Does Cartilage Down-regulate Growth Factor Expression in Tracheal Epithelium?

Wesley Hicks, Jr, MD; Lynn Sigurdson, PhD; Edward Gabalski, MD; Robert Hard, PhD; Leon Hall III, BSc; Joseph Gardella, PhD; Colin Powers, MD; Niranjan Kumar, PhD; Jamson Lwebuga-Mukasa, MD, PhD

Background: Maintaining tracheal integrity and restoring normal physiologic function after injury is complex. Some of the critical events in this process are deposition of a provisional extracellular matrix, tissue remodeling, and angiogenesis. These events are coordinated with epithelial migration and proliferation to restore the mucosal barrier. The ability of respiratory epithelial cells (REC) to migrate and proliferate and restore denuded areas of the large conducting airway after injury is poor.

Objective: To test the hypotheses that (1) the cartilaginous framework, underlying the extracellular matrix (submucosa) and epithelium, decreases the migratory ability of REC when compared with REC on the same provisional extracellular matrix (type I collagen) alone, and (2) this phenomenon is associated with a change in expression of transforming growth factor (TGF)-α and TGF-β, both of which have been demonstrated in cutaneous models to be important in epithelial migration and proliferation.

Design: We developed a culture system that reconstitutes the tracheal lumen in vitro, consisting of dissociated chondrocytes cultured in a manner to form cartilage, submucosa (type I collagen), and REC (termed a “composite culture”). Control cultures consisted of epithelial cells grown on type I collagen alone. Control and composite cultures were evaluated morphologically using scanning electron and light microscopy. Expression of TGF-α and TGF-β was determined in day 14 cultured epithelial cells from control and composite cultures by semiquantitative polymerase chain reaction.

Results: Epithelial cells from composite cultures did not spread and were less squamoid in morphological appearance than epithelial cells on type I collagen alone. Expression of both growth factors was reduced in epithelial cells from composite cultures compared with those on type I collagen.

Conclusions: Cartilage modulates TGF-α and TGF-β expression in REC, and may contribute to regulation of REC proliferation and differentiation.


Original Article

From the Department of Head and Neck Surgery, Roswell Park Cancer Institute (Drs Hicks and Sigurdson and Mr Hall), Departments of Otolaryngology (Dr Gabalski), Anatomy/Cell Biology (Dr Hard), Chemistry (Dr Gardella), and Surgery (Dr Powers), SUNY at Buffalo Medical School, and Department of Pulmonary and Critical Care Medicine, University of Buffalo (Drs Kumar and Lwebuga-Mukasa), Buffalo, NY, and Department of Otolaryngology, Stanford University Medical Center, Stanford, Calif (Dr Gabalski).

TRAUMA THAT disrupts intraluminal epithelium, regardless of its cause, often leads to aberrant repair. This process is pathologically manifested by the exuberant proliferation of granulation tissue and replacement of the normal respiratory epithelium with fibroblasts. This often leads to scar formation, airway stenosis, and eventual physiologic compromise of the host respiratory tract.

There is currently no effective way to study intraluminal events of reepithelialization after injury. Present approaches to tracheal repair include resection and reanastomosing the injured airway, replacement of the damaged portion by synthetic material, and use of autologous tissue for reconstruction of the tracheal defect. Recently, tissue engineering approaches have been taken, including forming an in vivo tracheal cartilaginous scaffolding by injecting dissociated chondrocytes into a preformed synthetic construct. Such devices were of limited success owing to lack of reepithelialization. In the case of synthetic replacement, migration of the prosthesis can occur and may result in chronic ulceration, and even fatal hemorrhage.

We have developed a 3-dimensional in vitro model system that incorporates both cartilaginous and epithelial elements, a feature unique among currently available models of upper airway injury and repair. Using this model, we hypothesized that communication between epithelial cells and underlying stroma would lead to modulation of epi-
thelial cell growth and differentiation through the release of growth factors. To test this hypothesis, we investigated expression of transforming growth factors α and β (TGF-α and TGF-β) in respiratory epithelial cells (REC) cultured on type I collagen or composite cultures of chondrocytes and type I collagen.

Transforming growth factor α is a member of the epidermal growth factor family and plays an important role in wound healing.\textsuperscript{9,11} Transforming growth factor β1 is a multifunctional polypeptide with differing cell-specific effects, including stimulation or inhibition of proliferation, and regulation of extracellular matrix production and remodeling.\textsuperscript{12,14}

Respiratory epithelial cells in a composite culture system with cartilage represents a 3-dimensional model of the tracheal luminal surface, which facilitates studies of the interactions between epithelium and its underlying stroma. Using this model, we have shown that cartilage may modulate the migration of REC, and changes in expression of TGF-α and TGF-β, when compared with REC grown on type I collagen alone.

**RESULTS**

Bovine chondrocytes established in primary culture were morphologically similar to in vivo bovine cartilage (Figure 1, A and B). Cartilage cultured for less than 2 months did not always form lacunae, but always produced an abundant extracellular matrix of type II collagen (Figure 1, C).

Respiratory epithelial cells grown on type I collagen formed a continuous sheet (Figure 2, A and B). In contrast, REC grown on composite cultures did not spread to confluence, but rather grew as patches of epithelium (Figure 2, C and D). The epithelial cell layer in both the composite cultures and on type I collagen was undifferentiated.
TGF-α AND TGF-β GENE EXPRESSION ON TYPE I COLLAGEN AND IN COMPOSITE CULTURES

In REC from day 14 composite cultures, expression of both TGF-α and TGF-β was reduced (Figure 3, lane 4, TGF-α; lane 6, TGF-β) compared with REC on type I collagen (Figure 3, lane 3, TGF-α; lane 5, TGF-β). Relative expression of β-actin was equal (Figure 3, lanes 1 and 2).

Paraffin sections of REC grown on type I collagen and on composites were immunostained for TGF-α and TGF-β. Both growth factors were expressed in chondrocytes, and to a lesser extent, in epithelial cells (data not shown).

COMMENT

A frequent problem seen in tracheal repair with synthetic or autologous materials is the failure of luminal surface reepithelialization. Failure of reepithelialization to reestablish luminal integrity is an important reason why no acceptable surgical procedure exists for the repair of extended segments of trachea compromised by inhalation injury, congenital anomalies, or neoplastic disease.

Why the rate of reepithelialization in the large conducting airway is different from that seen within other epithelial-lined or -covered surfaces is unclear. The phenomenon of “slowed” reepithelialization is seen after both ablative surgical reconstruction and denudation injury, where the epithelium and basement membrane are removed with an intact cartilaginous superstructure (eg, inhalation injury).

One of the difficulties in understanding the relationship between respiratory epithelium and its underlying substructure (cartilage and submucosa) is the inaccessibility of the tissue for direct observation. Our model facilitates the examination the tissue interactions and mechanisms resulting in REC migration.

Both TGF-α and TGF-β play crucial roles in new tissue formation and remodeling. Transforming growth fac-
tor α stimulates proliferation in cultured epithelial cells, fibroblasts, and endothelial cells. It is chemotactic for epithelial cells in vitro and enhances epithelial wound healing when applied topically. Transforming growth factor β is mitogenic for cells of mesenchymal origin and plays a role in repair through its ability to modulate extracellular matrix formation and tissue remodeling.

When isolated human REC were cultured on type I collagen, the cells spread to form a confluent layer as has been previously reported by other authors. When plated onto composite cultures with a layer of type I collagen on top of cartilage, the cells did not spread efficiently but formed epithelial nests. Complete reepithelialization of the surface did not occur, even after 3 weeks.

We examined TGF-α and TGF-β expression in REC from composite cultures and found that both of these growth factors were reduced in epithelial cells from 14-day composite cultures when compared with the expression of these factors in REC cultured on type I collagen alone. This suggests that the cartilage modulates the behavior of epithelial cells. One hypothesis for the observed diminished expression of TGF-α and TGF-β is the secretion of soluble factors from the cartilage. Transforming growth factor α was expressed in cartilage (not

Figure 2. Scanning electron micrographs of day 14 control (type I collagen) and composite cultures. A and B, Control; confluent epithelium (original magnification: A, ×32; B, ×100). C and D, Respiratory epithelial cells on composite culture. Arrows indicate underlying composite with nonconfluent epithelium (original magnification: C, ×32; D, ×100).
shown), where it may have acted on the epithelium in a paracrine manner to decrease its expression.

Patients with critical large conducting airway injury requiring medical intervention often succumb to their condition because no reliable means of restoring the large conducting airway exists. Lack of REC migration and proliferation remains the key physiologic and biologic issue underlying failure of surgical repair. Understanding the factors that influence epithelial migration and regeneration in the large conducting airway remains a central issue in solving this clinical problem. The model presented demonstrates one potential mechanism to explain the phenomenon of impaired reepithelialization seen in the large conducting airway after injury.

Accepted for publication March 31, 1999.

Reprints: Wesley L. Hicks, Jr, MD, Head and Neck Surgery, Roswell Park Cancer Institute, Elm and Carlton streets, Buffalo, NY 14263.