β₁-Integrins are involved in migration of human fetal tracheal epithelial cells and tubular morphogenesis

CHRISTELLE CORAUX, JEAN-MARIE ZAHM, EDITH PUCHELLE, AND DOMINIQUE GAILLARD

Institut National de la Santé et de la Recherche Médicale Unité 514, Institut Fédératif de Recherche 53, Université de Reims Champagne-Ardenne, Centre Hospitalier Universitaire Maison Blanche, 51092 Reims Cedex, France

Received 4 November 1999; accepted in final form 16 March 2000

Coraux, Christelle, Jean-Marie Zahm, Edith Puchelle, and Dominique Gaillard. β₁-Integrins are involved in migration of human fetal tracheal epithelial cells and tubular morphogenesis. Am J Physiol Lung Cell Mol Physiol 279: L224–L234, 2000.—Development of human fetal airways requires interaction of the respiratory epithelium and the extracellular matrix through integrins. Nevertheless, the specific roles of β₁-integrins during development and tubular morphogenesis are still unknown. To analyze β₁-integrin localization and influence during migration, we developed a model of human fetal tracheal explants growing on collagen and overlaid with a second layer of collagen to form a sandwich. In this configuration, cord and tubule formation proceeded normally but were inhibited by incubation with anti-β₁-integrin subunit antibodies. On a collagen matrix, β₁-integrins were immunolocalized on the entire plasma membrane of migrating epithelial cells and almost exclusively on the basal plasma membrane of nonmigratory epithelial cells. In a sandwich configuration, β₁-integrins became detectable in the cytoplasm of epithelial cells. Coating cultures with collagen transiently altered the morphology of migrating cells and their speed and direction of migration, whereas incubation with anti-β₁-integrin subunit antibodies irreversibly altered these parameters. These observations suggest that the matrix environment, by modulating β₁-integrin expression patterns, plays a key role during tubular morphogenesis of human fetal tracheal epithelium, principally by modulating epithelial cell migration.

TUBULAR MORPHOGENESIS of epithelial cells leads to the formation of human airways from the trachea to the distal bronchioles. This morphogenesis occurs during fetal development and depends on cell-matrix interactions (18) that are essential for cell proliferation, migration, and differentiation (15, 22, 28, 29, 31, 33). The cell-matrix interactions are mediated, at least partially, through epithelial cell surface receptors called integrins. The integrins are a large family of heterodimeric transmembrane glycoproteins composed of noncovalently linked α- and β-subunits. Until now, investigations have identified 16 α- and 8 β-subunits that combine to form at least 22 integrins. Each integrin recognizes more than one ligand, and each ligand can be recognized by more than one integrin (14, 23). Interactions between integrin receptors and ligands have been shown to activate intracellular signaling pathways involving mitogen-activated protein kinases, tyrosine protein kinases, or GTP-binding proteins that are thought to affect the cellular cytoskeleton (23, 35). The distribution of integrins has been studied in kidney and mammary glands where development is associated with tubular branched morphogenesis (3, 19) and more recently in fetal and adult human lungs (7, 34). The involvement of integrins during angiogenesis and tubulogenesis of kidney cells has been demonstrated (8, 10, 37) and partly elucidated from studies of murine airways (20, 26), but to date, no study has been devoted to the dynamics of epithelial cell migration during human fetal airway development due to the lack of a human in vitro model.

To assess the possible roles of β₁-integrins, the most prevalent integrin subunit forming complexes with subunits α₁-α₉ and α₇, and mediating cell adhesion to the majority of basement membrane proteins (2), we developed an in vitro model culturing human fetal tracheal explants on a type I collagen gel. The epithelial cells can grow, migrate, and form a network of branched epithelial cords and tubules when they are grown in a collagen sandwich. To determine whether this new cellular organization observed after modification of the matrix environment was dependent on cell receptors, we analyzed the cellular distribution of the β₁-integrin subunit and the effect of blocking antibodies to the β₁-integrin subunit on cell migration and tubular morphogenesis. The cellular distribution of the β₁-integrin subunit was evaluated immunocytochemically before and after modification of the matrix environment and during tubule formation. Moreover, anti-β₁-integrin subunit blocking antibodies totally inhibited the evolution of human fetal tracheal epithelial (HFTE) cell cultures as assessed morphologically and by the speed and direction of cell migration. These
findings suggest that β1-integrins mediate a critical role for human epithelial cell-matrix interactions during tubular morphogenesis of human airways.

**METHODS**

**Tissues.** Trachea from 12 normal human embryos and fetuses ranging from the pseudoglandular (7–17 wk gestation; n = 4) to the canalicular (18–24 wk gestation; n = 5) and alveolar (25 wk gestation to birth; n = 3) stages of development were obtained after spontaneous or medically induced abortions. The use of human fetal tissues was approved by the Regional Ethics Committee of Development and Reproduction. The trachea were dissected in the hours after the abortions and maintained in RPMI 1640 culture medium (Seromed, Biochrom, Berlin, Germany) containing penicillin (100 U/ml) and streptomycin (100 μg/ml) before preparation of the explants.

**Antibodies.** Monoclonal IgG1 clone P5D2 (9) raised against the β1-integrin subunit was used for immunocytochemistry. Monoclonal blocking IgG1 clone Lia 1/2 (5) raised against the β1-integrin subunit was used for the tubulogenesis blocking assay, cell morphology, and the migration studies. They were, respectively, provided by Dr. D. Sheppard (University of California, San Francisco, CA) and purchased from Immunotech (Marseille, France). Monoclonal antibodies raised against cytokeratin 13 (CK13) were purchased from Sigma (St. Louis, MO). Monoclonal antibodies raised against the Ki67 antigen (MIB-1 clone) were purchased from Immunotech. Negative controls consisted of incubation with monoclonal nonimmune IgG2a, κ fractions (Sigma).

**Preparation of collagen gel matrix.** The type I collagen used for explant cultures was extracted from rat tail tendons according to the method of Chambard et al. (6). Briefly, collagen was solubilized by stirring 1 g of Wistar rat tail tendons for 48 h at 4°C in 100 ml of a 0.1% acetic acid solution. The solution was then centrifuged at 16,000 g for 45 min at 4°C. Four volumes of supernatant containing 2 mg/ml of type I collagen without any other contaminant proteins (25) were mixed with 1.5 volumes of 5× concentrated RPMI 1640 medium and 0.15 volume of 1 N NaOH at 4°C; 2 ml of this collagen solution were then poured onto 35-mm culture dishes (Falcon, Becton Dickinson, Plymouth, UK) and allowed to polymerize at 37°C for 2 h.

**Fetal tracheal explant cultures.** The middle part of the trachea was cut into 1-mm² explants and plated onto collagen gel in 35-mm culture dishes. The explants were incubated in RPMI 1640 medium supplemented with 50 μg/ml of L-ascorbic acid, 50 U/ml of catalase, 0.2 μg/ml of glucagon, 10 μg/ml of apotransferrin, 0.6 ng/ml of 3,3',5-triiodo-L-thyronine, 4 ng/ml of epidermal growth factor, 4 ng/ml of sodium selenite, 1% of 100× MEM-nonsesssential amino acid solution, 15 ng/ml of retinoic acid, 4 ng/ml of hydrocortisone, 10 μg/ml of insulin, 200 U/ml of penicillin, and 200 μg/ml of streptomycin (Sigma-Aldrich Chemie, Steinheim, Germany) and incubated at 37°C in 95% air-5% CO₂.

**Collagen overlay to form a sandwich.** After 5–10 days of culture, an outgrowth of epithelial cells developed around the explants. The cultures were then overlaid with type I collagen at 4°C. The collagen sandwich was then incubated at 37°C in a 5% CO₂ atmosphere and allowed to polymerize for 30 min, and then culture medium was added. For β1-integrin-blocking assays, the explant cultures were incubated with 100 μg/ml of Lia 1/2 antibodies for 1 h at 37°C before the culture was covered with collagen. The cultures were examined under an inverted phase-contrast microscope (Zeiss IM35, Oberkochen, Germany).

**Transmission electron microscopy.** Collagen sandwich cultures were rinsed in 0.1 M, pH 7.2, phosphate-buffered saline (PBS, Sigma), fixed in 2.5% PBS-glutaraldehyde for 60 min, postfixed in 1% H₂O-osmium tetroxide for 2 h, dehydrated through graded concentrations of ethanol, and then embedded in agar resin 100 (Agar Scientific, Orsay, France). Semithin sections were stained with toluidine blue and observed under an Axiohot microscope (Zeiss). Ultrathin sections were stained with uranyl acetate and lead citrate and observed using a Hitachi 300 transmission electron microscope operating at 75 kV.

**Immunocytochemistry.** To determine the cellular localization of the β1-integrin subunit, the distribution of proliferative cells (Ki67-positive staining) and the epithelial nature of the cells (CK13-positive staining), fetal tracheal explant cultures and collagen sandwich cultures were rinsed in PBS, embedded in optimum cutting temperature compound (Tissue Tek, Sakura Finetek, Zoeterwoude, The Netherlands), and frozen in liquid nitrogen. Frozen sections (5 μm thick) were fixed in precooled methanol (−20°C). To saturate non-specific sites, the sections were incubated with PBS containing 3% bovine serum albumin (BSA). They were then sequentially treated as follows: exposed to the monoclonal antibody P5D2 raised against the β1-integrin subunit (1:50 in 1% PBS-BSA), raised against the Ki67 antigen (1:10 in 1% PBS-BSA) or raised against CK13 (1:400 in 1% PBS-BSA) for 90 min; washed; incubated with 3% PBS-BSA; exposed to biotinylated goat anti-mouse IgG (1:50 in 1% PBS-BSA; Vector Laboratories, Burlingame, CA) and then with horseradish peroxidase-conjugated streptavidin (1:400 in PBS-BSA; Dakopatts, Glostrup, Denmark) and visualized with diaminobenzidine and hydrogen peroxide. Sections were then dehydrated in graded concentrations of ethanol, and then embedded in agar resin 100 (Agar Scientific, Londres, France) and polymerized at room temperature before sectioning. Thin sections (80–100 nm) were cut on an LKB Ultratome 2 microtome (LKB-Produkter AB, Stockholm, Sweden), postfixed in 1% OsO₄ for 2 h, dehydrated in graded concentrations of ethanol, and then embedded in Spurr resin (Spurr, 1969). Semi-thin sections were stained with toluidine blue and observed under an Axiohot microscope (Zeiss). Ultrathin sections were stained with uranyl acetate and lead citrate and observed using a Hitachi 300 transmission electron microscope operating at 75 kV.

**Fig. 1.** Epithelial outgrowth development from human fetal tracheal explant. A: inverted phase-contrast microscopy observation of epithelial outgrowth of human fetal tracheal epithelial (HFTE) cells around the explant (Ex) on a type I collagen gel (Coll). B: migratory phenotype of HFTE cells at the forefront of the outgrowth is seen by their flattened aspect and lamellipodia (arrows). Bars: 650 μm in A; 4 μm in B.
Boehringer Mannheim) for 60 min; washed; and incubated with 3% PBS-BSA. The complexes formed were finally visualized with streptavidin-coupled fluorescein isothiocyanate for the $\beta_1$-integrin subunit and the Ki67 antigen or with streptavidin-coupled Texas Red for CK13 (1:50 in 1% PBS-BSA; Amersham Life Sciences, Poole, UK). The observations were made under an Axiophot microscope with epifluorescence and Nomarski differential interference illumination.

**Cell migration.** Cell migration was evaluated with a previously described technique (36). Briefly, fetal tracheal explant cultures were incubated for 30 min with Hoechst 33258 (0.1 mg/ml; Sigma) in culture medium, allowing incorporation of the fluorescent dye into the nuclei of living cells. The cultures were then washed twice with culture medium to remove excess dye and overlaid with type I collagen at 4°C or incubated with either fresh culture medium, an antibody raised against $\beta_1$-integrin (100 µg/ml of Lia 1/2 blocking antibody), or nonimmune IgG2a, κ for 1 h at 37°C. To measure cell migration, the culture dishes were placed on the stage of an inverted Zeiss IM35 microscope and enclosed in a transparent environmental chamber with 5% CO$_2$ in air at 37°C. The microscope was equipped with epifluorescence illumination (mercury lamp) through an excitation filter at 360 nm and an emission filter at 510 nm and with a low-level silicon-intensified target camera (Lhesa 4036, Cergy Pontoise, France). A shutter (Lambda 10-25) was placed in the excitation light path to illuminate the culture for short periods of time (1 s) and to simultaneously digitize the fluorescent images. The images were digitized every 15 min as $512 \times 512$ pixels and an 8-bit array with a Sparc-Classic (Sun Microsystems, Mountain View, CA) workstation equipped with an XVideo card (Parallax Graphics, Santa Clara, CA). Specific software was used to quantify cell migration through three main functions: the detection of individual cell nuclei,
the computation of their trajectories, and analysis of these trajectories. From the latter, the computer calculated the cell migration speed and the angular deviation (in degrees) from the horizontal of each successive movement of the nucleus. The migration speeds reported correspond to the mean migration speed of 12 HFTE cells at the migration front. A small variation in the angular deviation is characteristic of a uniform direction of cell migration.

Morphogenesis and cell morphology. To monitor the dynamics of cellular cord formation and the cell morphology alterations according to the culture conditions, cultures were placed in the environmental chamber on the stage of the Zeiss IM35 inverted microscope. To study the dynamics of cellular cord morphogenesis, phase-contrast images of the collagen sandwich cultures observed through a ×2.5 objective were recorded every 15 min for 24 h. Phase-contrast images of the cells located at the migrating front of the epithelial outgrowth in collagen sandwich cultures were digitized every minute for 2 h. To test the effect on cell morphology of pouring cold collagen gel (4°C) onto the cell culture, phase-contrast images of cell cultures on a collagen gel were digitized every 30 s for 5 min after incubation with culture medium either at 37°C or at 4°C. To analyze the effect of anti-β1-integrin subunit antibodies on cell morphology, immediately after Lia 1/2 antibody addition (100 μg/ml in culture medium) to the HFTE cell cultures, phase-contrast images were digitized every 15 s for 10 min.

Statistical analysis. The mean migration speeds of HFTE cells under the different experimental conditions were compared by unpaired Student’s t-test. Data are expressed as means ± SD. Linear regression test was used to determine the influence of the fetal age on the mean migration speed of HFTE cells. A P value < 0.05 was considered to be significant.

RESULTS

HFTE cells form differentiated tubular structures in type I collagen gel sandwich cultures. Whatever the age of the fetal trachea, cells of an epithelial nature, as demonstrated by CK13-positive immunostaining (data not shown), started to grow from the explants on the type I collagen gel after 2–7 days of culture, forming an outgrowth around the explants (Fig. 1A). The cells located at the advancing edge of the outgrowth had a migratory phenotype characterized by a flattened aspect and the presence of lamellipodia (Fig. 1B) and were nonproliferative as demonstrated by the absence of Ki67 antigen immunostaining (data not shown).

To analyze the behavior of the HFTE cells when covered with collagen, sandwich cultures were video recorded with phase-contrast illumination. A movie was made from 96 images recorded every 15 min for 24 h, allowing dynamic cellular cord morphogenesis to be observed. Sixteen typical frames were selected from the movie and are presented in Fig. 2. At the migratory front of the outgrowth, the HFTE cells became spherical within 15 min (Fig. 2A). After 30 min and during
the following 3 h, they started to spread (Fig. 2, B–D); then they moved, reorganizing themselves into thin cords and projecting small cellular branched ramifications (Fig. 2, E–H). After 24 h of culture, these branched epithelial cords had become thicker and longer (Fig. 2, I–P).

Within 2–5 days after the cultures were coated with collagen, tubular branched structures were clearly observable. In the spaces between the branches of the epithelial network, the fetal tracheal cell monolayer disappeared (Fig. 3A), and the HFTE cells regrouped to form epithelial tubules with a lumen, and immunolabeling detected the β1-integrin subunit at their plasma membrane. The β1-integrin subunit was also observed in the cytoplasm of the degenerative cells present around the tubule (H, arrows) and in the cytoplasm of the resting cells not involved in the tubule formation and localized at the interface of the two collagen layers (H, arrowheads).

Fig. 4. Immunocytochemical localization of the β1-integrin subunit during epithelial outgrowth development and tubular morphogenesis. B: β1-integrin subunit was detected on the entire plasma membrane of migratory HFTE cells at the advancing edge of the outgrowth, whereas it was almost exclusively expressed on the basal side of the stationary HFTE cells located behind the migration front (D). When the HFTE cells were in a collagen sandwich, the β1-integrin subunit was then detected in their cytoplasm (P). After 2 days, these cells formed epithelial tubules with a lumen, and immunolabeling detected the β1-integrin subunit at their plasma membrane. The β1-integrin subunit was also observed in the cytoplasm of the degenerative cells present around the tubule (H, arrows) and in the cytoplasm of the resting cells not involved in the tubule formation and localized at the interface of the two collagen layers (H, arrowheads). A, C, E, and G: corresponding phase-contrast micrographs. Bars: 46 μm in A–F; 92 μm in G and H.

β1-Integrin subunit is expressed by HFTE cells and its distribution is altered during outgrowth development and tubular morphogenesis. During development of the epithelial cell outgrowth on the collagen gel, the β1-integrin subunit was detected by immunocytochemical staining on the entire plasma membrane of the cells at the migrating edge of the culture (Fig. 4B), whereas an intense immunoreactivity was mainly observed on the basal side of the nonmigratory HFTE cells in contact with the collagen gel matrix (Fig. 4D). The plasma membranes of some cells located at basal
position were also weakly labeled (Fig. 4D). When the HFTE cells were covered with collagen, the outgrowth retracted, the cells became spherical, and the β₁-integrin subunit was detected in the cytoplasm of all the cells (Fig. 4F). After 2 days of sandwich culture, when the HFTE cells formed cords and tubules, the β₁-integrin subunit was localized at the plasma membrane of all the tubule-forming cells, in the cytoplasm of the degenerative cells present around the tubule, and in the cytoplasm of the resting cells not involved in the tubule formation, which are localized at the interface of the two collagen layers (Fig. 4H). All these alterations were similarly observed whatever the age of the trachea and the degree of differentiation of the HFTE cells used for cultures.

β₁-Integrins are involved in the tubular morphogenesis of HFTE cells. To determine the role of the β₁-integrins in the morphogenesis of HFTE cell branched cords and tubules, cultures of tracheal explants were incubated with blocking antibodies directed against the β₁-integrin subunit before being overlaid with collagen. Control cultures incubated with mouse nonimmune IgG developed cellular branched cords and tubules (Fig. 5A) similar to those observed in control collagen-overlaid cultures without antibodies (Fig. 3A). In contrast, when exposed to anti-β₁-integrin subunit antibodies, HFTE cells came to a standstill and failed to undergo cord formation. No further change could be observed after a 10-day period. This result was obtained in 100% of human fetal tracheal explant cultures incubated with anti-β₁-integrin subunit antibodies (Fig. 5B).

HFTE cell-matrix interactions lead to altered cell migration. The speed and angular deviation of HFTE cell migration were studied during outgrowth development when the cells were incubated with nonimmune IgG or antibodies directed against the β₁-integrin subunit and during the early phase of cellular cord formation. The nuclei could be observed with Hoechst fluorescent dye, and the study focused on the cells located at the migratory edge of the outgrowth. The cells were tracked for 1 h.

The trajectories of migrating cells, determined from the fields analyzed every 15 min, were fairly straight and unidirectional when the HFTE cells moved on collagen in culture medium or were incubated with nonimmune IgG (Fig. 6, A and C) as demonstrated by the uniformity of the angular deviations measured from one cell to another and by the small SD of the mean angular deviations (Fig. 6, B and D). In contrast, when HFTE cells were incubated with blocking anti-β₁-integrin subunit antibodies or after they were covered with collagen, the trajectories became extremely erratic, losing their uniform direction of migration (Fig. 6, E and G). These altered migration patterns were characterized by wide variations in the angular deviations measured from one cell to another and by the elevated SD of the mean angular deviations (Fig. 6, F and H).

In control cultures, no significant relationship was demonstrated between the age of the trachea and the mean migration speed of the HFTE cells. The mean migration speeds of HFTE cells on collagen gel in fresh medium or incubated with mouse nonimmune IgG were not significantly different (36.2 ± 14.2 and 35.7 ± 15.1 μm/h, respectively). In contrast, the mean migration speed of HFTE cells after incubation with blocking anti-β₁-integrin subunit antibodies (20.5 ± 5.9 μm/h) was significantly lower compared with those in control cultures incubated with fresh medium (36.2 ± 14.2 μm/h) or mouse nonimmune IgG (35.7 ± 15.1 μm/h; P < 0.05). When comparing the mean migration speeds of HFTE cells on collagen and in a collagen sandwich, the cells moved significantly more slowly during the first hour after the overlay of collagen gel (16.1 ± 2.7 μm/h) compared with HFTE cells on collagen in fresh medium (36.2 ± 14.2 μm/h; P < 0.05). To evaluate the cell migration alteration after cell-matrix interactions were modified, we compared the mean migration speeds of HFTE cells in a collagen sandwich. Control cultures of HFTE cells incubated with mouse nonimmune IgG developed cellular cords (A), whereas HFTE cells incubated with blocking anti-β₁-integrin subunit antibodies became stagnant and failed to undergo tubulogenesis (B). Bars, 650 μm.

Fig. 5. Involvement of β₁-integrins in cord and tubule formation by HFTE cells in a collagen sandwich. Control cultures of HFTE cells incubated with mouse nonimmune IgG developed cellular cords (A), whereas HFTE cells incubated with blocking anti-β₁-integrin subunit antibodies became stagnant and failed to undergo tubulogenesis (B). Bars, 650 μm.
first minute, the cells extended lamellipodia (Fig. 7A), and thereafter they progressively retracted these cytoplasmic extensions (lamellipodia). This morphological change was accompanied by retraction of the overall outgrowth, leaving some individual cells stranded (Fig. 7B). After 10 min, the HFTE cells became spherical (Fig. 7C). The collagen overlay was polymerized within 30 min, and 90 min later, the cell morphology changed again with the extension of new visible lamellipodia (Fig. 7D).

Because the second layer of collagen was cold (4°C), we controlled the effect of the temperature change on HFTE cell morphology. Cells cultured on collagen were observed by phase-contrast microscopy after the addition of cold medium (4°C) alone. Under these conditions, the cells remained flattened, and lamellipodia were also observed (data not shown).

Altered HFTE cell morphology at the advancing edge of the outgrowth on collagen after incubation with anti-β₁-integrin subunit antibodies was observed in phase-contrast micrographs (Fig. 8). During the first minute, the HFTE cells extended lamellipodia (Fig. 8, A and B), and thereafter, they progressively retracted these cytoplasmic extensions (Fig. 8C). Five minutes later, the lamellipodia disappeared and filopodia started to become visible (Fig. 8, D and E); these latter disappeared within 10 min at the periphery of the HFTE cells, which had lost their migratory phenotype (Fig. 8F). Similar observations were made whatever the age of the fetal trachea used.

**DISCUSSION**

β₁-Integrins play an important role in cell-cell and cell-matrix interactions, acting as regulators of cell phenotype, survival, migration, proliferation, and/or differentiation (1, 12, 27, 33). In the present study, we developed an in vitro human fetal model to assess the role of the matrix environment via the β₁-integrin family receptor during epithelial cell migration leading to human airway development. Our results show that matrix environment modification can act through β₁-integrins to modulate the epithelial cell migration and to induce cord and tubule formation by HFTE cells. Until now, only a few studies have described in vitro models for studying tubular morphogenesis of human respiratory epithelial cells (4, 16). However, those studies always used dissociated adult cells mixed with type I collagen, and such a technique, which requires numerous cells, was not appropriate for HFTE cells.
and does not permit an analysis of the dynamic migratory process observed during in vivo fetal airway development.

In our model, the tubular morphogenesis phenomenon is obtained after two steps of cell culture involving two steps of cell-matrix interactions. In the first step, the basal plasma membranes of all the cell monolayers cultured on type I collagen interact with the matrix. The HFTE cells grow out from the explant, and this outgrowth contains two populations: at the leading edge, those with a migratory phenotype characterized by flattening and lamellipodia and expressing the $\beta_1$-integrin subunit on their entire plasma membrane; and behind this migration front or near the explant, cells that do not spread, do not have any lamellipodia, and express the $\beta_1$-integrin subunit almost exclusively on their basal plasma membranes. The $\beta_1$-integrins detected in basolateral plasma membranes were previously shown to be involved in the stable attachment of stationary epithelial cells to the matrix and in the maintenance of cell-cell interactions (30, 32). The pericellular distribution of $\beta_1$-integrins was reported in processes involving respiratory cell migration, such as wound repair, when epithelial cells at the edge of the wound migrated to reepithelialize a denuded area (12). Coraux et al. (7) also previously described this differential expression of the $\beta_1$-integrins during the development of human fetal airways in vivo. The $\beta_1$-integrin subunit was expressed on the entire plasma membrane of cells at the tip of the bronchial epithelial buds growing and migrating into the mesenchyme of human fetal lungs, whereas it was localized at the basolateral plasma membrane of anchored and polarized epithelial cells along the proximal branches. In our model, blocking antibodies directed against the $\beta_1$-integrin subunit demonstrated the implication of these glycoproteins in the migratory process because they altered cell morphology by inducing retraction of the lamellipodia. The impaired migration of respiratory and retinal pigment epithelial cells in the presence of antibodies to $\beta_1$-integrins was previously described (12, 13), but Hergott et al. (13) did not observe any cell phenotype change. The phenotype alteration that we described can explain the slowed speed and angular deviation variations of the migration. Cell migration is known to be a process involving cytoplasmic extensions at the leading edge of migratory cells (lamellipodia), with cytoskeleton modifications and cell-matrix interactions via integrins (21). During this process, the cell nuclei seem to be passive and swept along by the cytoplasmic movements. This postulate can explain the relatively straight trajectories of the peripheral HFTE cell nuclei.
on collagen. Adding blocking antibodies caused the lamellipodia to retract by partially inhibiting the cell-matrix interactions, and the nuclear trajectories became erratic.

Cell morphology and migration alterations could also be observed during the second step of cell culture, i.e., the addition of a second layer of collagen, so that the entire surface, not only the basal plasma membrane of HFTE cells, came in contact with the collagen matrix. The cell migratory phenotype was temporarily lost and associated with a significant slowing of the mean migration speed of the advancing HFTE cells, a disorientation of their migration direction, and a slightly delayed cord and tubule formation. The second collagen layer caused a depolarization and a redistribution of the β₁-integrin subunit from the basal side to the cytoplasm of HFTE cells. The relationships between the cells and the matrix seem to be disturbed because the cells redistributed part of their surface receptors. Consequently, the cells became spherical, leading to altered migration. Chambard et al. (6) described the same phenomenon of disorganization of polarized thyroid epithelial cell monolayers and alteration of cell migration that was triggered by contact of the apical plasma membranes of the cells with collagen and led to the complete reorganization of the cell population. This migration stopped when a new apical pole was formed by the cells lining the collagen-free lumen. In our model, unlike the observations made with anti-β₁-integrin subunit antibodies, perturbation of the cell-matrix interactions by the second collagen layer was transient; lamellipodia formation, migration, and ramifications proceeded after a short delay. Studies using polarized Madin-Darby canine kidney cells (24, 37) also showed that contact of the apical domain with a collagen matrix modified the β₁-integrin expression pattern, demonstrating a polarity reversion. In our culture model on a collagen matrix, nonmigratory HFTE cells were polarized because β₁-integrins were detected in their basal plasma membrane. When coated with a second layer of collagen, the cells were transiently depolarized. When epithelial tubules were formed, the HFTE cells edging the lumen were polarized, showing apical microvilli and cilia as reported during human fetal airway development in vivo (11). The reorganization of HFTE cell monolayers in tubular structures led to a new polarization of the HFTE cells facing the lumen.

These different roles of β₁-integrins can be explained by their cellular localization but also by the α-chain involved in the heterodimeric receptor formation. The β₁-integrin subunit is known to form complexes with numerous α-subunits to form some extracellular matrix receptors differentially expressed during airway...
development (7). Moreover, the contributions of integrin heterodimers to the morphogenesis of branched tubular organs have been reported (10, 17, 20).

In conclusion, we demonstrated that the tubular morphogenesis of HFTE cells in a collagen sandwich is dependent on cell-matrix interactions and cell-migration regulation. According to their cellular distributions, β1-integrins appear to be involved in the cell migration and tubule formation of HFTE cells. Our assay could also be used to examine the role of specific integrins using anti-α-subunit antibodies and to analyze whether other extracellular matrix components may assist or inhibit the process.

This work was supported in part by European Community Network no. BIO-CT 95-0284 and by the Association Française de Lutte contre la Mucoviscidose (Paris, France). C. Coraux is the recipient of a fellowship funded by the Ministère de L’Enseignement Supérieur et de la Recherche.

REFERENCES


β1-INTRGRINS AND FETAL AIRWAY TUBULOGENESIS

L233

This article is copyrighted as indicated in the article. Reuse of AJP--Lung Cell Mol Physiol content is subject to the terms at http://ajplung.physiology.org/site/misc/terms.xhtml