Fibronectin Matrix Deposition and Cell Contractility*

Implications for Airway Remodeling in Asthma

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The adhesion of cells to the extracellular matrix (ECM) protein, fibronectin, is important in the regulation and coordination of such complex processes as cell growth, migration, differentiation, and ECM organization. The deposition of fibronectin into the ECM is a cell-dependent process that is normally tightly regulated to ensure controlled matrix deposition. Increased deposition of fibronectin and collagen into the subepithelial space of the airways is observed in all forms of asthma and occurs early in the progression of the disease. Experimental evidence suggests a model in which fibronectin matrix accumulation contributes to the progression of asthma by altering both the structural properties of the airways and the functional properties of cells of the airway wall.

Key words: asthma; collagen; contractility; extracellular matrix; fibronectin; migration

Abbreviations: ECM = extracellular matrix; GST = glutathione-S-transferase; III-1H = cryptic, heparin-binding, C-terminal III-1 fragment

One of the prominent structural changes associated with asthma involves enhanced matrix deposition and remodeling within the subepithelial region of the airway wall. In patients with severe, persistent asthma, the architecture of the airway wall undergoes prominent, often permanent, structural changes, including the increased deposition of fibronectin and collagen types III and V into the subepithelial layer and loss of airway epithelium. Studies of asthmatic patients have repeatedly demonstrated a twofold to threefold increase in the thickness of the basement membrane, even in patients undergoing treatment with corticosteroids. Furthermore, histologic studies have demonstrated increased deposition of extracellular matrix (ECM) proteins, including fibronectin, into the subepithelium of patients with mild, atopic asthma. Clinical evidence has indicated a correlation between airway hyperresponsiveness and the structural changes associated with airway inflammation, leading researchers to propose that airway remodeling is a fundamental abnormality involved in the pathogenesis of asthma.

ECM Organization and Asthma

The mechanism by which enhanced ECM deposition contributes to the pathogenesis of asthma is not known. It has been proposed that the increased deposition of fibronectin and collagen into the subepithelial matrix alters the precise molecular composition of the airway and thereby affects both the geometric and biomechanical...
properties of the airway. Mathematical models suggest that the accumulation of subepithelial ECM will increase the thickness of the airway wall and thereby enhance airway narrowing in response to smooth muscle contraction. In addition, collagen fibrils within the subepithelial matrix appear thicker and more densely packed in asthmatic patients than in control subjects. Other studies have identified irregularly arranged collagen fibrils in the ECM of asthmatic patients. It has been proposed that alterations in collagen deposition and organization increase the tensile stiffness of the airways and, in turn, decrease airflow distensibility. Immunoelectron microscopy data suggest that the presence of fibronectin in wounds is associated with type III collagen fibers. Other studies have demonstrated colocalization of fibronectin and collagen fibers in cultured fibroblasts. In addition, in vitro evidence indicates that fibronectin polymerization into the ECM is required for subsequent collagen deposition. Thus, in patients with asthma, the enhanced deposition of fibronectin into the subepithelial space may alter the deposition and/or arrangement of collagen fibrils and, in turn, modify the mechanical properties of the airway wall.

**Fibronectin and Cell Contractility**

Fibronectin is a high-molecular-mass, multidomain glycoprotein that circulates in a soluble form in the plasma and is also found in an insoluble, multimeric form within the ECM. The polymerization of soluble fibronectin into insoluble fibrils within the ECM is a dynamic, cell-dependent process that is mediated by a series of events involving the actin cytoskeleton and integrin receptors. Tight control over the process of fibronectin deposition ensures that protomeric fibronectin remains soluble in the blood and that this soluble fibronectin is polymerized into the ECM only at appropriate sites. In certain chronic inflammatory diseases, including asthma, the loss of this regulation gives rise either to excess or to inappropriate fibronectin deposition that parallels the development of tissue fibrosis. The development of tension in contracting granulation tissue is mediated primarily by fibroblasts and requires an organized cytoskeleton. Tension generation due to increases in cytoskeletal contractility may be determined directly by imbedding cells into three-dimensional, floating collagen gels and measuring the degree of gel contraction over time. In this well-characterized model of wound contraction, cells anchored in the collagen matrix organize and contract collagen fibrils. To determine the role of ECM fibronectin deposition on actin organization and collagen contraction, fibronectin-null cells were imbedded into floating, native type I collagen gels, and the ability of fibronectin to stimulate collagen gel contraction was assessed. Fibronectin-null cells were cultured in defined media to precisely control the levels of matrix fibronectin. The addition of fibronectin to fibronectin-null cells imbedded in collagen gels triggered a dose-dependent increase in collagen gel contraction. The addition of either vitronectin or laminin to collagen gels did not increase gel contraction, suggesting that the stimulation of cell contractility is not a general property of ECM proteins. Fibronectin-stimulated contractility was dependent on integrin ligation, however, integrin ligation by fibronectin fragments was not sufficient to induce collagen contraction. Fibronectin-induced contractility was blocked by agents that inhibit fibronectin polymerization, suggesting that fibronectin deposition into the ECM is critical in triggering collagen contraction. Thus, excess fibronectin matrix deposition in the subepithelial space of the airways may contribute to abnormal tissue remodeling by enhancing the cell-mediated contraction of collagen fibrils.

**Fibronectin and Cell Migration**

The restoration of the epithelial barrier following airway injury involves the migration of epithelial cells at the leading edge of the wound over a fibronectin-containing provisional matrix. Cell migration is a dynamic process that requires adhesive events that coordinate interactions between integrin receptors and the actin cytoskeleton to regulate the formation and release of focal adhesions. Previous studies have correlated a decrease in actin stress fiber formation and a disruption of focal adhesions with growth factor-stimulated increases in cell motility. In addition, Chinese hamster ovary cells expressing αIIbβ3 integrin mutants, which increase the organization of actin filaments, display a decrease in the rate of cellular migration on a fibrinogen-coated substrate. The ability of ECM fibronectin to increase cell contractility suggests the possibility that increased fibronectin deposition in the subepithelial space of airways may contribute to the development of asthma by altering the rate or extent of epithelial cell migration after injury. Our preliminary in vitro studies indicate that the migration of small airway epithelial cells requires ECM fibronectin deposition. However, with the addition of increasing amounts of fibronectin to these cells, the rate of migration subsequently decreases, suggesting that excess fibronectin deposition into the subepithelial space may inhibit reepithelialization. Interestingly, it has been proposed that reepithelialization of the airway modulates ECM remodeling, as prostaglandin E2 derived from airway epithelial cells can inhibit fibroblast proliferation, collagen and fibronectin production, and collagen gel contraction. Taken together, these data suggest that a dynamic, reciprocal relationship exists between epithelial cell migration and ECM fibronectin deposition such that cell migration and matrix remodeling following airway injury are temporally regulated by the rate and/or extent of fibronectin deposition into the ECM.

**Mechanism of ECM Fibronectin Effects**

The mechanism by which the ECM form of fibronectin specifically affects cell contractility and migration is not known. Current models of fibronectin matrix assembly propose that the binding of fibronectin to the cell surface triggers a series of conformational changes that result in the exposure of homophilic binding sites and the subsequent elongation of fibronectin fibrils. Thus, one way in which ECM fibronectin may differentially affect cell
behavior is through the exposure of conformation-dependent epitopes that arise during fibronectin matrix polymerization. We previously identified a conformation-dependent binding site within the III-1 module of fibronectin that is involved in the polymerization of fibronectin on cell surfaces. This cryptic binding site is not exposed in soluble fibronectin and, thus, may serve to trigger cellular responses that are unique to the matrix form of fibronectin. Moreover, proteolytic cleavage of isolated III-1 at Ile-597 exposes a cryptic heparin-binding activity in III-1, suggesting a potential mechanism by which this region of fibronectin may interact with cell surfaces. Studies were conducted to determine whether the interaction of cells with this cryptic region of III-1 affects cell contractility. To construct a recombinant fibronectin with properties of matrix fibronectin, we engineered a glutathione-S-transferase (GST)-tagged fusion protein in which the cryptic, heparin-binding, C-terminal III-1 fragment (III-1H) was linked directly to the integrin-binding III-8–10 modules (GST/III-1H,8–10). GST/III-1H,8–10 specifically stimulated an increase in collagen gel contraction; integrin-ligation alone was ineffective. III-1H-containing constructs as well matrix fibronectin colocalized with caveolin on cell surfaces and fractionated with low-density membrane complexes by a mechanism that required heparin sulfate proteoglycans. Moreover, the disruption of caveolae inhibited the fibronectin- and III-1H-mediated increases in collagen gel contraction. These data suggest a model in which a heparin-binding neopeptope in the III-1 module of fibronectin is exposed during fibronectin matrix deposition. The subsequent interaction of this matrixcryptic site with heparin sulfate proteoglycans directs a portion of ECM fibronectin into lipid rafts where it alters cytoskeletal organization.

SUMMARY

The formation of a fibronectin matrix is a dynamic, cell-dependent process that is normally tightly regulated. In patients with asthma, the loss of this regulation gives rise to an increase in the deposition of fibronectin and other ECM molecules in the subepithelial matrix. Our studies indicate that a matrixcryptic site in fibronectin modulates cell contractility, collagen gel contraction, and cell migration. As such, the increased or inappropriate exposure of this site in matrix fibronectin may contribute to abnormal tissue remodeling by enhancing and/or prolonging cell contractility and by altering the rate of reepithelialization. Developing methods to control the extent or duration of the exposure of cells to matrix-specific epitopes may provide a useful approach for promoting normal tissue remodeling in asthma.

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Damage to the airway epithelium is one prominent feature of chronic asthma. Mucosal damage includes gap openings, partial denudation, and loss of ciliated cells. Apoptosis of the airway epithelium is increasingly recognized as a potential mechanism by which damage may occur. Corticosteroids (CSs) induce apoptosis in inflammatory cells, which in part explains their ability to suppress airway inflammation. However, CS therapy does not necessarily reverse epithelial damage. We examined whether CS therapy actually could induce airway epithelial apoptosis using culture models of primary airway epithelial cells and cell lines. The administration of CSs in low-micromolar concentrations induces apoptosis that involves the disruption of mitochondrial polarity, the activation of caspases, and the involvement of Bcl-2. Clear differences exist between CS-induced apoptosis in the cultured epithelium vs cultured hematopoietic cells in regard to time course and resistance to apoptosis. Our data suggest that the use of CSs, in concentrations that could be attained in vivo with the inhalation of potent preparations or with systemic administration, may be one factor in the airways remodeling and epithelial damage that is seen in many patients with chronic, persistent asthma.

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Key words: airway epithelium; asthma; apoptosis; corticosteroids; remodeling

Abbreviations: ASL = airway surface liquid; CS = corticosteroid; GR = glucocorticoid receptor; TUNEL = terminal deoxynucleotidyl transferase mediated dUTP

The airway epithelium is a target of inflammatory and physical stimuli in the patient with asthma. Injury to the epithelium is a common finding in patients with asthma, even when the clinical state is mild.¹,² Epithelial damage correlates with airway hyperreactivity in asthmatic patients,³ is seen in about half of subjects with mild asthma and in almost all subjects with persistent asthma,⁴ and may be seen in patients with newly diagnosed asthma.⁵ This suggests that occult inflammation has already initiated the process of mucosal damage and remodeling. Asthmatic subjects shed fourfold as many epithelial cells as do healthy subjects after bronchial washing.⁶ Many of these are ciliated epithelial cells, which are shed preferentially.⁷,⁸ The epithelial layer disruption that has been described in bronchial biopsy specimens obtained during fiberoptic bronchoscopy has been attributed in part to biopsy artifacts.⁹ However, the increased expression of CD44 cells in the areas of damage,¹⁰ and epithelial growth factor receptor expression (c-erbB-1)¹¹ as markers of repair,¹² along with extensive denudation of the epithelium that is seen in patients with severe and fatal asthma,¹³ indicates that epithelial damage does occur in vivo. While asthma is an inflammatory disorder of the airways, inflammation alone is insufficient to explain both the chronic nature of the disease and its progression over time. Inflammation must occur in an airway that is susceptible to damage. Epithelial damage, along with other changes such as smooth muscle hypertrophy, basement membrane thickening, and submucosal fibrosis, are cardinal features of chronic airway remodeling, which is the culmination of this combination of inflammation plus structural changes in patients with long-standing, persistent asthma.

Corticosteroid-Induced Apoptosis of Airway Epithelium*

A Potential Mechanism for Chronic Airway Epithelial Damage in Asthma

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Damage to the airway epithelium is one prominent feature of chronic asthma. Mucosal damage includes gap openings, partial denudation, and loss of ciliated cells. Apoptosis of the airway epithelium is increas-

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