Biosynthesized matrix provides a key role for survival signaling in bronchial epithelial cells

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Wadsworth, Sam J., Anette M. Freyer, Randolph L. Corteling, and Ian P. Hall. Biosynthesized matrix provides a key role for survival signaling in bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 286: L596–L603, 2004. First published November 14, 2003; 10.1152/ajplung.00217.2003.—The extracellular matrix (ECM) influences a variety of cellular functions, including survival, adhesion molecule expression, differentiation, and migration. The ECM composition of the epithelial basement membrane is altered in asthmatics. In this study, we elucidate the major survival signals received by bronchial epithelial cells in vitro by studying the effects of a variety of ECM factors and soluble growth factors on bronchial epithelial cell survival. Our findings indicate that the insulin family of soluble growth factors provides important survival signals but also that adhesion to ECM is a crucial determinant of bronchial epithelial cell survival. In the BEAS-2B bronchial epithelial cell line, collagens I and IV, laminin, fibronectin, and vitronectin provide significant levels of protection from apoptosis. Tenascin-C has no effect, whereas elastin and collagen V increase apoptosis to above control levels. BEAS-2B cells secrete their own biosynthesized matrix (BSM), which also provides rescue from apoptosis. Protection by collagen I, fibronectin, and vitronectin was found to be via an RGD domain. Laminin-, collagen IV-, and BSM-mediated survival is not RGD dependent. Primary bronchial epithelial cells exhibit a similar pattern of apoptosis rescue to the BEAS-2B cell line, although we did not observe any vitronectin-mediated protection in the primary cells. These data indicate that bronchial epithelial cell survival is dependent both on soluble growth factors and on a variety of ECM-derived signals.

BEAS-2B; apoptosis; basement membrane; insulin; integrins

AN INTACT RESPIRATORY EPITHELIUM is important for normal airway function. The integrity of the airway epithelium depends on adhesion of epithelial cells either to each other or to the extracellular matrix (ECM)/basement membrane, which is in close juxtaposition with the basolateral layer of cells. One feature of inflammatory airway diseases, including asthma, is significant loss of columnar epithelial cells, which leads to loss of epithelial integrity (12, 16). In addition to a vital barrier function, the airway epithelium has a number of other important functions, including solute transport and secretion of key mediators. The latter function has led to the concept of the epithelium and underlying airway myofibroblasts functioning as an integrated unit (9). Thus disruption of this unit will alter a number of potentially important functions.

Whereas epithelial cells can be shed from the epithelium by direct mechanical damage (e.g., at bronchoscopy), a second potential mechanism for the disruption of the epithelium is cell death. Epithelial cell death may be brought around by necrosis, usually secondary to inhalation of toxins, or potentially by apoptosis. Epithelial cells from many epithelial surfaces will die through apoptosis if removed from their cell-matrix attachments, a phenomenon first observed in mammary epithelium, termed “anoikis” (6). A similar mechanism of apoptosis induction is also apparent in the bronchial epithelium (2). However, the background rate of apoptosis in intact epithelia in vivo appears to be very low (24), suggesting that in this setting, epithelial cells receive a strong survival signal acting to prevent the onset of apoptosis. The aim of the current study was to extend earlier observations on the nature of the survival signals that airway epithelial cells receive and, in particular, to determine the role of matrix in these responses. Here, we report that a number of important mediators/cytokines can promote survival signaling in airway epithelial cells and also that a key role for matrix exists in airway epithelial cell survival signaling. Furthermore, airway epithelial cells are able to synthesize matrix that provides a key survival signal. These general mechanisms operate in both a model cell line (BEAS-2B) and in cultured primary bronchial epithelial cells.

METHODS

Cell culture. The BEAS-2B, simian virus 40-transformed, immortalized bronchial epithelial cell line used for part of this study (18) was a gift from Dr. Raymond Penn (Philadelphia, PA). Cells were routinely cultured and passed in 75-cm² cell culture flasks (Corning Costar) in DMEM (Sigma) supplemented with 0.4 mM L-glutamine and bovine pituitary extract (0.004 ml/ml), transferrin (10 μg/ml), triiodothyronine (10 nM), hydrocortisone (0.2 μM), all-trans retinoic acid (33 nM), and epinephrine (0.5 μg/ml) (all Sigma).

Primary human bronchial epithelial cells (HBECS) were purchased from Clonetics and were cultured to passage 4, using bronchial epithelial growth medium (BEGM, Clonetics). BEGM consisted of bronchial epithelial basal medium (BEBM) with growth factor supplements (as used in SRM) supplied by Cambrex. All experiments were conducted at passage 4 with cells seeded in BEBM without growth factor supplements onto uncoated or ECM-coated 96-well plates. Cells were cultured for 48 h as described above.

ECM preparation. ECM-coated dishes were produced by incubating wells of a 96-well plate with 50 μl of 10 μg/ml of fibronectin (Sigma), collagen IV (Sigma), laminin I (Calbiochem), vitronectin (Calbiochem), collagen V (Sigma), tenasin-C (Chemicon), and elastin (Sigma) overnight. In other experiments, we coated wells with a biosynthesized matrix using a previously published method (7). Briefly, cells were seeded in serum-free SRM media.

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(BEAS-2B) or BEGM (HBECs) onto plastic, flat-bottomed 96-well plates and incubated until confluent. Cells were then removed using 0.02 M NH4OH to leave behind an intact, biosynthesized matrix (BSM). Wells were also coated with serum proteins by incubating with 10% FCS in DMEM without cells present for 48 h.

**BSM protein quantification.** To quantify BSM protein content, flat-bottomed 96-well plates were coated with BSM as described above; control wells were coated with fibronectin at a range of concentrations. A Bradford-based protein assay kit (Bio-Rad) was used to stain protein adsorbed to the bottom of BSM- or fibronectin-coated wells. Wells were washed with PBS, and the absorption at 595 nm of BSM-coated wells was compared with the fibronectin standards to provide a quantitative value for BSM protein content.

**Apoptosis assays.** Cells were seeded at a density of 0.8–1.2 × 10^5 cells/ml into 96-well flat-bottomed dishes (Nunc) in DMEM with serum or growth factors added as indicated and cultured for 48 h at 37°C and 5% CO2. In some experiments, the protein synthesis blocker cycloheximide (CHX; 50 μM, Sigma) was added to wells before cell seeding, as used in previous studies, to prevent de novo synthesis of ECM (5).

**Integrin-matrix interaction.** For relevant experiments before seeding onto matrix-coated wells, cells were kept in suspension for 15 min at 37°C with either the RGD-blocking GRGDSP peptide (200 μM; Calbiochem) or the inactive analog GRADS (200 μg/ml; Novabiochem).

**Evaluation of apoptosis by fluorescence microscopy.** Cells in 96-well plates were fixed in situ for 30 min with 4% paraformaldehyde and stained with 1.5 μg/ml of propidium iodide (PI; Sigma). Stained cells were observed by fluorescence microscopy (×200; Nikon Diaphot 300), and apoptotic cells were morphologically identified by the presence of condensed, brightly stained nuclei and reduced cytoplasm. The total number of healthy and apoptotic cells was calculated for three random fields per well, in triplicate wells in three separate plates, and the percentage of apoptotic cells was calculated. All counts were performed with the observer blinded to treatments.

**Evaluation of apoptosis by DNA fragmentation.** To confirm the results of the PI staining assay for apoptosis, we used a TdT-mediated dUTP nick end labeling (TUNEL)-based assay. Briefly, a TiterTACS kit (R&D Systems) was used to measure DNA fragmentation, a late sign of apoptosis. Cells were cultured as outlined earlier in a variety of apoptosis-stimulating and survival-inducing conditions. After 48 h, cells were fixed and DNA breaks in apoptotic cells were detected using a TdT enzyme linked to a horseradish peroxidase amplification system. The number of apoptotic cells in a well was proportional to the absorbance at 450 nm. We also performed cell counts by counting total cell number in three random fields per condition in triplicate wells. Over the course of the incubation period, a significant increase in cell number had occurred in healthy cultures. We therefore corrected the results for cell number by dividing the absorption value obtained from the TiterTACS assay by the total cell number counted for each experimental condition, the resulting value being proportional to the percentage of apoptotic cells present in the sample.

**RESULTS**

To study survival signaling in airway epithelial cells, we used two model systems. First, we performed experiments in the bronchial epithelial cell line BEAS-2B. We then extended our studies into HBECs grown under nondifferentiating conditions to model the basal epithelial cells present in the normal respiratory epithelium. Throughout our experiments, proliferating HBECs maintained a classic “cobblestone” appearance, suggesting squamous cell differentiation had not occurred.

**Effect of serum and soluble growth factors on BEAS-2B apoptosis.** Our initial experiments were designed to confirm the previously observed dependence of airway epithelial cell survival on the presence of serum in the culture medium. Figure 1 shows that BEAS-2B cells, when deprived of serum at seeding, exhibit increased levels of apoptosis up to a maximum at 48 h of 19.0 ± 1.4% (n = 3). However, when these experiments were performed in the presence of the protein synthesis inhibitor CHX (50 μM), apoptosis rates were markedly higher (81.4 ± 3.3%, n = 3, P < 0.001). The CHX-induced cell death response was inhibited (although not back to baseline levels) by the presence of serum (Fig. 1), suggesting that factors present in serum could, to a large extent, provide the necessary survival signal required to prevent the onset of apoptosis.

We next examined the survival effects of a range of mediators and cytokines to which airway epithelial cells are likely to be exposed in vivo. PDGF, transforming growth factor-β, serotonin, histamine, and bradykinin all failed to significantly prevent serum deprivation-induced cell death (data not shown). In contrast, IGF-I, IGF-II, and insulin all provided strong concentration-dependent survival signals (Fig. 2, A–C). The survival signals from EGF and FGF were less potent but still significant (Fig. 2, D and E).

The logEC50 ± SE values for inhibition of apoptosis for these agents were: IGF-I = −7.28 ± 0.15, insulin = −6.42 ± 0.31, IGF-II = −7.34 ± 0.28, EGF = −9.56 ± 0.45, and FGF = −8.96 ± 0.40 (all units log g/ml, n = 3).

To verify the survival effects observed using our PI staining counting method, we used a TiterTACS (R&D systems) TUNEL-based assay to detect DNA breaks in cells undergoing late-stage apoptosis. Results were corrected for cell number and expressed as a % of serum withdrawal (Fig. 3). A similar pattern of results was observed using both PI counting and TiterTACS methods.

Together, these results demonstrate that a number of survival factors inhibits apoptosis induced by serum withdrawal. However, despite the above effects, we found these soluble growth factors were unable to reverse CHX-induced cell death as effectively as serum (data not shown). We also observed that confluent monolayers treated with CHX did not undergo a significant increase in apoptosis (Fig. 4), complementing previous studies in a mammary epithelial cell line that show CHX treatment does not influence anoikis levels of detached cells (26). Because an intact monolayer will already possess an ECM substrate, we hypothesized that an ECM component of serum may be responsible for the greater protective effect.

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**Fig. 1. Effect of serum on apoptosis.** BEAS-2B cells were seeded onto tissue culture plastic in DMEM at various FCS concentrations with or without 50 μM cycloheximide (CHX). After 48 h in culture, cells were fixed and stained with 1.5 μg/ml of propidium iodide (PI, Sigma). Stained cells were observed by fluorescence microscopy (×200; Nikon Diaphot 300), and apoptotic cells were morphologically identified by the presence of condensed, brightly stained nuclei and reduced cytoplasm. The total number of healthy and apoptotic cells was calculated for three random fields per well, in triplicate wells in three separate plates, and the percentage of apoptotic cells was calculated. All counts were performed with the observer blinded to treatments.

**Fig. 2. Effect of soluble growth factors on BEAS-2B cell survival.** To study survival signaling in airway epithelial cells, we used two model systems. First, we performed experiments in the bronchial epithelial cell line BEAS-2B. We then extended our studies into HBECs grown under nondifferentiating conditions to model the basal epithelial cells present in the normal respiratory epithelium. Throughout our experiments, proliferating HBECs maintained a classic “cobblestone” appearance, suggesting squamous cell differentiation had not occurred.

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observed with serum exposure. Therefore, we next studied the effect of matrix present in serum and of matrix secreted by cells on survival in the presence of CHX.

Effect of insoluble factors on BEAS-2B apoptosis. First, we observed that coating wells with serum protein provided similar levels of protection to those seen with the addition of serum into culture medium, with CHX-induced cell death being inhibited by 69.0 ± 4.7% (Fig. 5). However, pretreatment with SRM, which contains a variety of growth factors (see Methods), failed to replicate this survival effect, suggesting that a component of serum not present in SRM was responsible. We also found that chemically stripping confluent cells from culture plates left behind a BSM secreted by these cells attached to the bottom of the wells. This BSM replicated the survival effect of serum when cells were replated onto it (all n = 3, Fig. 5). Interestingly, the amount of matrix secreted by BEAS-2B cells was high after 48 h in culture, being 25 ± 0.4 µg/ml (equivalent to a protein concentration of 3.91 ± 0.06 µg/cm²; n = 5). Together, these data suggest that airway epithelial cells in culture are able to rapidly modify their extracellular environment by secreting matrix factors that provide a key survival signal for the cells.

Effects of individual matrix factors on BEAS-2B apoptosis. To define the key matrix factors that provide the survival signal for BEAS-2B cells, we used the model of protection from CHX-induced apoptosis described above. We precoated wells with single matrix factors at concentrations (10 µg/ml) similar to that of biosynthesized matrix we had previously observed to be secreted and examined apoptosis rates when cells were seeded in the presence of CHX (Fig. 6A). Under these conditions, apoptosis rates on uncoated plastic wells were high (95.5 ± 1.1%). A significant survival effect was observed with fibronectin (54.5 ± 3.3%), vitronectin (48.3 ± 2.8%), collagen I (32.9 ± 3.3%), collagen IV (53.7 ± 4.4%), BSM (54.5 ± 3.3%), and laminin (31.3 ± 2.8%), which all provided rescue from apoptosis (n = 3). Elastin, tenascin-C, and collagen V did not confer any protection (all n = 3). The pattern of survival effects of matrix was similar when serum deprivation (in the absence of CHX) was used as the apoptotic stimulus (Fig. 6B), although as shown in Fig. 1, the overall rates of apoptosis were...
lower than experiments in which CHX was included. In these experiments, collagen V and elastin increased apoptosis levels by 74.6 ± 6.6% and 40.0 ± 7.7%, respectively (n = 3).

Effect of RGD-blocking peptide on ECM rescue from apoptosis. Airway epithelial cells such as the BEAS-2B cell line are known to express a range of integrin receptors (25). Based on the pattern of survival effects observed with matrix factors, we hypothesized that the strongest candidate interaction for the survival signal seen in these experiments was interaction of RGD motifs present on matrix with cell surface integrins. We therefore studied the effect of the RGD blocking, short peptide antagonist GRGDS, and a relevant inactive control peptide (GRADS) on rescue from CHX-induced cell death provided by matrix factors. In keeping with a role of interaction with RGD-binding integrins, GRGDS peptide inhibited survival signaling for several of the matrix factors studied (Fig. 7, A and B). GRGDS inhibition of ECM-mediated survival reached statistically significant levels on fibronectin (84.9 ± 4.0% inhibition of rescue, n = 5, P < 0.001), vitronectin (95.5 ±

![Fig. 3. TdT-mediated dUTP nick end labeling confirmation of PI apoptosis assay. BEAS-2B cells were seeded serum free (except where stated) with individual growth factors added at the time of seeding. DNA fragmentation was quantified using a TiterTACS kit, and absorption values were corrected for cell number using average total cell counts for each condition. For unlabeled cells, the TdT labeling enzyme was excluded. Positive control (Pos control) was created by addition of the kit’s endonuclease enzyme to 10% FCS cells. SRM, serum replacement medium. Values are expressed as % of sfm control.](image1)

![Fig. 5. Effect of biosynthesized matrix (BSM) and serum protein (FCS) on apoptosis. Plastic wells were coated with BSM or incubated overnight with DMEM + 10% FCS or with serum replacement medium (SRM). BEAS-2B cells were then seeded onto the precoated wells in serum-free conditions with or without 50 μM CHX. After 48 h in culture, cells were fixed, and apoptosis was assessed by PI staining, expressed as % ± SE. **P < 0.01 compared with plastic control by ANOVA, n = 3.](image2)

![Fig. 4. Effect of CHX on apoptosis levels of confluent cells. BEAS-2B cells were seeded onto tissue culture plastic in serum-free bronchial epithelial growth medium, CHX (50 μM final) was added at the time points stated, and cells were incubated for a total of 48 h. Cells were fixed and stained with PI. Apoptosis is expressed as % ± SE of all cells counted. Cells were confluent at 20 h postseeding, n = 3.](image3)

![Fig. 6. Extracellular matrix (ECM) regulation of BEAS-2B apoptosis. Cells were seeded in serum-free DMEM onto plastic wells precoated with individual matrix factors at 10 μg/ml in the presence (A) or absence (B) of 50 μM CHX. After 48 h in culture, apoptosis was assessed by PI staining and expressed as % ± SE of all cells counted. **P < 0.01, *P < 0.05 compared with plastic control by ANOVA, n = 3.](image4)
growth factors confirmed that like BEAS-2B cells, HBECs also receive a survival signal from the insulin family of growth factors, although the level of protection is weaker than in the cell line. Insulin, IGF-I, and IGF-II (Fig. 8, A–C, respectively) all showed concentration-dependent protective effects. EGF (Fig. 8D) demonstrated a weaker survival effect. FGF did not show any effect on cell survival in our experiments (data not shown). Interestingly, adhesion experiments showed insulin did not affect the adherence of HBECs to uncoated plastic wells at an early time point (5 h postseeding), thus suggesting insulin promotes HBEC survival without promoting cell-substratum adherence (data not shown).

**Effect of ECM on HBEC apoptosis.** The overall pattern of ECM-mediated rescue observed in the HBEC system was similar to that in the BEAS-2B cell system (Fig. 9). As before, growth factor withdrawal induced a higher level of apoptosis in the HBECs (67.9 ± 4.8% in this set of experiments) compared with the transformed cell line. Collagen IV (78.7 ± 5.9%) and BSM (74.4 ± 5.2%) conferred the highest level of rescue. Fibronectin (42.1 ± 6.5%), laminin (51.3 ± 6.0%), and collagen I (40.7 ± 7.1%) showed statistically significant but less effective protection (n ≥ 3, P < 0.01). Tenasin-C and elastin did not show statistically significant levels of protection, whereas collagen V induced an increase in HBEC apoptosis to above control levels (43.1 ± 4.8% increase, n = 4, P < 0.01). Although vitronectin provided a survival signal for BEAS-2B cells, we did not observe any protection by this matrix factor in the primary HBEC system.

**DISCUSSION**

Maintenance of an intact respiratory epithelium is a critically important determinant of normal respiratory function. Disruption of the airway epithelium has been reported in a number of conditions, including asthma (10). In asthma, epithelial shedding may be present, leading to altered airway responses that may play a role in maintaining airway inflammation (8, 9). The aim of the current study was to determine some of the key survival signals received by airway epithelial cells. With both a representative human bronchial epithelial cell line (BEAS-2B) and also undifferentiated HBECs, we were able to show that in addition to survival signals from insulin/IGF-I and IGF-II pathways, airway epithelial cells also receive a key survival signal from matrix factors with which the cell may come into contact.

We found primary HBECs were in general more prone to undergo apoptosis than cells of the BEAS-2B cell line, perhaps unsurprising considering the potentially unlimited subculture capacity of the cell line compared with the very limited lifespan of the primary cells in vitro. The insulin family of growth factors brought about an increased survival rate in both the primary cells and transformed cell line without having any effect on early stage adhesion to the substratum. Levels of protection were lower in primary cells, again reflecting their greater tendency to undergo apoptosis. We also observed profound survival effects for a number of matrix factors, including fibronectin, collagens I and IV, laminin, and vitronectin. Cell survival resulting from the integration of signals from matrix-binding integrins and signaling from the insulin receptor are in keeping with previous observations in other epithelial cell systems (4, 13).
These matrix responses are highly likely to be mediated through interaction between cell surface integrins and matrix motifs. One of the most common integrin-binding matrix motifs is the RGD sequence. Many integrins bind to this motif, including the vitronectin receptor $\alpha_5\beta_1$, the vitronectin/fibronectin receptor $\alpha_5\beta_3$, the fibronectin/tenascin-C receptor $\alpha_5\beta_6$, and the fibronectin receptor $\alpha_5\beta_1$. The laminin/collagen integrin receptors $\alpha_2\beta_1$ and $\alpha_3\beta_1$ show only weak RGD interactions (21). Our data suggest a key role for the RGD motif present on a number of key matrix factors such as fibronectin, vitronectin, and collagen I. The RGD motif does not seem to play a crucial role in adhesion to laminin, collagen IV, or biosynthesized matrix. This contrasts with previous work demonstrating survival signaling by BSM is RGD dependent (2). In our experiments, we did observe a small inhibition of BSM-mediated rescue with the RGD-blocking peptide, but not to statistically significant levels. As it is likely that BSM consists of several different matrix factors signaling through different recognition sequences, it is possible a small proportion of the BSM survival signal may be via an RGD-dependent factor such as fibronectin or vitronectin. However, the lack of a major RGD-dependent survival signal on BSM suggests the matrix secreted by BEAS-2B cells consists mainly of the normal basement membrane proteins, laminin, and/or collagen IV. This supports previous work demonstrating the ability of HBECs to secrete and adhere to laminin-5 in culture (14).

The pattern of matrix-mediated cell survival was similar in the two cell types we tested but not identical. Vitronectin provided a strong survival signal in the BEAS-2B cells but had no effect in the primary HBEC system. A more subtle difference was the greater protective effect of collagen IV compared with other ECM factors in HBECs. Previous work has shown differences in integrin expression profile between cultured HBECs and the BEAS-2B cell line, both of which differ from normal bronchial epithelium in vivo (17, 25). These data could provide a likely explanation for the different ECM effects observed in the primary cells and the cell line. The absence of vitronectin-mediated rescue in cultured HBECs is probably due to the lack of expression of the $\alpha_5\beta_1$-integrin. The $\alpha_5\beta_1$-integrin acts as a vitronectin receptor, is expressed in the BEAS-2B cell line (25), and most likely mediates protection from apoptosis in BEAS-2B cells, as previously observed in an $\alpha_5\beta_1$-transfected HEK-293 cell line (3). The strong survival effect of collagen IV in HBECs may also be due to a difference in integrin expression as the laminin/collagen IV-binding $\alpha_5\beta_1$-integrin is expressed by HBECs in vitro but not by BEAS-2B cells (25). Although this receptor predominantly interacts with laminin, the strong survival effect of collagen IV observed in HBECs may be due to the presence of this integrin.
The observation in the primary and transformed cell systems that collagen V causes a significant increase in apoptosis demonstrates that, depending on the ligand engaged, matrix signaling can be both pro- and anti-apoptotic.

The previous variations observed in integrin expression profile between primary HBECs and the BEAS-2B cell line, in conjunction with the differences we observed in matrix rescue, suggest that conclusions drawn from matrix studies using transformed epithelial cell lines should be made carefully since responses may be different than those seen with primary cells. Because there is also some variation in integrin expression between the epithelium in vivo and primary cells in culture [for example, the tenasin-C receptor α6β4-integrin is present in vivo but not in vitro (25)], caution also needs to be exercised when relating specific matrix effects observed in the primary systems in vitro to the intact bronchial epithelium.

In vivo, cells at the basolateral surface of the airway are in close contact with the basement membrane. Immunohistochemical studies have shown little matrix to be present in the airway epithelium above the level of the true basement membrane, but epithelial cells maintain cell-cell contact via tight junctions, adherens junctions, and desmosomes (20). Therefore, it seems likely that the integrity of the airway epithelium depends first on interaction of cells in the basal layer of the epithelium with the basement membrane and second on maintenance of cell-cell contact. The relative proportion of matrix factors changes during airway inflammation. For example, tenascin-C, which is normally absent from the airway epithelium, is present in the basement membrane of asthmatics, with expression levels correlated to disease severity (11). These matrix changes may lead to an alteration in the ability of the immediate cell environment to provide the requisite survival signals. Laminin isoforms are also altered in the asthmatic cell environment to provide the requisite survival matrix changes may lead to an alteration in the ability of the epithelium to protect airway epithelial cells from apoptosis. For example, the tenasin-C receptor α6β4-integrin is present in vivo but not in vitro (25)], caution also needs to be exercised when relating specific matrix effects observed in the primary systems in vitro to the intact bronchial epithelium.

In summary, therefore, we have defined a key role for matrix in the protection of airway epithelial cells from apoptosis. Airway epithelial cells are able to synthesize their own matrix that contributes to this survival signal. Changes in the matrix factors present within the airway wall during inflammation may alter survival signaling in both airway epithelial cells and other important structural cells in the airway. Indeed, this may represent a generic protective mechanism designed to maintain the integrity of the airway wall during chronic inflammatory insults.

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REFERENCES


