

Histopathologic features of early and progressive asthma

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During the last decade, morphologic studies on bronchial biopsy specimens have led to our present understanding of asthma as an inflammatory airways disease. However, little knowledge exists about the sequence of cellular events during the disease or of possible mucosal changes early in asthma. So far the primary cause, the site of damage, and the mechanisms inducing the inflammatory reaction remain to be elucidated. A multifactorial genetic susceptibility may be important for the development of asthma. Suggested factors that may trigger changes in the cells' morphologic and functional phenotype are viral infections, allergen exposure, maternal factors, diet, and smoking. Current evidence has implied that interactions between epithelial cells and the subepithelial connective tissue in the mucosa are important for normal homeostatic balance. Changes in airway epithelial phenotype possibly resulting from altered gene expression in its lining cells may be very important even as a first line change in asthma. (*J Allergy Clin Immunol* 2000;105:S509-13.)

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The health and integrity of the airway epithelium may be important factors regulating the functions of connective tissue cells and the composition of the extracellular matrix (ECM).¹⁻⁴ There is increasing evidence that epithelial cells perform multiple functions related to modulation of local inflammation, wound healing, differentiation, and establishment of polarity that require interactions of their cell surface receptors with the ECM.⁴⁻⁶ Evaluated by both light and electron microscopy, the first studies showed that patients with mild-to-severe asthma show damage of the airway epithelium.⁷⁻⁹ In addition to the shedding of the columnar epithelial cells, the ciliated cell-line has been reported to be the most elaborately affected cell type.^{7,10} The mechanisms behind airway epithelial cell damage or its repair mechanisms are not well known.⁵ Because airway epithelial cells closely interact with ECM, altered expression of ECM proteins may occur in asthma. Cellular migration, differentiation,

Abbreviations used

BM:	Basement membrane
ECM:	Extracellular matrix
LF:	Lamina fibroreticularis
MCP-1:	Monocyte chemoattractant protein-1

proliferation, and remodeling are events that occur during embryonic and postnatal development and reoccur during wound healing.^{1-3,5,6} To better understand the underlying pathophysiologic condition behind the development and progression of asthma, it is crucial to understand the type of interactive mechanisms that take place at the airway epithelial and stromal interface during normal airway branching morphogenesis and in pathologic processes modified by inflammation.

RECOGNITION OF PATHOLOGIC CHANGES IN THE AIRWAY MUCOSA

Airway epithelium

In specimens from patients dying in status asthmaticus, it has been difficult to find areas of normal bronchial epithelium. Autopsy specimens have shown marked airway edema with the shedding of airway columnar epithelial cells that leaves a layer of basal cells on the basal lamina.¹¹ The use of endoscopic bronchial biopsy techniques has led to recognition of structural changes in the airway epithelium of living patients with asthma.⁷⁻¹⁰

The methods of taking bronchial biopsy specimens by means of rigid tube bronchoscopy and the use of electron microscopy^{7,10} allows the study of airway mucosa of patients with asthma with less artifactual loss of the epithelial cells than with the fiberoptic technique. To establish epithelial structure in the airways of patients with newly diagnosed asthma in biopsy specimens, the number of cells touching the lumen was counted. The most discernible finding was that of goblet cell hyperplasia. The epithelium was made up of a greater than normal ratio of goblet to ciliated cell, reflecting a change in the epithelial phenotype already early in asthma.¹⁰ Epithelial shedding may be related to a more progressive chronic type of asthma.⁷

Inflammation of the airway mucosa

Recently many studies have suggested that epithelial cells may orchestrate the recruitment and activation of several different types of inflammatory cells by releasing

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chemotactic factors with proinflammatory properties and by expressing adhesion molecules.¹²⁻¹⁵ Immunohistochemical and cell culture studies have shown that epithelial cells from patients with asthma can express many potent inflammatory cytokines, such as GM-CSF, IL-6,^{12,16,17} and chemokine monocyte chemoattractant protein-1 (MCP-1),¹⁸ suggesting that they are in an activated state contributing to the inflammatory pathology of asthma. Bronchial epithelial cells can be induced to express IL-1, IL-6, IL-8, GM-CSF and MCP-1^{12,17} by treatment with IL-1 and tumor necrosis factor produced by activated macrophages. MCP-1 is a chemoattractant and activating agent for monocytes¹⁸ suggesting that it contributes to accumulation of macrophages in the bronchial epithelium. A paracrine feed-back pathway demonstrates that interactive mechanisms exist between epithelial cells producing GM-CSF and MCP-1 and macrophages in the stroma capable of producing IL-1.

A recent study showed that inflammatory cytokines could have a role even in altering airway epithelial cell morphologic condition.¹⁹ IL-4 has been recognized as an important pleiotropic cytokine that participates in asthmatic lung injury and inflammation. In 4- to 52-week-old transgenic mice in which IL-4 was expressed in the bronchial and bronchiolar epithelial cells under the control of the Clara cell secretory protein promoter, IL-4 caused progressive pulmonary infiltration of many inflammatory cells including lymphocytes and eosinophils. Interestingly, airway epithelial cell hypertrophy and mucus cell metaplasia were observed in the Clara cell secretory protein promoter IL-4 mice at all ages.¹⁹ These alterations were similar to morphologic changes that were earlier recognized in adult patients with asthma.^{10,20}

The great variability in the number of mast cells, eosinophils, lymphocytes, and neutrophils in the airway mucosa of patients with asthma gives important clues to disease process. The regular occurrence of mast cells in the airway epithelium, even in patients with mild asthma of short disease duration, supports the importance of mast-cell involvement in the initial stage of the disease.^{7,15,21,22} Pesci et al²² showed that there is progressive mast-cell degranulation when the cells move toward the airway lumen, both in patients with asthma and in control subjects, but that this degranulation is more evident in patients with asthma. The number of degranulated mast cells in the airway epithelium is significantly higher in patients with asthma than in control subjects. In both groups, granulated mast cells were absent from the epithelium; whereas in the lamina propria, granulated mast cells formed about one third of the total mast cells in patients with asthma and two thirds of the total mast cells in normal subjects.²² Increased numbers of eosinophils have been found both in patients with asthma with stable disease and in patients with evidence of disease activity.^{14,23-25} An increase in the number of eosinophils in the mucosa has been related to an increase in asthma symptoms^{26,27} and may contribute to the pathophysiologic process responsible for a change from stable asthma into asthma exacerbation.

Airway epithelial basement membrane and ECM

The basement membrane (BM) is a thin layer of specialized ECM between the epithelium and stroma that not only provides mechanical support but also influences cell-specific functions.^{28,29} Several studies have suggested that the subepithelial BM in patients with asthma is thickened,^{7,11,30-32} and it has been proposed that such thickening could cause the disease to become more persistent. The BM of the airways consists of a basal lamina and the lamina fibroreticularis (LF).²⁹ The constituents of the basal lamina, type IV collagen, laminin, and some proteoglycans are uniformly found in different isoforms in all basal laminae in different organs of the body.²⁸ In asthma, changes in the BM have mainly been described to occur in the LF.³⁰⁻³³ The thickness of the BM has been evaluated at electron microscopy level or in light microscopy with routine staining of the specimens.^{30,31} At the epithelial side, the border of the BM can be clearly seen, but at the stromal side there is a gradual change of the composition of the LF to the stromal matrix, which makes it difficult to define the inner border of the BM. Thus it has become more important to reveal the molecular components of the BM and, by their specific accumulation in the BM area, to define its thickness. However, a thickened BM in asthma can be seen, even without the use of specific monoclonal antibodies, both in electron micrographs and in light microscopy in hematoxylin and eosin-stained sections.^{30,31}

Collagenous proteins are the major constituents of ECMs. At least 18 different collagen types have been identified that contain at least 1 collagen triple helix as a structural motive. In the airways, the main contributors to the airway wall structure are interstitial collagen types I, III, and V.³²⁻³⁴ Collagen type IV is the main component of the BM and forms networks by self-assembly. Laminin-nidogen complexes self-associate into less-ordered aggregates.^{28,29} Laminin and type IV collagen serve as substrates for migration and attachment for epithelial cells.^{28,35} Roche et al³³ showed that in asthma the abnormally dense lamina reticularis beneath the bronchial epithelium is composed of interstitial collagen types I, III, and V and fibronectin and suggested that it is produced by myofibroblasts lying beneath the epithelium.³⁶

Special interest has recently been focused on laminins.³⁵ The laminins form a family of asymmetric cross-shaped glycoproteins containing 1 α chain (α 1- α 5), 1 β chain, (β 1- β 3), and 1 γ chain (γ 1- γ 2). The first laminin characterized, Ln-1 (α 1, β 1, γ 1) was isolated from a murine tumor, and at least 11 different Lns are now known.³⁵ In the adult human bronchi, immunoreactivity against Ln chains α 1, β 1, and γ 1 has been demonstrated in the BM, blood vessels, and smooth muscle cells. Ln α 3 and β 3 chains are also expressed in the bronchial BM. The Ln α 2 chain is seen occasionally in nerves, and the Ln β 2 chain is found only in smooth muscle cells and blood vessels.³⁷ These studies suggest that

normal adult bronchial BM contains both Ln-1 and Ln-5 ($\alpha 3$, $\beta 3$, $\gamma 2$). Several Ln-binding integrins such as $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 6\beta 1$, and $\alpha 6\beta 4$ have been detected on human bronchial epithelial cells.³⁸ The Ln chain composition in asthmatic airways has recently been studied in a biopsy study that shows no difference in Ln $\alpha 1$ distribution and thickness among the patients with seasonal and chronic asthma and control subjects.³⁹ The Ln $\beta 2$ chain, which usually is negative in adult airways, stained positively in the BM of chronic and occupational asthma. The Ln $\alpha 2$ chain, which is negative in normal adult airways, stained positive in the BM of patients with occupational and chronic asthma but not in mild asthma or control subjects.³⁹ Because the Ln $\alpha 2$ and $\beta 2$ chain appear to function only during morphogenesis of the lungs,³⁷ some mechanism in the asthmatic process seems to activate the expression of these Ln chains. The normally occurring Ln-1 combination possibly becomes shifted to a different Ln combination. This change could disturb epithelial integrity and binding because the assembly forms of Ln (Ln-2, $\alpha 2\beta 1\gamma 1$, and Ln-4, $\alpha 2\beta 2\gamma 1$) containing the Ln $\alpha 2$ chain do not normally bind or bind with low affinity to the common Ln integrin receptor integrin $\alpha 2\beta 1$. Increased expression of these Lns in asthma could lead to weakened cellular attachment and the loss of epithelial cells. Loss of BM attachment and integrin signaling is known to lead to apoptotic death of epithelial cells.

The glycoproteins fibronectin and tenascin occur in some BMs, but their role is unclear.^{40,41} Tenascin is a large hexameric ECM glycoprotein, which is highly expressed during embryonic development in a spatially and temporally restricted manner, especially at the epithelial-mesenchymal border.⁴¹ It has a variety of biologic activities, associated with specific domains of the molecule, such as cellular adhesion and signaling, growth, differentiation, and morphogenesis of certain tissues. In adult tissues its expression is absent or markedly downregulated, but it re-emerges in pathologic conditions such as wound healing, inflammatory, and neoplastic processes.⁴¹ Tenascin and fibronectin have opposite functions in many cell models: tenascin is inhibitory, whereas fibronectin promotes adhesion (for instance of bronchial epithelial cells).^{40,41} Cultured human bronchial epithelial cells can be stimulated to produce both of these ECM glycoproteins.⁴²

Tenascin has been extensively studied in various organs⁴¹ and has been described to be variably expressed in the BM zone of bronchi.^{43,44} A recent study on bronchial biopsy specimens⁴⁵ showed an increase in tenascin immunoreactivity in the bronchial subepithelial BM layer in patients with chronic and with seasonal asthma compared with control subjects. The tenascin immunoreactivity appearing as an intense wide subepithelial band in asthma was seen only occasionally in the BM of control specimens. Instead, in contrast to earlier published results by Roche et al,³³ no accumulation of any fibronectin isoform (extradomain A and extradomain B cellular fibronectins, oncofetal fibronectin, and total fibronectin) could be detected in the airway BM area. A

diffuse immunoreaction against both total fibronectin and locally produced extradomain A-fibronectin was similarly visible in the airway mucosa of both patients and control subjects.⁴⁵ A similar type of immunostaining is recognized with monoclonal antibodies against collagen type III. The used monoclonal antibodies stain the BM and stroma, but no clear accumulation to the BM can be recognized (unpublished observations). Because of the diffuse staining of collagen type III and fibronectin, we propose that these components do not contribute to the special BM-related thickening. In contrast to the results mentioned earlier, tenascin staining appears as a brightly fluorescent band with clear localization in the BM zone. Thus it could contribute to the formation of the increased width of BM zone in asthma.

RECAPITULATION OF DEVELOPMENTAL MECHANISM IN EARLY AND PROGRESSIVE ASTHMA

The epithelial BM compartmentalizes airway mucosa separating the epithelium, which is of endodermal origin, from the mesenchyme. The BM is considered a specialized form of the ECM. Its role has become important both in airway developmental processes related to the branching morphogenesis of the airways and epithelial cell differentiation.^{3,6,28,29} The ECM components play a significant role in regulating the behavior of cells. Different matrix proteins induce changes in cell shape, polarity and movement. They bind soluble messengers, such as growth factors and cytokines, and facilitate cell-cell-matrix interactions.⁶ Matrix induces differentiation that includes commitment of epithelial cells to their determined phenotype. The 3 main ways of communication are soluble factors, cell surface receptors (the most important of which are integrin receptors), and tensile forces.⁶ These activities contribute to the phenotypic changes that are related to alterations in the expression of specific genes which occur in the cells. These properties are important during embryogenetic events and in determining the function of cells in different parts of the airways.^{6,46} Abnormal expression of these properties may underlie pathogenetic processes in the airways.^{1,6,46,47} Considerable data supports an important role for ECM glycoproteins, such as laminin and fibronectin, in receptor-mediated interactions with cells.^{35,40}

Epithelial damage and inflammation in asthma may be associated with wound-repair processes in the airways.^{4,5,45} Wound healing is characterized by the appearance of many ECM components in a spatial and sequential pattern.^{1-5,48} Such repair and developmental processes share common features indicating that, during inflammation and wound healing, mechanisms functioning during embryogenesis may be recapitulated, repeating the processes of development.¹⁻⁶ Accordingly we have recently shown re-expression of Ln subchains $\alpha 2$ and $\beta 2$ in asthmatic airways, normally expressed only for a short period during the branching morphogenesis of the fetal airways.³⁹ Also tenascin, which is usually scarce in adult tissues but

is abundant during embryogenesis and in healing wounds, is highly accumulated in asthma at the epithelial mesenchymal border.^{41,45} Re-expression of tenascin may be related to the inflammatory process in the airways or re-expression of similar type of mechanisms normally functioning during the embryonic development of the conducting airways. Recent studies have also shown similar type of re-expression of fetally important bombesin-like peptides such as gastrin-releasing peptide in airway repair processes.⁴⁹ The mechanisms of epithelial cell commitment during airway development may be primarily changed in patients with asthma, leading to disturbances in formation of normal morphologic and functional epithelial phenotype. However, the study by Jain-Vora et al¹⁹ shows that inflammatory mediators could postnatally sustain phenotypic changes in the epithelium. In the postnatal mouse lung, IL-4 was capable of altering epithelial cell differentiation. In asthma already at a clinically early stage, epithelial damage and inflammation can be recognized in the mucosa. Different inflammatory mediators involved in the asthmatic pathologic process are likely to interfere with normal epithelial healing and differentiation mechanisms. Epithelial-mesenchymal interactions may be further disturbed, leading to more pronounced changes in the structure of the airway wall.

CONCLUSIONS

In asthma, airway epithelial cell damage and inflammation are important factors contributing to the pathogenetic process of the progression of the disease. In between these processes, there are simultaneously activated repair-remodeling-healing cascades, including synthesis and deposition of new matrix and degradation of many matrix components. Airway epithelial phenotypic changes may depend on early altered commitment of the epithelial cells in patients with asthma caused by genetic susceptibility. This could lead to abnormal functional cellular responses to triggering factors. It will be important to know these cascades and trace changes in epithelial cell gene expression to find new markers to detect asthma at an earlier stage of the process.

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