Fibronectin matrix polymerization regulates small airway epithelial cell migration

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Submitted 31 October 2002; accepted in final form 6 March 2003

Hocking, Denise C., and Cecilia H. Chang. Fibronectin matrix polymerization regulates small airway epithelial cell migration. Am J Physiol Lung Cell Mol Physiol 285: L169–L179, 2003. First published March 14, 2003; 10.1152/ajplung.00371.2002.—The continuous conversion of soluble fibronectin into extracellular matrix fibrils occurs through a dynamic, cell-dependent process. As the extracellular matrix is assembled, changes in the conformation of matrix proteins may expose biologically active, matricryptic sites that alter cell behavior. In this study, an in vitro model of wound healing was used to determine the role of matrix fibronectin in airway epithelial cell motility. Our findings indicate that, under basal conditions, small airway epithelial cell (SAEC) migration requires active fibronectin matrix polymerization. Furthermore, SAEC migration is increased significantly by the interaction of cells with a recombinant construct containing fibronectin’s matricryptic III-1 site. In contrast, addition of increasing amounts of fibronectin to SAECs significantly decreased the rate of cell migration. This fibronectin-induced inhibition of cell migration was overcome by blocking excess fibronectin matrix deposition. These data indicate that SAEC migration is regulated in a biphasic manner by the polymerization of fibronectin in the extracellular matrix and suggest a stimulatory role for fibronectin’s matricryptic III-1 site in cell motility.

extracellular matrix; wound healing; cell motility; lung repair

ONE OF THE PROMINENT STRUCTURAL changes associated with asthma involves enhanced extracellular matrix (ECM) deposition and remodeling within the subepithelial region of the airways (29). Early in the development of the disease, the region immediately beneath the epithelial basement membrane undergoes a consistent, two- to threefold increase in thickness (3, 28, 30). In patients with severe, persistent asthma, the architecture of this region undergoes prominent, often permanent structural changes, including increased deposition of fibronectin and collagen types I, III, and V in the subepithelial matrix (29). Studies have also shown increased deposition of ECM proteins, including fibronectin, in the subepithelium of patients with mild, atopic asthma (30). Clinical evidence demonstrates a correlation between airway hyperresponsiveness and the structural changes associated with airway remodeling (1, 19), suggesting that abnormal ECM deposition is a fundamental abnormality involved in the pathogenesis of asthma. The functional consequences of this altered ECM deposition are incompletely understood.

Histological changes in the airway epithelium of asthmatics have also been well documented (28). In subclinical cases of asthma, airway epithelial cell shedding, as evidenced by the recovery of epithelial cells in bronchial lavage fluid, has been demonstrated (1). In cases of mild asthma, epithelial cell damage is evident at all levels of the airway (20), whereas in chronic, severe asthma, loss of the pseudostratified columnar epithelial cells can be a prominent feature (28). With ECM remodeling, epithelial loss has been shown to correlate with airway hyperreactivity (1, 19), suggesting that incomplete reepithelialization may play a prominent role in the pathogenesis of asthma (28).

The restoration of the airway epithelial barrier involves the migration of epithelial cells at the leading edge of the wound over a fibronectin-containing provisional matrix (5). Fibronectins are a family of multidomain glycoproteins that circulate in a soluble form in the plasma and are deposited in a fibrillar form in the ECM (18). The continuous conversion of soluble fibronectin into ECM fibrils occurs through a dynamic, yet tightly controlled, cell-dependent process (22). As the ECM is assembled and remodeled, changes in the conformation of matrix proteins may expose biologically active, “matricryptic” sites (8). Our studies indicate that the ECM form of fibronectin triggers changes in cytoskeletal organization that are distinct from those of soluble fibronectin (15). We have localized this activity to a conformation-dependent site in the III-1 module of fibronectin (14). This cryptic epitope is not exposed in soluble fibronectin (21) but may become exposed as cells assemble and stretch ECM fibronectin fibrils (34, 36). Cell migration requires ECM-mediated adhesion to coordinate interactions between integrin receptors and the actin cytoskeleton (17). As such, these studies suggest the possibility that changes in ECM fibronectin deposition or conformation may contribute to the pathogenesis of asthma by altering the extent of reepithelialization after airway injury.

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In the present study, we have used an in vitro model of wound healing to determine the role of ECM fibronectin in the regulation of airway epithelial cell motility. Our findings indicate that, under basal conditions, small airway epithelial cell (SAEC) migration requires active fibronectin matrix polymerization. Furthermore, SAEC migration is increased significantly by the interaction of cells with a recombinant fibronectin construct containing the matricryptic III-1 site. In contrast, exogenous addition of increasing amounts of fibronectin to SAECs significantly decreases the rate of cell migration. This fibronectin-induced inhibition of cell migration can be overcome by blocking excess fibronectin matrix deposition. These data indicate that SAEC migration is regulated in a biphasic manner by the polymerization of fibronectin into the ECM and suggest a stimulatory role for fibronectin’s matricryptic III-1 site in cell motility.

MATERIALS AND METHODS

Reagents. Unless otherwise indicated, chemical reagents were obtained from Sigma (St. Louis, MO). Tissue culture plates and supplies were from Corning/Costar (Cambridge, MA). Collagen I was obtained from Upstate Biotechnology (Lake Placid, NY). Human plasma fibronectin was isolated from Cohn’s fraction I and II, as described previously (23). Anti-fibronectin III-1 IgG (9D2; see Ref. 4) was a gift from Dr. Deane Mosher, University of Wisconsin (Madison, WI). Nonimmune mouse IgG was obtained from Sigma and further purified by protein A-Sepharose (Amersham Biosciences, Piscataway, NJ) chromatography. F(ab)’ fragments were generated from 9D2 and nonimmune IgG as previously described (15). The proteolytic 70- and 40-kDa fibronectin fragments were generated as previously described (15).

Cell culture. Mouse embryo cells, derived from mouse embryo cells, were cultured on collagen-coated dishes in a 1:1 mixture of Cellgro (Mediatech, Herndon, VA) and Aim V (Invitrogen, Carlsbad, CA). These media do not require serum supplementation. Thus the fibronectin-null cells are cultured under conditions where no exogenous source of fibronectin is present.

Human SAECs were obtained from BioWhittaker (Walkersville, MD) and cultured in serum-free, SAEC growth media (SAGM, BioWhittaker). SAECs were seeded in flasks at 2.5 × 10^4 cells/cm^2 and grown at 37°C in a 5% CO_2 incubator to ~85% confluence (~3 days). SAECs were subcultured using Trypsin/EDTA (BioWhittaker) followed by Trypsin Neutralization Solution (BioWhittaker). Experiments were performed using cells between passage 2 and 5.

Preparation of recombinant fibronectins. Figure 1 shows the recombinant fibronectin constructs used in this study. The generation of these constructs has been described previously (14). Proteins were isolated from lysates of transfected DH5a bacteria using glutathione agarose (Amersham Biosciences). Proteins were dialyzed extensively against PBS and stored at −80°C until use. Purity of the recombinant fibronectin constructs was assessed by SDS-PAGE.

Cell migration assays. Cell migration was determined using an in vitro wound repair assay (11). Fibronectin-null cells were seeded on collagen-coated 35-mm dishes in AimV/Cellgro at 5 × 10^4 cells/cm^2. SAECs were seeded on 35-mm dishes in SAGM growth media at 4.5 × 10^4 cells/cm^2. Cells were grown for 2 days to confluence. A thin section in the center of the monolayer was gently removed with the tapered end of a sterile, plastic microspatula. The average width of the denuded region was ~450 μm. Cells were washed three times with either AimV/Cellgro (fibronectin-null cells) or SAEC basal media to remove cellular debris. Five nonoverlapping regions of the denuded area (“wound”) were viewed with an Olympus inverted microscope using a ×2 objective (time = 0 min). Phase-contrast images were obtained using a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI). At various times after wounding, the denuded areas were rephotographed, and wound areas were measured using ImagePro-Plus software (Media Cybernetics, Silver Spring, MD) calibrated with a stage micrometer. To identify each region of the wound, a numbered grid was marked on the bottom of each plate before cell seeding. Cell migration area was calculated as cleared area (time = 0 min) − cleared area (time = 2, 4, 6, 8, or 10 h).

The effect of fibronectin on fibronectin-null cell migration was determined by treating cells with intact fibronectin (1–80 nM) or the recombinant fibronectin construct, glutathione-S-transferase (GST) fusion proteins. Relevant type III modules are numbered.

Fig. 1. Fibronectin fusion proteins. Schematic representation of a fibronectin subunit and recombinant glutathione-S-transferase (GST) fusion proteins. Relevant type III modules are numbered.
one-S-transferase (GST)/III1H, 8–10 (50–1,000 nM), immediately after wounding. To determine the role of fibronectin polymerization in fibronectin-null cell migration, fibronectin was pretreated with F(ab')2 fragments derived from either the anti-III-1 antibody, 9D2, or nonimmune IgG (10 μg/ml; 400 nM) for 15 min before its addition. To determine the effect of increased fibronectin matrix deposition on SAEC migration, cells were treated with intact fibronectin (20–80 nM) in the absence and presence of either 9D2 or IgG (50 μg/ml; 300 nM) for 40 h before wounding.

Immunofluorescence microscopy. SAECs were seeded on glass coverslips at 3.8 × 10^4 cells/cm² in the presence of either 50 μg/ml 70-kDa fibronectin fragment or an equal volume of PBS. Cells were incubated for 24 h at 37°C with 5% CO₂, fixed with 2% paraformaldehyde, and permeabilized with 0.5% Triton X-100. Fibronectin was visualized using an anti-fibronectin monoclonal antibody (mAb; clone CCBD; Chemicon, Temecula, CA) followed by a Texas red-labeled anti-bronectin monoclonal antibody (mAb; clone CCBD; Chemicon, Temecula, CA) followed by a Texas red-labeled goat anti-mouse secondary antibody. After being stained, cells were examined with an Olympus BX60 microscope equipped with epifluorescence and photographed using a Spot digital camera.

Cell adhesion assays. Tissue culture dishes (96 wells) were coated with 10 μg/ml fibronectin in PBS at 37°C overnight. Wells were then washed with PBS. SAECs were seeded at 4 × 10⁴ cells/ml in 0.1 ml/well SAGM in the presence of 50 μg/ml of either the anti-III-1 antibody (9D2 mAb), the anti-cell binding domain antibody (clone Fn12–8; PanVera, Madison, WI), or nonimmune mouse IgG. Cells were allowed to attach for 30 min at 37°C in 5% CO₂. Cells were then washed with PBS and fixed with 1% paraformaldehyde for 30 min. Cells were stained with 0.5% crystal violet and then air-dried. The dye was solubilized with 1% SDS, and the absorbance at 590 nm was determined using a Bio-Rad Benchmark Plus plate reader (Hercules, CA). Background absorbance of protein-coated wells in the absence of cells was subtracted from each data point.

Statistical analysis. Data are expressed as the mean area ± SE of five nonoverlapping wound regions and represent one of at least two independent experiments. Data were analyzed using one-way ANOVA or the Student’s t-test for unpaired samples (Prizm software; GraphPad, San Diego, CA). Differences <0.05 were considered significant.

RESULTS

Fibronectin induces a biphasic effect on cell migration. Distinguishing the effects of ECM fibronectin from those of soluble fibronectin has been difficult because of the presence of fibronectin in the serum used to culture cells and the ability of most adherent cells to constitutively synthesize and deposit fibronectin in the ECM. To overcome these issues, fibronectin-null cell lines were established from fibronectin-null mouse embryos and adapted to grow under serum-free conditions where no exogenous source of fibronectin is present (33). This fibronectin-null background provides a mechanism for determining cell behavior in the complete absence of fibronectin and for distinguishing the effects of soluble vs. matrix fibronectin (14, 15, 31–33). Using these cells, we have shown that fibronectin that has been polymerized in ECM fibrils stimulates actin cytoskeletal organization (15). Actin filament reorganization is necessary for both cell extension and traction during cell migration (17), suggesting a possible role for ECM fibronectin in regulating cell motility.

To directly assess the effect of fibronectin on cell migration, initial studies were conducted using fibronectin-null cells. As shown in Fig. 2, fibronectin-null cells adherent to a substrate of type I collagen exhibited a biphasic response to fibronectin such that peak stimulation of migration occurred at a fibronectin concentration of ~10 nM. As the exogenous fibronectin concentration was increased from 20 to 80 nM fibronectin, there was a dose-dependent decrease in cell migration back to basal levels (Fig. 2). The rate of cell migration in the presence of 80 nM fibronectin was not significantly different from that observed in the absence of fibronectin (Fig. 2). The increase in cell migration in response to 10 nM fibronectin was apparent within 2 h of fibronectin addition (Fig. 3A). By 8 h, the fibronectin-null cell wound areas treated with 10 nM fibronectin were nearly closed, whereas wounded areas of nontreated cells remained open (Fig. 3B).

Stimulation of cell migration requires fibronectin polymerization. To determine whether the fibronectin-induced increase in cell migration requires the formation of ECM fibronectin fibrils, fibronectin polymerization was inhibited with the 9D2 mAb. Previous studies have demonstrated that 9D2 mAb binds to the III-1 module of fibronectin and inhibits fibronectin matrix polymerization but does not block the initial association of fibronectin with cell surfaces (4). The 9D2 mAb has been shown to inhibit fibronectin polymerization in a variety of cell types, including dermal fibroblasts (4),

![Fig. 2. Effect of fibronectin on cell migration. Fibronectin-null cell monolayers were wounded, and images of nonoverlapping denuded areas were obtained as described in MATERIALS AND METHODS. Cells were then incubated in the absence or presence of increasing concentrations of fibronectin. After 6 h, the wound areas were reimaged. Data are presented as average area migrated (area (time = 0 min) – area (time = 6 h)) ± SE and represent 1 of 3 experiments. *Significantly different from minus fibronectin, P < 0.001.](http://ajplung.physiology.org/)

AJPL-Lung Cell Mol Physiol • VOL 285 • JULY 2003 • www.ajplung.org
aortic smooth muscle cells (31), microvascular endothelial cells (31), and the fibronectin-null cells (33). Fibronectin-null cell monolayers were wounded and then incubated with 5 nM fibronectin in the absence and presence of either 9D2 or nonimmune mouse F(ab') fragments. Control cell monolayers received an equal volume of PBS. As shown in Fig. 4, addition of the 9D2 F(ab')2 fragments to fibronectin-treated cells inhibited cell migration in response to fibronectin, suggesting a role for fibronectin matrix polymerization in cell motility. As expected, 9D2 F(ab') fragments did not affect cell migration in the absence of fibronectin (Fig. 4).

The fibronectin matrix mimetic GST/III1H,8–10 stimulates cell migration. As cells polymerize their fibronectin matrix, the exposure of new epitopes may serve to initiate responses that are unique to matrix fibronectin. We previously demonstrated that the effects of ECM fibronectin on cell contractility can be mimicked by a recombinant fusion protein in which the cryptic, heparin-binding, COOH-terminal III-1 fragment (III-1H) is linked directly to the integrin-binding III8–10 modules (GST/III1H,8–10; see Ref. 14). The cryptic epitope in III-1 is not exposed in soluble fibronectin (21) but may become exposed as cells assemble and stretch ECM fibronectin fibrils (34, 36). To determine whether the increase in cell migration induced by ECM fibronectin could be mimicked by GST/III1H,8–10, fibronectin-null cell monolayers were wounded and then treated with increasing concentrations of GST/III1H,8–10. As shown in Fig. 5A, GST/III1H,8–10 stimulated a dose-dependent increase in the rate of cell migration, resulting in a fivefold increase in area migrated compared with controls (6 h; Fig. 5A). Unlike intact fibronectin, the stimulatory effect of GST/III1H,8–10 on cell migration was not biphasic, since cells treated with up to 10 μM GST/III1H,8–10 demonstrated a sustained increase in the rate of cell migration (Fig. 5A and data not shown).

We previously showed that the GST/III1H,8–10-mediated increase in cell contractility is specific to the

Fig. 3. Time course of fibronectin (FN) response. Migration assays were performed on fibronectin-null cell monolayers as described in the legend to Fig. 2. After being wounded, cells were treated with 10 nM fibronectin or an equal volume of PBS. Wound areas were obtained at various times after fibronectin addition. A: data are presented as average area migrated [area (time = 0 min) – area (time = 2, 4, 6, or 8 h)] ± SE and represent 1 of 3 experiments. *Significantly different from minus fibronectin, P < 0.001. B: phase-contrast images of monolayers at 0 and 8 h. Bar = 100 μm.

Fig. 4. Fibronectin polymerization stimulates cell migration. Migration assays were performed on fibronectin-null cell monolayers as described in the legend to Fig. 2. After being wounded, cells were treated with 5 nM fibronectin or an equal volume of PBS in the presence of either 9D2 or nonimmune F(ab') fragments (10 μg/ml). Wound areas were remeasured 6 h after fibronectin addition. Data are presented as average area migrated ± SE and represent 1 of 3 experiments performed. #Significantly different from +mFab', P < 0.01.
heparin-binding III-1 fragment, since a fibronectin construct in which the COOH-terminal heparin-binding domain of fibronectin (III-13) is substituted for III-1H (GST/III8–10,13) does not increase cell contractility (14). Similarly, treatment of fibronectin-null cells with GST/III8–10,13 did not increase cell migration (Fig. 5B), suggesting that the stimulation of cell motility is a specific function of the cryptic, heparin-binding region of III-1. Furthermore, treatment of cells with a fibronectin construct in which the III2–4 modules were substituted for the integrin-binding III8–10 modules did not increase cell migration (GST/III1H,2–4; Fig. 5B), suggesting that integrin ligation is necessary for the migration response to GST/III1H,8–10. However, treatment of cells with an integrin-binding construct in which the III-1H fragment was absent (GST/III8–10; Fig. 5B) failed to increase cell migration, suggesting that integrin ligation alone is not sufficient to trigger cell migration.

To determine whether the adhesive substrate modulates the stimulatory effect of GST/III1H,8–10, fibronectin-null cells were seeded on either collagen I-, laminin-, or vitronectin-coated dishes and grown to confluence. The cell monolayers were wounded and then treated with either 250 nM GST/III1H,8–10 or an equal volume of PBS. As shown in Fig. 5C, the basal level of migration of cells adherent to either laminin- or vitronectin-coated substrates was greater than that observed with cells adherent to collagen I. Nonetheless, addition of GST/III1H,8–10 to either collagen I- or laminin-adherent cells triggered a significant increase in migration (Fig. 5C). Interestingly, addition of GST/III1H,8–10 to vitronectin-adherent cells resulted in only a small, but significant, increase in the extent of cell migration (Fig. 5C). These results may reflect the ability of the heparin-binding domain of vitronectin to antagonize the effects of the III-1H (14).

Fibronectin polymerization is required for SAEC migration. Migrating airway epithelial cells continuously secrete and polymerize fibronectin into insoluble ECM fibrils (13). In an initial step of fibronectin matrix formation, cells bind the NH2-terminal region of fibronectin in a specific and saturable manner (22). Thus fibronectin polymerization can be blocked in a variety of cell types by the addition of excess NH2-terminal 70-kDa fragments of fibronectin (22). Similarly, in the present study, addition of 70-kDa fibronectin fragments to SAECs inhibited the formation of fibronectin fibrils (13).
Fig. 6. Effect of fibronectin polymerization on small airway epithelial cell (SAEC) migration. SAECs were seeded in defined media in the presence of either 50 μg/ml of the 70-kDa fibronectin fragment (B) or an equal volume of PBS (A). After a 24-h incubation, cells were processed for immunofluorescence as indicated in MATERIALS AND METHODS. Fibronectin was visualized using a monoclonal anti-fibronectin antibody followed by a Texas red-labeled goat anti-rabbit antibody. Bar = 10 μM. C: SAECs were seeded in the presence of either 50 μg/ml of the 70-kDa fibronectin fragment, an equal molar concentration of the non-blocking 40-kDa fragment, or an equal volume of PBS. Migration assays were performed as described in the legend to Fig. 2. The wound areas were reimaged at 2, 4, and 6 h. Data are presented as average area migrated ± SE and represent 1 of 2 experiments performed. *P < 0.001 and +P < 0.05, significantly different from PBS. D: phase-contrast images of monolayers at 0 and 6 h. Bar = 100 μm.
matrix fibrils (Fig. 6A). To determine the role of fibronectin polymerization in SAEC migration, cells were seeded in defined media in the presence of either the 70-kDa fragment or the nonblocking 40-kDa fragment and grown to confluence. The cell monolayers were wounded and then retreated with either the fibronectin fragments or an equal volume of PBS. As shown in Fig. 6B, addition of the 70-kDa fibronectin to SAECs resulted in a significant inhibition of cell migration. In contrast, addition of the 40-kDa fragment did not significantly affect cell migration.

To further examine the role of fibronectin polymerization in SAEC migration during wound closure, the anti-III-1 antibody 9D2 mAb was added to cells after wounding. As shown in Fig. 7A, treatment of SAEC monolayers with the 9D2 mAb after wounding resulted in an inhibition of SAEC migration. The addition of the 9D2 mAb to SAECs did not block integrin-mediated adhesion to fibronectin (Fig. 7B). Taken together, these data indicate a key role for fibronectin polymerization in airway epithelial cell migration.

**GST/III1H,8–10 stimulates SAEC migration.** To determine whether the matricryptic III-1 fibronectin fragment could enhance SAEC motility over the levels induced by endogenous fibronectin polymerization, the migration of SAECs was determined in the absence and presence of 250 nM GST/III1H,8–10. As shown in Fig. 8, treatment of SAECs with GST/III1H,8–10 significantly increased cell migration by ~1.5-fold at 6 h. In contrast, treatment of SAECs with an equal molar concentration of GST/III8–10,13 did not increase cell migration (Fig. 8), providing further evidence that stimulation of cell motility is specific to the matricryptic heparin-binding site in the III-1 module.

To determine whether GST/III1H,8–10 could overcome the 9D2 mAb-mediated inhibition of SAEC migration, cells were pretreated with either GST/III1H,8–10 or the integrin-binding fragment, GST/III8–10. After wounding, cells were treated with the 9D2 mAb to inhibit fibronectin polymerization. Control
monolayers were treated with nonimmune IgG. As shown in Fig. 9, pretreatment of SAECs with GST/III1H,8–10 reversed the inhibitory effects of 9D2 mAb on cell migration. This effect was specific to GST/III1H,8–10, since treatment of SAECs with the integrin-binding construct, GST/III8–10, did not promote the migration of 9D2 mAb-treated cells (Fig. 9).

Increased fibronectin matrix deposition inhibits SAEC migration. Our studies indicate that fibronectin-null cells demonstrate a biphasic migration response to fibronectin (Fig. 2) and that the stimulatory effect of low doses of fibronectin requires fibronectin polymerization (Fig. 4). The ability of the fibronectin polymerization inhibitors to suppress SAEC migration (Figs. 6 and 7) suggests that, under basal conditions, SAECs function within the range of fibronectin that normally promotes migration. If so, the addition of increasing levels of exogenous fibronectin may subsequently inhibit SAEC motility. Thus, to further assess the effect of ECM fibronectin on SAEC migration, SAECs were treated for 40 h with increasing amounts of fibronectin to allow for increased ECM fibronectin deposition. As shown in Fig. 10A, exogenous addition of fibronectin to SAECs resulted in a dose-dependent decrease in SAEC migration. To determine whether the decrease in cell migration was the result of fibronectin deposition in the ECM, SAECs were seeded in the presence of 80 nM fibronectin and in the absence and presence of the fibronectin polymerization inhibitor 9D2 mAb. The migration assay was then performed in the absence of additional fibronectin or 9D2 mAb. As shown in Fig. 10B, the inhibitory effect of 80 nM fibronectin was blocked by the presence of 9D2 mAb but not by the addition of nonimmune IgG. The data suggest that increased fibronectin matrix deposition by SAECs decreases the extent of cell migration. In addition, these data indicate that SAECs respond to fibronectin polymerization with a biphasic response that is similar to that observed with the fibronectin-null cells.

**DISCUSSION**

Repair and remodeling after lung injury involves the coordinated interactions of numerous cell types with ECM molecules, including fibronectin, which functions to promote cell attachment, migration, and differentiation (6). One of the early responses to airway injury is the migration of the remaining epithelial cells over a fibronectin-containing provisional matrix (5). Previous in vitro studies have demonstrated that fibronectin promotes the migration of airway epithelial cells (13). In the present study, we have extended these observations by demonstrating that airway epithelial cell migration requires the formation of fibronectin matrix fibrils. In addition, we have shown that a recombinant fibronectin fragment that contains the matricryptic III-1 site of fibronectin enhances basal SAEC migration and promotes SAEC migration under conditions in which fibronectin polymerization is blocked. In contrast, ligation of integrin receptors alone with an integrin-binding fragment of fibronectin was not sufficient to stimulate cell migration. Evidence indicates that a cryptic epitope in the III-1 module of fibronectin becomes exposed as cells assemble and stretch ECM fibronectin fibrils (34, 36). Taken together, these data suggest a model in which the polymerization of ECM fibronectin fibrils exposes a matricryptic site in the III-1 module of fibronectin that serves to stimulate airway epithelial cell migration. Furthermore, these studies suggest that cell motility may be regulated by the structural organization of the ECM.

Fibronectin circulates in a soluble form at high concentrations in plasma (~300 μg/ml) and is also found in its insoluble, multimeric form within ECM through-

**Fig. 9.** GST/III1H,8–10 promotes cell migration in the absence of fibronectin matrix assembly. SAEC monolayers were incubated in the absence and presence of 300 nM GST/III1H,8–10 or GST/III8–10 for 18 h. Monolayers were wounded as described in the legend to Fig. 2. Cells were then treated with 25 μg/ml 9D2 or IgG. Data are presented as average area migrated ± SE and represent 1 of 2 experiments performed. #P < 0.01.
out the body (18). Fibrillar fibronectin may be derived either from cellular fibronectin synthesized locally in tissues or from circulating plasma fibronectin (18). The continuous conversion of soluble fibronectin into ECM fibrils occurs through a dynamic, yet tightly controlled, receptor-mediated process (22). Although it has long been thought that the ECM form of fibronectin is its primary functional form in vivo (18), few studies have addressed potential differences in the response of cells to soluble vs. ECM fibronectin. Our previous studies demonstrated that the ECM form of fibronectin stimulates fibronectin-null cell growth (33), contractility (15), and ECM organization (31) by mechanisms that are distinct from those of soluble fibronectin. In the present study, we have shown that fibronectin polymerization also regulates cell motility. Thus the ability of cells to rapidly alter the rate or extent of fibronectin fibril assembly (22) provides a unique mechanism by which cells may modify cell-matrix signaling events and, hence, behavior.

Tight control over the process of fibronectin polymerization ensures that protomeric fibronectin remains soluble in the blood and that soluble fibronectin is polymerized into an insoluble matrix only at appropriate sites. In asthma, loss of this regulation gives rise to an increase in the deposition of fibronectin and other ECM molecules in the subepithelial matrix (29). Our studies indicate that increased fibronectin matrix polymerization by SAECs decreases their rate of migration. Previous studies have demonstrated a decrease in the rate of cell migration when Chinese hamster ovary (CHO) cells are plated on fibronectin multimers polymerized in vitro compared with cells seeded on protomer fibronectin (24). Furthermore, overexpression of the α5β1-integrin in CHO cells results in increased fibronectin deposition and a decrease in the rate of cell migration (12). We previously demonstrated that fibronectin polymerization specifically increases cytoskeletal organization and mechanical tension generation by cells; ligation of integrin receptors in the absence of fibronectin polymerization does not (15). CHO cells expressing α1β1-integrin mutants that increase the organization of actin filaments also display a decrease in the rate of cellular migration on a fibrinogen-coated substrate (16). Taken together, these studies suggest that formation of fibronectin matrix fibrils generates tension between the ECM and the actin cytoskeleton, which promotes strong adhesive contacts, enhances tissue contraction, and inhibits cell migration. In this manner, cell migration and ECM remodeling during normal wound repair are temporally regulated by the rate and extent of fibronectin deposition in the ECM. Excess fibronectin deposition after injury would alter this relationship, triggering abnormal tissue remodeling by inhibiting cell migration and enhancing tissue contraction.

It has been proposed that the increased deposition of fibronectin and collagen in the subepithelial matrix of asthmatics alters the precise molecular composition of the airway and thereby affects both the geometric and biomechanical properties of the airway (26). 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 (26). Interestingly, it has been proposed that reepithelialization of the airways also modulates ECM remodeling (27, 28), since PGE2 derived from airway epithelial cells can inhibit fibroblast proliferation (25), collagen and fibronectin production (10), 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 ECM remodeling is downregulated after fibronectin-mediated reepithelialization of the airways. Excess or inappropriate fibronectin deposition after injury would alter this relationship, triggering abnormal tissue remodeling by inhibiting epithelial cell migration and subsequently perpetuating fibroblast-mediated ECM production.

The reconstruction of the epithelial barrier is critical to restoring airway integrity after injury (35). Epithelial cell injury is associated with the accumulation of subepithelial and intraepithelial inflammatory cells (1, 19) and the release of cytotoxic inflammatory mediators, including eosinophil granule proteins, tumor necrosis factor, and reactive oxygen species (2). The degree of epithelial loss has been shown to correlate with airway hyperreactivity (1, 19) and is considered to be a key factor in the development of airway hyperresponsiveness in asthma (28). In the absence of an intact epithelium, smooth muscle cells demonstrate an increased sensitivity to various mediators, including histamine, ACh, platelet-activating factor, and antigen (7). Afferent nerve endings, which are normally protected by an intact epithelium, may also become exposed upon epithelial injury and thus become susceptible to the bronchoconstricting effect of certain inflammatory mediators (7). Additionally, loss of airway epithelium may contribute to the pathogenesis of asthma through the loss of epithelial-derived relaxing factors that contribute to normal smooth muscle tone, including nitric oxide and PGE₂ (28).

Therapeutic approaches designed to stimulate or accelerate airway epithelial cell migration may restore epithelial barrier function and decrease airway hyperresponsiveness in asthma. Our studies indicate that a recombinant fibronectin construct containing the matricryptic III-1 site of fibronectin enhances SAEC migration. Moreover, the stimulatory effect of GST/III1H, 8–10 on SAEC migration was not biphasic, since cells treated with up to 10 μM GST/III1H, 8–10 demonstrated a sustained increase in cell migration. Unlike intact fibronectin (31), this construct does not become incorporated into ECM fibrils and does not promote the copolymerization of collagen in the matrix (D. C. Hocking and J. Sottile, unpublished observations). Hence, these studies suggest a novel pharmacological approach to lung repair in which a small, ECM fibronectin mimetic may be used to promote epithelial cell migration without increasing ECM density.

We thank Dr. Jane Sottile for providing the fibronectin-null cells and critically reading the manuscript. This work was supported by National Heart, Lung, and Blood Institute Grants HL-60181 and HL-64074.

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AJP-Lung Cell Mol Physiol • VOL 285 • JULY 2003 • www.ajplung.org


