelphi, American Thoracic Society, AstraZeneca Pharmaceuticals, Discovery, Genentech, Novartis, Omnicare, RAND Corporation, Respiratory Medicine, Respiratory Research, and SanofiAventis. He has also participated in physician education programs (including speakers' bureaus) sponsored by the American College of Chest Physicians; American Lung Association; American Academy of Allergy, Asthma and Immunology; AstraZeneca; Merck; Genentech; Novartis; and the Respiratory and Allergic Disease (RAD) Foundation.

Received for publication June 10, 2005; revised July 12, 2005; accepted for publication July 15, 2005.

Available online August 8, 2005.

Reprint requests: Rodolfo M. Pascual, MD, Wake Forest University School of Medicine, Center for Human Genomics, Medical Center Boulevard, Winston-Salem, NC 27157. E-mail: rpascual@wfubmc.edu.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.07.011

Abbreviations used

AHR: Airways hyperresponsiveness
ASM: Airway smooth muscle
 β_2 -AR: β_2 -Adrenergic receptor
MUC: Mucin glycoprotein
RBM: Reticular basement membrane
STAT: Signal transducer and activator of transcription
VEGF: Vascular endothelial growth factor

elements, bronchial epithelium, and subepithelium and describe their importance for the physiologic changes observed in asthmatic subjects.

MODELS OF AIRWAY REMODELING

A number of models have been used to characterize airway remodeling in asthma. Examination of lung tissues available at autopsy have shown that profound changes in airway morphology and structure have occurred in those that have died from asthma when compared with decedents with asthma dying from other causes and those without asthma.¹ *In vivo*, characterization of airway biopsy specimens from living subjects with asthma have been compared with those from control subjects. Many studies demonstrate stereotypical histopathologic changes associated with asthma, reversibility of some changes with corticosteroids,⁵ changes in noninflammatory airway wall structures, and alterations in inflammatory cell distribution.^{6,7} These studies are limited by the fact that bronchoscopic biopsy specimens are small and mostly limited to more proximal airway wall. The use of bronchoscopy and lung challenge models in human subjects has shown persistent changes in bronchoalveolar lavage components,⁸ whereas in animals it has been shown that persistent changes in airway histopathology might occur after chronic airway challenge.⁹

Transgenic and knockout mouse models have revolutionized our understanding of airway remodeling by demonstrating that loss or gain in function of a gene product can correlate with airway structural and inflammatory changes that closely resemble those seen in asthma. Experimental systems involving cultured airway cells, including primary smooth muscle cells, fibroblasts, and epithelial cells, have been developed, and various end points have been examined that are related to airway remodeling. Among the functions examined are the regulation of cellular proliferation and cytokine and matrix component release and some of the relevant signaling pathways regulating those events.

ASM REMODELING

Airflow obstruction and AHR are a result of the contraction of ASM and hence indicate alterations in smooth muscle function. Several studies of patients dying from severe asthma have reported an increase in the

apparent muscle mass within the airway wall in subjects with severe asthma when compared with control subjects.^{1,10,11} In histologic studies an increase in mass is assumed when there is an increase in the cross-sectional area occupied by ASM. Subjects with fatal asthma have been reported to exhibit greater degrees of luminal narrowing and ASM mass when compared with patients with asthma experiencing nonasthma-related death. A reduction in the lumen area could be due to differences in lung inflation at the time of death, a concern voiced by critics of these studies. However, typically there is also an increase in the percentage of the airway wall area occupied by ASM in subjects with severe asthma, which also suggests an increase in muscle mass in these subjects. A direct relationship between duration of asthma or severity of asthma and the amount of ASM mass also appears to be present. For example, Bai et al¹ reported that older patients with fatal asthma had more muscle in the airway wall than younger patients with fatal asthma. Assuming that ASM mass is increased in asthma, several possible mechanisms could explain this finding: an increase in cell number (hyperplasia) resulting from increased cell proliferation or a reduction in apoptosis, cellular hypertrophy, and/or differentiation of other cell types into ASM cells, with subsequent migration into the muscle bundle.

Most of the available evidence supports ASM hyperplasia as an important mechanism leading to increased ASM mass in the asthmatic airway. A partial list of potential airway mitogens and factors that can affect ASM mass is presented in Table I.¹²⁻¹⁴ Growth factors that engage receptor tyrosine kinases, a variety of agents that engage heterotrimeric G protein-coupled receptors, and other agents have been shown to be capable of inducing ASM hyperplasia. The majority of such studies that have identified these agents have been performed with cell culture models, and such models have allowed for the characterization of the signaling pathways mediating mitogen-induced ASM proliferation. These substances might be produced by resident airway cells, secreted by infiltrating inflammatory cells, or secreted by ASM, which could then act in an autocrine manner.¹² Most of these substances have been identified in bronchoalveolar lavage fluid and airway biopsy specimens or have been produced by airway cells collected from subjects with asthma. Cell culture models have allowed for the *in vitro* analysis of signaling pathways that regulate ASM proliferation.¹³ Mitogenesis mediated by cytokines, receptor tyrosine kinase ligands, and G protein-coupled receptor ligands occurs through the phosphoinositide 3'-kinase¹⁵ or the extracellular signal-regulated kinase¹⁶ pathways. Accumulating evidence suggests that combinations of ligands can induce ASM proliferation *in vitro* in a synergistic manner.¹⁷

β_2 -Adrenergic receptor (β_2 -AR) agonists appear to attenuate ASM proliferation elicited by a variety of mitogenic stimuli. Additionally, ligands activating other G protein-coupled receptors coupled to Gs/adenylyl cyclase and hence promoting cyclic AMP generation appear to also attenuate mitogen-stimulated ASM proliferation.^{18,19} Glucocorticoids have broad activity against a variety of

TABLE I. Factors affecting smooth muscle mass^{12,13}

Type	Examples	Mechanisms
Peptide growth factors	Epidermal growth factor, insulin-like growth factors, platelet-derived growth factors, fibroblast growth factor, TGF- β	Act through receptor tyrosine kinases and signal through mitogen-activated protein kinase cascades.
Inflammatory cell mediators	α -Thrombin, tryptase, sphingosine 1-phosphate, endothelin 1, substance P, thromboxane A ₂ , serotonin, LTD ₄ , LTE ₄ , PGE ₂ , IL-1 β , TNF- α	Many engage G protein-coupled receptors and can act synergistically with growth factors. Cytokines act through mitogen-activated protein kinases, nonreceptor tyrosine kinases, nuclear factor κ B, and Janus kinase-signal transducer and activator of transcription.
Extracellular matrix components	Collagens I, IV, and V; fibronectin; laminin	Might be antiapoptotic and appear to signal through integrins. ¹⁴
Pharmacologic agents	β_2 -Adrenergic receptor agonists (short-long acting), glucocorticoids, phosphodiesterase inhibitors	Agents promoting cyclic AMP inhibit mitogenesis. Steroids antagonize both mitogenic and antimitogenic pathways.

LT, Leukotriene; PG, prostaglandin.

mitogenic stimuli; however, they also can inhibit mitogen-induced cyclooxygenase 2/prostaglandin E₂ induction, a potentially important endogenous anti-proliferative pathway. The inhibition of endogenous antiproliferative pathways by both steroids and β_2 -AR agonists could potentially limit the usefulness of such agents as modulators of airway remodeling. Moreover, a recent study demonstrated that chronic β_2 -AR overexpression resulted in increased AHR in mice and appeared to modulate procontractile signaling pathways.²⁰

Whether ASM cells are larger in asthma (cellular hypertrophy) is not clear, and it has been surprisingly difficult to address this issue. In one report, in which a stereology technique was applied to bronchial biopsy specimens obtained from living subjects, the number of cells in subjects with mild-to-moderate asthma was increased compared with that seen in control subjects, but the cell size was similar.²¹

The regulation of vascular smooth muscle cell migration is better understood than the regulation of ASM cell migration, but the literature regarding ASM migration is growing. Many of the same substances that are mitogens in ASM have been shown to promote ASM migration in model systems; examples include platelet-derived growth factor, TGF- β , IL-1 β , and vascular endothelial growth factor (VEGF). Moreover, agents used to treat asthma (including corticosteroids), cyclic AMP-mobilizing agents (including the β_2 -adrenergic agonist salmeterol), and phosphodiesterase 4 inhibitors have been shown to inhibit mitogen-stimulated ASM migration *in vitro*.²² In addition to the extracellular signal-regulated kinase and phosphoinositide 3'-kinase pathways, the p38 mitogen-activated protein kinase pathway and Rho-kinase pathways also appear to play a role in ASM migration.¹³

Interestingly, airway biopsy studies have failed to demonstrate alterations in markers of apoptosis (activated caspase expression or through terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay) in ASM.²³ Such studies have also failed to demonstrate changes in proliferative markers. This is not surprising for

several reasons: such methods lack sensitivity, it is likely that ASM cells divide infrequently, and programmed cell death is a rare event. Consistent with the last possible mechanism, ASM cells in culture appear to be resistant to apoptosis and are capable of undergoing numerous cell passages. Additionally, extracellular matrix components produced by ASM and present in ASM bundles *in vivo* might have antiapoptotic effects.¹⁴ Although there is little direct evidence addressing this issue, resistance of ASM to apoptosis is plausible and could partially explain the increased ASM mass in the airways of asthmatic subjects.

Total airways resistance and nonspecific AHR are increased in asthma. How changes in ASM mass translate into increases in airways resistance remains under investigation. Potential mechanisms for the presumed ASM hypercontractility could include changes in the balance of contractile-relaxant receptors, changes in signaling, or changes in the actin-myosin force transduction apparatus. Histopathologic studies demonstrate that the entirety of the airway wall (mucosa, submucosa [including ASM], and adventitia) is thickened,⁷ and therefore intuitively one would expect that a greater force would need to be generated by the ASM contractile apparatus in an asthmatic airway.

However, *in vitro* studies have not consistently shown that asthmatic ASM generates more force than normal ASM.^{24,25} ASM force generation has usually been studied by treating ASM tissue strips or bronchial rings *ex vivo* with a variety of agents while measuring isometric tension. There are obvious limitations to this approach because it is known that smooth muscle bundles are not oriented in a perfect circumferential manner about the airway lumen, but rather ASM bundles spiral around the longitudinal axis, thus making it quite difficult to measure tension in a way that reflects force vectors generated *in vivo*. Perhaps the load imposed on ASM is reduced by changes in airway elastance or altered interactions with the lung parenchyma.²⁶⁻²⁸ It is also possible that the increased total mass results in more total force generation that is not reflected by studying airway segments.

Another interesting hypothesis is that airway wall thickening in asthmatic subjects results in a greater reduction in luminal cross-section when normal force is applied.²⁹ This hypothesis has been modeled mathematically by James et al.³⁰ Using an *in vivo* mouse model, Wagers et al.³¹ examined various parameters of respiratory impedance in allergen-challenged animals and concluded that increased AHR could be explained by increased airway closure and increased mucosal thickening without any change in smooth muscle shortening. Much attention has recently been focused on the dynamic aspects of airway narrowing and AHR. Indeed, early studies suggested that dynamic events like deep inspiration profoundly affect airways resistance. One might surmise that deep inspiration, by stretching ASM, breaks the actin-myosin cross-bridges that are responsible for force generation. Fish et al.³² observed that bronchomotor tone appeared to be regulated by changes in lung volume. A deep inspiration abolished AHR in healthy subjects, but this effect was attenuated in subjects with asthma. The authors suggested that perhaps a major problem in asthma was an inability to stretch the ASM. In a follow-up study Skloot et al.³³ demonstrated that healthy subjects have asthmatic responses and nonspecific AHR when they are prohibited from taking a deep inspiration. Since then it has been shown that the velocity of shortening of asthmatic ASM is greater,³⁴ and this and other studies led to the postulate that actin filaments are longer in asthmatic ASM²⁹; that factor, in part, prevents muscle lengthening during stretching (ie, during deep inspiration).

In another interesting study, Irvin et al.³⁵ showed that during sleep, thoracic gas volume decreases and pulmonary resistance increases. They also observed that despite the restoration of thoracic gas volume, pulmonary resistance remained high, an effect that would be consistent with what is observed clinically, namely that asthma exacerbations often occur at night. These observations are also consistent with an exaggerated loss of airway-parenchymal interdependence (the ability of the lung parenchyma to "splint open" airways) in asthmatic subjects at night, which could also contribute to nocturnal worsening of asthma.

Stimulated ASM cells in culture have been shown to be capable of producing a wide array of inflammatory mediators that might contribute to remodeling^{12,36}; however, a detailed discussion of ASM synthetic functions is beyond the scope of this review.

CHANGES IN INFLAMMATORY CELLS IN AIRWAY REMODELING

Despite the fact that airway inflammation has been known to be a prominent feature of asthma for decades, it still is not known whether inflammation drives the remodeling response in the airway wall or whether an intrinsic abnormality of airway structure or function drives inflammation. It has been widely assumed that allergic inflammation is the initiating event. A partial list of

various asthma inflammatory mediators is presented in Table II.^{18,29,37-44} There is no doubt, on the basis of *in vivo* studies, including airway challenge models, that airway inflammation is strongly associated with symptoms. Moreover, treatment that reduces inflammation effectively reduces morbidity and can reverse some aspects of remodeling.⁵ More recently, transgenic technology has identified inflammatory mediators and signaling pathways that directly regulate airway remodeling.

It has been well established that CD4⁺/T_H2 lymphocytes (ie, those that produce IL-13, IL-4, and IL-5) play a central role in allergic diseases. These cells are increased in the airways of asthmatic subjects, as are their repertoire of cytokines. The requirement of T_H2 cytokines in the development of the asthma phenotype has been most convincingly demonstrated in mouse models. Transgenic mice overexpressing IL-13 or IL-5 in the lungs have airway fibrosis and AHR, both phenotypic features of asthma in human subjects.^{37,45} Grunig et al.⁴⁶ demonstrated the central role of IL-13 using a mouse model of asthma, showing that IL-13 mediated AHR, goblet cell hyperplasia, and airway eosinophilia and that those effects occurred irrespective of whether T and B lymphocytes were present. Conversely, it is believed that CD4⁺/T_H1 cells (ie, those that produce IFN- γ) are generally antagonistic toward T_H2 cell function and proliferation. Mice lacking in T-bet, a transcription factor critical to T_H1 cell development, have AHR, eosinophilia, mucus overproduction, and other features consistent with an asthma phenotype.⁴⁷ Importantly, mice that lack signaling components (signal transducer and activator of transcription [STAT] 6^{-/-}) required for a response to antigenic stimulation are resistant to the development of allergic inflammation.⁴⁸

The key role of antigen-presenting cells in T_H2-mediated inflammation (also reviewed by Kuipers and Lambrecht⁴⁹) was recently demonstrated in a study using ovalbumin-challenged mice, showing that depletion of CD11⁺ cells abolishes AHR and eosinophilic inflammation.⁵⁰ Taken together, these and a wealth of other data show that T_H2-derived inflammatory mediators drive changes in airway structure, the functions of resident and infiltrating cells, and hence remodeling.

Eosinophils are prominent in the airways of patients with asthma.⁵¹ Airway and lung eosinophilia is highly regulated by the T_H2 cytokines IL-4, IL-5, and IL-13. As effector cells, eosinophils are capable of producing T_H2 and other cytokines, chemokines, lipid mediators, and growth factors and are also capable of causing an increase in mucus production, causing subepithelial fibrosis, and altering ASM contractility (AHR). Whether eosinophils are required to mediate T_H2 effects on airway remodeling has been an area of intense debate. Corry et al.⁵² demonstrated in mice that treatment with anti-IL-4 antibodies abrogated ovalbumin-mediated AHR without affecting airway eosinophilia, whereas treatment with anti-IL-5 antibodies did not affect ovalbumin-mediated AHR, although it effectively inhibited airway eosinophilia. In contrast, Foster et al.⁵³ found that IL-5-deficient mice were

TABLE II. Inflammatory factors in asthma^{29,38}

Class	Examples	Functions in remodeling
Cytokines	IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-11, IL-13, IL-16; GM-CSF, TNF- α	IL-13 is a major product of T _H 2 lymphocytes. ^{37,39} IL-5 drives eosinophilia and causes subepithelial fibrosis. ⁴⁰
Chemokines	Eotaxin, eosinophilic cationic protein, macrophage inflammatory protein 1 α , monocyte chemotactic protein 1, 3, and 5	Monocyte chemotactic protein 1 mediates TGF- β release.
Growth factors	TGF- β , connective tissue growth factor, basic fibroblast growth factor, platelet-derived growth factor, neurotransmitters (NK-A, NK-B, substance P), platelet activating factor, insulin-like growth factor, vascular endothelial growth factor	TGF- β mediates T-cell activation by dendritic cells and stimulates fibroblasts to produce matrix. ⁴¹
Lipid mediators	LTD ₄ , LTC ₄ , LTB ₄ ; PGE ₂ , PGF _{2α} , PGD ₂ ; thromboxane A ₂	PGE ₂ inhibits ASM proliferation, ¹⁹ (leukotrienes reviewed by Holgate et al ⁴²).
G protein-coupled receptor ligands	Prostanoids, leukotrienes, endothelin, histamine, adenosine, thrombin	Adenosine mediates IL-13 activity. ⁴³
Metalloproteinases	A disintegrin and metalloproteinase 33, MMP-9, MMP-3, MMP-12, MMP-2, TIMP-1, plasminogen activator inhibitor 1	MMP-9/TIMP-1 ratio increased in asthma, ⁴⁴ MMPs modulate extracellular matrix.

LT, Leukotriene; PG, prostaglandin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases.

resistant to ovalbumin-mediated AHR. However, recent evidence from studies in which targeted deletion of eosinophils was carried out suggests that eosinophils are required for ovalbumin-induced AHR in mice.^{54,55} Thus it appears that eosinophils are key effector cells contributing to both airway inflammation and remodeling in asthma. However, the lack of clinical efficacy for antibodies that inhibit IL-5 and reduce circulating and sputum eosinophil numbers in human asthmatic subjects continues to fuel this debate.⁵⁶⁻⁵⁸ Because current therapeutic strategies have only been shown to partially deplete eosinophils in the lungs of asthmatic subjects, the lack of efficacy for anti-IL-5 treatment of asthmatic subjects cannot be taken as definitive evidence for a lack of an important role for IL-5 in human asthma.

Neutrophils have been demonstrated in the airways of asthmatic subjects, especially in the presence of severe disease with severe airflow obstruction.⁵⁹ Although patients with severe airflow obstruction often have impressive bronchodilator reversibility, their airflow obstruction is often not fully reversible, implying that fixed structural changes in the airways have occurred. However, the role of the neutrophil in this form of asthma is not clear.

Mast cell degranulation and activation by allergen-mediated cross-linking of IgE results in the release of a variety of mediators capable of remodeling ASM, including prostaglandin D₂,⁶⁰ cysteinyl leukotrienes,⁶¹ and tryptase.⁶² A recent *in vivo* study of bronchial biopsy specimens demonstrated mast cells infiltrating ASM bundles. Evidence for an effect of mast cells on remodeling was suggested by the fact that mast cell infiltration of ASM correlated with AHR.⁶³ Other examples of the potential role of mast cells in remodeling are studies demonstrating that mast cells are a source of plasminogen activator inhibitor 1 in the asthmatic airway, whereas plasminogen activator inhibitor 1-deficient mice demonstrate reduced

matrix production, probably through modulation of matrix metalloproteinase 9.^{64,65}

The importance of the distal airway and airspace inflammation has been suggested by the fact that distal airway inflammation appears to be more common in those with nocturnal asthma.⁶⁶ Nocturnal symptoms are an important contributor to morbidity and are a marker of poorly controlled disease. In addition, distal airway inflammation seems to be prominent in more severe disease that is often steroid resistant.⁵⁹

One potential consequence of distal airway and airspace inflammation is surfactant dysfunction. Surfactant assists in maintaining airflow in the smaller airways, and several studies have shown that the components of surfactant might be altered in asthma. In one such study, antigen challenge resulted in a change in surfactant surface tension in several subjects, with evidence that the lipid components of surfactant were altered.⁶⁷ Further evidence of the role of surfactant was suggested by a small, clinical crossover trial in which pretreatment with a synthetic surfactant abolished the early asthmatic response to inhaled antigen in atopic asthmatic subjects.⁶⁸

EPITHELIAL CELL REMODELING

Asthmatic airway biopsies, autopsy studies, and animal models all show that the epithelium is remodeled in asthma. Epithelial cells are found in increased amounts in asthmatic sputum, and epithelial detachment from the basement membrane is frequently observed in the various models of asthma. Moreover, there are a higher proportion of goblet cells in the asthmatic epithelium *in vivo*, and subepithelial mucus glands are enlarged.⁶⁹

The epithelium forms the outer cellular barrier and serves important regulatory functions in immunity and

airway clearance. A body of literature indicates that the epithelial barrier immune function is abnormal in asthma (reviewed by Holtzman et al⁷⁰). It has been demonstrated that the transcription factor STAT1 is constitutively activated in epithelial cells from asthmatic subjects but not in those from healthy subjects or those with chronic bronchitis. STAT1 is a key transcription factor in IFN-mediated signaling, which is an important mechanism in viral airway infection, a known cause of asthma exacerbation. Notably, IFN- γ levels were not found to be increased in tissue from asthmatic subjects. Further analysis showed that other transcription factors, including nuclear factor κ B, STAT3, and activator protein-1, were also not activated. Epithelial STAT1 levels correlated with intercellular adhesion molecule 1 expression, which in turn correlated with T-lymphocyte accumulation in tissue.⁷¹ Taken together, these data suggest that the asthmatic epithelial functions that might regulate subsequent immune events are abnormal, implicating the epithelium as potentially driving asthmatic inflammation.

Mucus production is an important feature of asthma and contributes substantially to morbidity and mortality, especially in more severe disease. Many case reports and autopsy series demonstrate that in most cases of fatal asthma, many of the small airways are completely obstructed with mucus. Goblet cell hyperplasia has been consistently demonstrated in asthma, irrespective of whether the disease is mild or severe. Similarly, enlargement and hyperplasia of mucus glands found in the subepithelium is also a frequent finding. Goblet cells and mucus glands are the sources of mucin glycoproteins (MUCs) in the airways.

The predominant constituent of mucus is mucin, and it is responsible for most of the viscoelastic properties of mucus. Other substances that contribute to sputum elasticity and viscosity include albumin and other plasma-derived proteins and DNA and actin, both of which are byproducts of cellular death. The regulation of mucin production has been reviewed⁶⁹ and will be discussed briefly here. Twelve *MUC* genes have been described. Whereas the expression of several *MUC* genes in the airway can be detected, *MUC5AC* gene expression appears to predominate in both asthmatic and nonasthmatic airways but is probably greater in the asthmatic airway. Goblet cell hyperplasia was also confirmed in subjects with mild-to-moderate asthma in this study by using stereology techniques on airway biopsy specimens.⁷² Mouse models have also consistently shown goblet cell hyperplasia when T_H2 cytokines (IL-4, IL-5, IL-9, and IL-13) are overexpressed.⁶⁹ Similarly, adoptive transfer of T_H2 cells induces goblet cell hyperplasia in ovalbumin-challenged mice.⁷³

In addition to mucus-producing cells, the epithelium also consists of basal cells and pseudostratified cells that are equipped with cilia. Basal cells are important for epithelial regeneration and hence its integrity. Ciliated cells move mucus in a general cephalad direction, providing an important mechanism for clearing secreted mucus, particulate matter, allergen particles, and infec-

tious agents. The central role of IL-13 in asthma discussed above also extends to the regulation of the airway epithelium. IL-13 increased the proportion of secretory cells, an effect mediated by *MUC5AC*, and altered epithelial cell morphology, reducing the number of ciliated epithelial cells in a culture model of primary epithelial cells.⁷⁴

ASTHMATIC SUBEPITHELIUM AND EPITHELIAL DAMAGE AND REPAIR

Underlying the bronchial epithelial cell layer is the lamina reticularis, also known as the reticular basement membrane (RBM; reviewed by Jeffery⁷). Several studies have reported that this layer is thickened, and this is a characteristic feature of airway remodeling in asthma. For example, Payne et al⁷⁵ compared the thickness of the RBM in children with asthma who required moderately high doses of inhaled steroids with that in healthy children and adults with asthma. Interestingly, the thickness of the RBM in children was similar to that in adults with asthma and nearly twice as thick as that in control subjects, indicating that this feature of airway remodeling occurs early in the course of asthma. However, because an increase in the RBM has recently been described in at least some patients with allergic rhinitis without asthma, the specificity of this finding as a marker for asthma has been questioned.⁷⁶

The interaction among components of the subepithelium and the epithelial cells has been a recent area of intensive study. It is widely held that the epithelium is denuded in the asthmatic airway, meaning that the epithelial cells lose their attachment to the inner airway wall. Increased numbers of airway epithelial cells are found in the sputum of asthmatic subjects, especially when asthma is exacerbated. Moreover, numerous histopathologic studies show clusters of sloughed epithelial cells known as creola bodies in the lumen, whereas other biopsy studies have shown a loss of surface epithelium. However, there has been a great deal of variability within these studies because intact epithelium is frequently observed, and many studies have been done postmortem. Whether the epithelium is denuded in asthma remains controversial because one recent biopsy study done in living subjects demonstrated no difference in epithelial integrity when comparing subjects with milder asthma with control subjects.⁷⁷

Characteristic of an epithelial surface is the fact that epithelial injury results in a repair response in which the epithelium proliferates from the outside of the wound inward, and cells and mediators facilitate repair and cellular attachment. If the airway epithelium is injured as an integral part of asthma, an injury-repair cycle would be expected in the bronchial wall. An interaction between the airway epithelium and mesenchymal elements in the airway is essential for normal airway repair, and an abnormal functioning of this epithelial-mesenchymal trophic unit has been proposed to be central to the airway pathology and physiology characteristic of asthma.⁷⁸ The currently

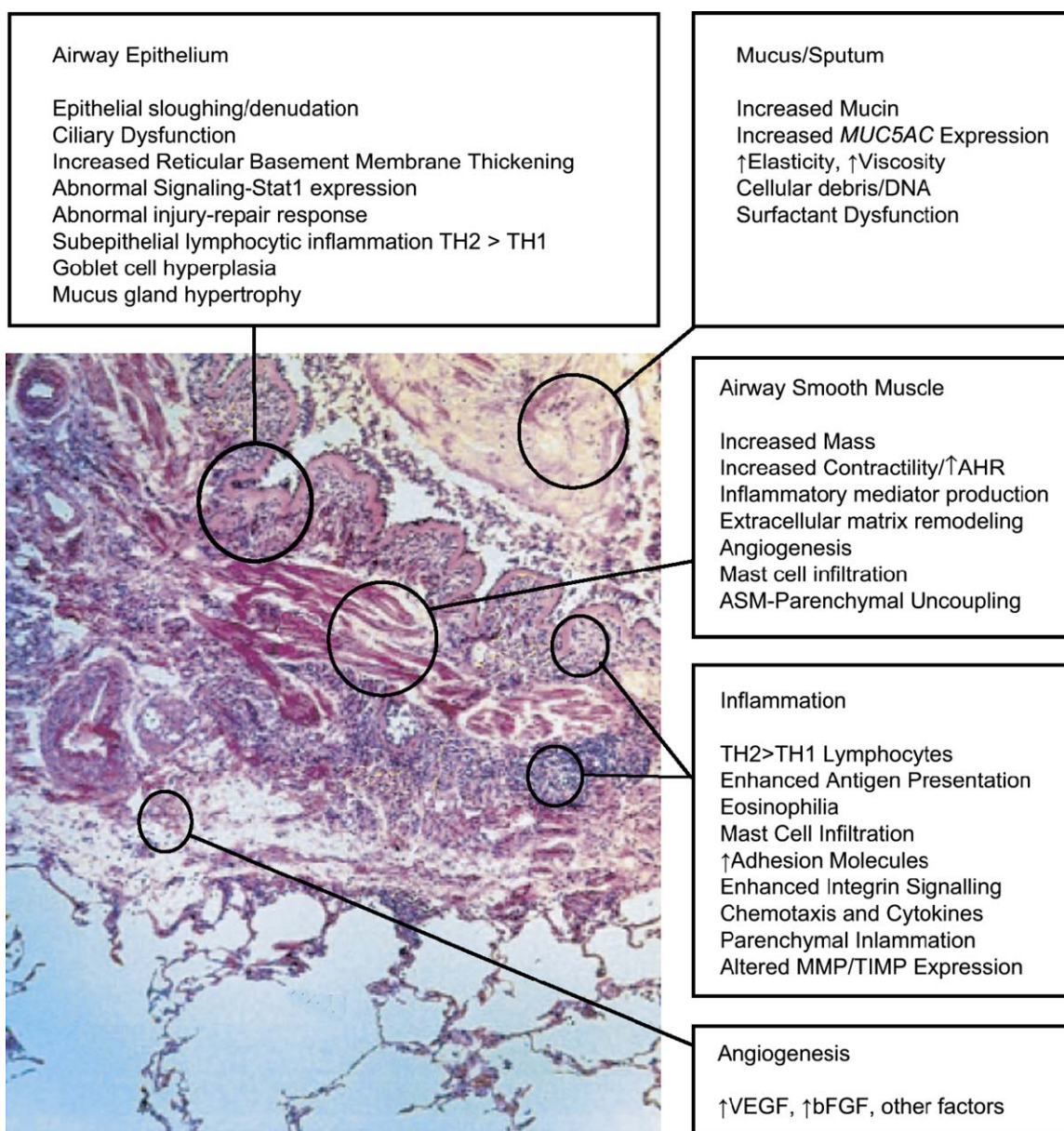


FIG 1. Airway remodeling in long-standing asthma. An airway from a 70-year-old patient with asthma is shown. Examples of the structural and functional changes of chronic asthma are highlighted and are discussed throughout the text. *MMP*, Matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinases; *bFGF*, basic fibroblast growth factor.

avored model describes a defective repair response or recurrent injury that leads to a self-perpetuating cycle of chronic inflammation modulated by various cytokine and growth factors, including TGF- β and epidermal growth factor.⁷⁹ Additionally, it has been shown that such growth factors can modulate epithelial proliferation *in vitro*.⁸⁰ Some investigators have speculated that an abnormality of repair could drive inflammation. This represents a novel concept because the prevailing wisdom has been that inflammation causes injury and remodeling.

In addition to the changes observed in the RBM, there is evidence of remodeling of the matrix components in

deeper layers of the airway, including those that surround the smooth muscle bundles. Bramley et al²⁶ have speculated that modification of the matrix surrounding ASM could have the effect of reducing airway elastance and hence the imposed load. If this were the case, the asthmatic airway would contract to a greater extent than the normal airway, even if the force generated by ASM was similar.

Fibroblasts and myofibroblasts appear to be key effector cells in both normal repair responses and in diseases in which fibrosis occurs, including asthma. Myofibroblasts behave like inflammatory cells in that they produce cytokines and growth factors and might be distinguished

from other fibroblasts by the expression of α -smooth muscle actin. Batra et al⁸¹ have shown that the T_H2 cytokine IL-4, as well as TGF- β , induced fibroblasts to express α -smooth muscle actin, hence adopting a myofibroblast phenotype. In addition, IL-4 was also shown to stimulate collagen type III production by these human lung myofibroblasts.

Matrix metalloproteinases are key regulators of the components of matrix and regulate the deposition of collagen and cytokine release (listed in Table II and reviewed by Kelly and Jarjour⁸²). Tissue inhibitor of metalloproteinases are endogenously expressed inhibitors of metalloproteinases and exist in equilibrium with the metalloproteinases in the airway wall. An imbalance between these factors could favor matrix deposition and hence affect remodeling. For example, Vignola et al⁸³ demonstrated increased tissue inhibitor of metalloproteinases 1 levels in the sputum of subjects with severe asthma *in vivo* when compared with those in subjects with milder asthma, and this finding was associated with airway remodeling assessed by means of computed tomography. As mentioned above, IL-13 appears to be a major factor driving airway inflammation that leads to airway remodeling. Many of the effects of IL-13 appear to be mediated by the metalloproteinases. In one study the effects of IL-13 overexpression were found to be mediated by TGF- β , and TGF- β activation was matrix metalloproteinase 9 dependent.⁸⁴

On the basis of observations from histopathologic studies, it has been hypothesized that the vascular bed is enlarged in asthma and thus also contributes to airway remodeling. The vascular bed might contribute to airflow obstruction by increasing wall thickness caused by more vessels or distended vessels or through edema formation. Salvato⁸⁵ studied proximal airway biopsy specimens and found that biopsy specimens from subjects with asthma had more vessels located in the lamina propria that occupied a greater cross-sectional area than biopsy specimens obtained from control subjects. Several complementary studies have demonstrated potential mechanisms for this finding. VEGF levels are increased in tissues obtained in asthmatic subjects. VEGF overexpression led to endothelial sprouts (angiogenesis) in the airway in transgenic animals.⁸⁶ Hoshino et al⁸⁷ found more vessels in bronchial biopsy specimens from atopic asthmatic subjects when compared with those found in control subjects and furthermore found that asthmatic bronchial walls contained more cells with immunoreactivity to the angiogenesis factors VEGF, basic fibroblast growth factor, and angiogenin. A photomicrograph of an asthmatic airway with a comprehensive list of alterations in the airway epithelium, mucus, ASM with increased inflammation, and angiogenesis is presented in Fig 1.

CONCLUSIONS

The clinical findings of asthma were initially identified many years ago and include reversible airflow obstruction,

AHR, and mucus hypersecretion. Subsequently, histopathologic studies of airways from both animals and human subjects provided a structural basis for these clinical observations and led to the development of the concept of airways remodeling. Longitudinal studies of patients with asthma suggest that a progressive loss of lung function occurs in at least some asthmatic subjects. Changes in the structure and function of the airway associated with remodeling can explain the time-dependent changes in airway physiology that have been observed. More recently, molecular biology approaches, including the application of transgene technology, have identified mechanisms that might explain the structural changes seen in the asthmatic airway. To date, the accumulated evidence strongly suggests that airway remodeling results in the progressive loss of lung function; however, this remains a hypothesis. Proof of this concept would require that a therapy that modifies one or more of the structural changes observed, such as mucus hypersecretion, smooth muscle hypertrophy, or airways inflammation, would also prevent loss of lung function or prevent irreversible airway obstruction.

REFERENCES

1. Bai TR, Cooper J, Koelmeyer T, Pare PD, Weir TD. The effect of age and duration of disease on airway structure in fatal asthma. *Am J Respir Crit Care Med* 2000;162:663-9.
2. Grol MH, Gerritsen J, Vonk JM, Schouten JP, Koeter GH, Rijcken B, et al. Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. A 30-year follow-up study. *Am J Respir Crit Care Med* 1999;160:1830-7.
3. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998;339:1194-200.
4. Bleeker ER. Similarities and differences in asthma and COPD. The Dutch hypothesis. *Chest* 2004;126(suppl):93S-5S.
5. Laitinen A, Altraja A, Kampe M, Linden M, Virtanen I, Laitinen LA. Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. *Am J Respir Crit Care Med* 1997;156:951-8.
6. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;140:1745-53.
7. Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 2001;164(suppl):S28-38.
8. Shaver JR, Zangrilli JG, Cho SK, Cirelli RA, Pollice M, Hastie AT, et al. Kinetics of the development and recovery of the lung from IgE-mediated inflammation: dissociation of pulmonary eosinophilia, lung injury, and eosinophil-active cytokines. *Am J Respir Crit Care Med* 1997;155:442-8.
9. McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin Exp Allergy* 2004;34:497-507.
10. Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study. *Am Rev Respir Dis* 1993;148:720-6.
11. Kuwano K, Bosken CH, Pare PD, Bai TR, Wiggs BR, Hogg JC. Small airways dimensions in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1993;148:1220-5.
12. Howarth PH, Knox AJ, Amrani Y, Tliba O, Panettieri RA Jr, Johnson M. Synthetic responses in airway smooth muscle. *J Allergy Clin Immunol* 2004;114(suppl):S32-50.
13. Hirst SJ, Martin JG, Bonacci JV, Chan V, Fixman ED, Hamid QA, et al. Proliferative aspects of airway smooth muscle. *J Allergy Clin Immunol* 2004;114(suppl):S2-17.
14. Freyer AM, Johnson SR, Hall IP. Effects of growth factors and extracellular matrix on survival of human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 2001;25:569-76.

15. Page K, Li J, Wang Y, Kartha S, Pestell RG, Hershenon MB. Regulation of cyclin D(1) expression and DNA synthesis by phosphatidylinositol 3-kinase in airway smooth muscle cells. *Am J Respir Cell Mol Biol* 2000;23:436-43.
16. Orsini MJ, Krymskaya VP, Eszterhas AJ, Benovic JL, Panettieri RA Jr, Penn RB. MAPK superfamily activation in human airway smooth muscle: mitogenesis requires prolonged p42/p44 activation. *Am J Physiol Lung Cell Mol Physiol* 1999;277:L479-88.
17. Krymskaya VP, Orsini MJ, Eszterhas AJ, Brodbeck KC, Benovic JL, Panettieri RA Jr, et al. Mechanisms of proliferation synergy by receptor tyrosine kinase and G protein-coupled receptor activation in human airway smooth muscle. *Am J Respir Cell Mol Biol* 2000;23:546-54.
18. Maruno K, Absood A, Said SI. VIP inhibits basal and histamine-stimulated proliferation of human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 1995;268:L1047-51.
19. Pascual RM, Billington CK, Hall IP, Panettieri RA Jr, Fish JE, Peters SP, et al. Mechanisms of cytokine effects on G protein-coupled receptor-mediated signaling in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L1425-35.
20. McGraw DW, Almoosa KF, Paul RJ, Kobilka BK, Liggett SB. Antithetic regulation by beta-adrenergic receptors of Gq receptor signaling via phospholipase C underlies the airway beta-agonist paradox. *J Clin Invest* 2003;112:619-26.
21. Woodruff PG, Dolganov GM, Ferrando RE, Donnelly S, Hays SR, Solberg OD, et al. Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. *Am J Respir Crit Care Med* 2004;169:1001-6.
22. Goncharova EA, Billington CK, Irani C, Vorotnikov AV, Tkachuk VA, Penn RB, et al. Cyclic AMP-mobilizing agents and glucocorticoids modulate human smooth muscle cell migration. *Am J Respir Cell Mol Biol* 2003;29:19-27.
23. Benayoun L, Letuve S, Druihe A, Boczkowski J, Dombret MC, Mechighel P, et al. Regulation of peroxisome proliferator-activated receptor gamma expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *Am J Respir Crit Care Med* 2001;164:1487-94.
24. Bai TR. Abnormalities in airway smooth muscle in fatal asthma. A comparison between trachea and bronchus. *Am Rev Respir Dis* 1991;143:441-3.
25. Whicker SD, Armour CL, Black JL. Responsiveness of bronchial smooth muscle from asthmatic patients to relaxant and contractile agonists. *Pulm Pharmacol* 1988;1:25-31.
26. Bramley AM, Thomson RJ, Roberts CR, Schellenberg RR. Hypothesis: excessive bronchoconstriction in asthma is due to decreased airway elastance. *Eur Respir J* 1994;7:337-41.
27. Macklem PT. Mechanical factors determining maximum bronchoconstriction. *Eur Respir J Suppl* 1989;6:516s-9s.
28. Macklem PT. A theoretical analysis of the effect of airway smooth muscle load on airway narrowing. *Am J Respir Crit Care Med* 1996;153:83-9.
29. Solway J, Fredberg JJ. Perhaps airway smooth muscle dysfunction contributes to asthmatic bronchial hyperresponsiveness after all. *Am J Respir Cell Mol Biol* 1997;17:144-6.
30. James AL, Pare PD, Hogg JC. The mechanics of airway narrowing in asthma. *Am Rev Respir Dis* 1989;139:242-6.
31. Wagers S, Lundblad LK, Ekman M, Irvin CG, Bates JH. The allergic mouse model of asthma: normal smooth muscle in an abnormal lung? *J Appl Physiol* 2004;96:2019-27.
32. Fish JE, Ankin MG, Kelly JF, Peterman VI. Regulation of bronchomotor tone by lung inflation in asthmatic and nonasthmatic subjects. *J Appl Physiol* 1981;50:1079-86.
33. Skloot G, Permutt S, Togias A. Airway hyperresponsiveness in asthma: a problem of limited smooth muscle relaxation with inspiration. *J Clin Invest* 1995;96:2393-403.
34. Ma X, Cheng Z, Kong H, Wang Y, Unruh H, Stephens NL, et al. Changes in biophysical and biochemical properties of single bronchial smooth muscle cells from asthmatic subjects. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1181-9.
35. Irvin CG, Pak J, Martin RJ. Airway-parenchyma uncoupling in nocturnal asthma. *Am J Respir Crit Care Med* 2000;161:50-6.
36. Panettieri RA Jr. Airway smooth muscle: immunomodulatory cells that modulate airway remodeling? *Respir Physiol Neurobiol* 2003;137:277-93.
37. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999;103:779-88.
38. Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004;22:789-815.
39. Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev* 2004;202:175-90.
40. Blyth DI, Wharton TF, Pedrick MS, Savage TJ, Sanjar S. Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. *Am J Respir Cell Mol Biol* 2000;23:241-6.
41. Clark DA, Coker R. Transforming growth factor-beta (TGF-beta). *Int J Biochem Cell Biol* 1998;30:293-8.
42. Holgate ST, Peters-Golden M, Panettieri RA, Henderson WR Jr. Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. *J Allergy Clin Immunol* 2003;111(suppl):S18-34.
43. Blackburn MR, Lee CG, Young HW, Zhu Z, Chunn JL, Kang MJ, et al. Adenosine mediates IL-13-induced inflammation and remodeling in the lung and interacts in an IL-13-adenosine amplification pathway. *J Clin Invest* 2003;112:332-44.
44. Mautino G, Capony F, Bousquet J, Vignola AM. Balance in asthma between matrix metalloproteinases and their inhibitors. *J Allergy Clin Immunol* 1999;104:530-3.
45. Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med* 1997;185:2143-56.
46. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
47. Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 2002;295:336-8.
48. Mathew A, MacLean JA, DeHaan E, Tager AM, Green FH, Luster AD. Signal transducer and activator of transcription 6 controls chemokine production and T helper cell type 2 cell trafficking in allergic pulmonary inflammation. *J Exp Med* 2001;193:1087-96.
49. Kuipers H, Lambrecht BN. The interplay of dendritic cells, Th2 cells and regulatory T cells in asthma. *Curr Opin Immunol* 2004;16:702-8.
50. van Rijt LS, Jung S, Kleinjan A, Vos N, Willart M, Duez C, et al. In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J Exp Med* 2005;201:981-91.
51. Bousquet J, Chané P, Lacoste JY, Barneon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
52. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996;183:109-17.
53. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195-201.
54. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, et al. A critical role for eosinophils in allergic airways remodeling. *Science* 2004;305:1776-9.
55. Lee JJ, Dimina D, Macias MP, Ochkur SI, McGarry MP, O'Neill KR, et al. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004;305:1773-6.
56. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199-204.
57. Kips JC, O'Connor BJ, Langley SJ, Woodcock A, Kerstjens HA, Postma DS, et al. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003;167:1655-9.
58. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.

59. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999;160:1001-8.
60. Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K, et al. Prostaglandin D2 as a mediator of allergic asthma. *Science* 2000;287:2013-7.
61. Vargaftig BB, Singer M. Leukotrienes mediate murine bronchopulmonary hyperreactivity, inflammation, and part of mucosal metaplasia and tissue injury induced by recombinant murine interleukin-13. *Am J Respir Cell Mol Biol* 2003;28:410-9.
62. Berger P, Perng DW, Thabrew H, Compton SJ, Cairns JA, McEuen AR, et al. Trypsin and agonists of PAR-2 induce the proliferation of human airway smooth muscle cells. *J Appl Physiol* 2001;91:1372-9.
63. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002;346:1699-705.
64. Cho SH, Tam SW, Demissie-Sanders S, Filler SA, Oh CK. Production of plasminogen activator inhibitor-1 by human mast cells and its possible role in asthma. *J Immunol* 2000;165:3154-61.
65. Cho SH, Ryu CH, Oh CK. Plasminogen activator inhibitor-1 in the pathogenesis of asthma. *Exp Biol Med* (Maywood) 2004;229:138-46.
66. Kraft M, Djukanovic R, Torvik J, Cunningham L, Henson J, Wilson S, et al. Evaluation of airway inflammation by endobronchial and trans-bronchial biopsy in nocturnal and nonnocturnal asthma. *Chest* 1995;107(suppl):162S.
67. Hite RD, Seeds MC, Bowton DL, Grier BL, Safta AM, Balkrishnan R, et al. Surfactant phospholipid changes after antigen challenge: a role for phosphatidylglycerol in dysfunction. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L610-7.
68. Babu KS, Woodcock DA, Smith SE, Staniforth JN, Holgate ST, Conway JH. Inhaled synthetic surfactant abolishes the early allergen-induced response in asthma. *Eur Respir J* 2003;21:1046-9.
69. Fahy JV. Goblet cell and mucin gene abnormalities in asthma. *Chest* 2002;122(suppl):320S-6S.
70. Holtzman MJ, Morton JD, Shornick LP, Tyner JW, O'Sullivan MP, Antao A, et al. Immunity, inflammation, and remodeling in the airway epithelial barrier: epithelial-viral-allergic paradigm. *Physiol Rev* 2002;82:19-46.
71. Sampath D, Castro M, Look DC, Holtzman MJ. Constitutive activation of an epithelial signal transducer and activator of transcription (STAT) pathway in asthma. *J Clin Invest* 1999;103:1353-61.
72. Ordonez CL, Khashayar R, Wong HH, Ferrando R, Wu R, Hyde DM, et al. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 2001;163:517-23.
73. Cohn L, Tepper JS, Bottomly K. IL-4-independent induction of airway hyperresponsiveness by Th2, but not Th1, cells. *J Immunol* 1998;161:3813-6.
74. Laoukili J, Perret E, Willems T, Minty A, Parthoens E, Houcine O, et al. IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells. *J Clin Invest* 2001;108:1817-24.
75. Payne DN, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, et al. Early thickening of the reticular basement membrane in children with difficult asthma. *Am J Respir Crit Care Med* 2003;167:78-82.
76. Braunstahl GJ, Fokkens WJ, Overbeek SE, Kleinjan A, Hoogsteden HC, Prins JB. Mucosal and systemic inflammatory changes in allergic rhinitis and asthma: a comparison between upper and lower airways. *Clin Exp Allergy* 2003;33:579-87.
77. Ordonez C, Ferrando R, Hyde DM, Wong HH, Fahy JV. Epithelial desquamation in asthma: artifact or pathology? *Am J Respir Crit Care Med* 2000;162:2324-9.
78. Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J Allergy Clin Immunol* 2000;105:193-204.
79. Puddicombe SM, Polosa R, Richter A, Krishna MT, Howarth PH, Holgate ST, et al. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. *FASEB J* 2000;14:1362-74.
80. Puddicombe SM, Torres-Lozano C, Richter A, Bucchieri F, Lordan JL, Howarth PH, et al. Increased expression of p21(waf) cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. *Am J Respir Cell Mol Biol* 2003;28:61-8.
81. Batra V, Musani AI, Hastie AT, Khurana S, Carpenter KA, Zangrilli JG, et al. Bronchoalveolar lavage fluid concentrations of transforming growth factor (TGF)-beta1, TGF-beta2, interleukin (IL)-4 and IL-13 after segmental allergen challenge and their effects on alpha-smooth muscle actin and collagen III synthesis by primary human lung fibroblasts. *Clin Exp Allergy* 2004;34:437-44.
82. Kelly EA, Jarjour NN. Role of matrix metalloproteinases in asthma. *Curr Opin Pulm Med* 2003;9:28-33.
83. Vignola AM, Paganin F, Capieu L, Scichilone N, Bellia M, Maakel L, et al. Airway remodelling assessed by sputum and high-resolution computed tomography in asthma and COPD. *Eur Respir J* 2004;24:910-7.
84. Lee CG, Homer RJ, Zhu Z, Lanone S, Wang X, Kotliansky V, et al. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor beta(1). *J Exp Med* 2001;194:809-21.
85. Salvato G. Quantitative and morphological analysis of the vascular bed in bronchial biopsy specimens from asthmatic and non-asthmatic subjects. *Thorax* 2001;56:902-6.
86. Lee CG, Link H, Baluk P, Homer RJ, Chapoval S, Bhandari V, et al. Vascular endothelial growth factor (VEGF) induces remodeling and enhances TH2-mediated sensitization and inflammation in the lung. *Nat Med* 2004;10:1095-103.
87. Hoshino M, Takahashi M, Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol* 2001;107:295-301.