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Alveolar Epithelial Cells Express Both Plasminogen Activator and Tissue Factor* Potential Role in Repair of Lung Injury

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The formation of fibrin is a part of the normal tissue repair process. Fibrin not only serves as a hemostatic barrier and limiting factor in the exudative process, but also provides a matrix for the tissue repair that follows injury. The plasmin/plasminogen activator system is a key component in the fibrinolysis that accompanies tissue repair.

The adult respiratory distress syndrome (ARDS) is a common medical problem associated with a high mortality. Intraalveolar fibrin deposition is a prominent feature of the early phase of this syndrome. Recent studies suggest that the procoagulant/fibrinolytic balance is altered in the early phase of ARDS such that fibrin deposition is favored. The extensive remodeling process that occurs in survivors leads to the eventual return to near-normal lung anatomy and physiologic function in most individuals.

The specific cell types that contribute to the procoagulant/fibrinolytic balance in the alveolar space are not known. The alveolar macrophage has been recognized as a potentially relevant cell in that it has the capacity to express procoagulants, plasminogen activator, and plasminogen activator inhibitors. Since alveolar epithelial cells play a central role in the repair process following acute lung injury, we hypothesized that they might also be a source of procoagulant and/or fibrinolytic components. To test this hypothesis we studied rat alveolar epithelial cells in vitro, isolated by enzyme dissociation followed by differential adherence to IgG-coated plates.

We recently reported that these cells synthesize and secrete a plasminogen activator with the catalytic properties of a urokinase-type plasminogen activator (u-PA) rather than a tissue-type plasminogen activator. Studies of regulation of alveolar epithelial cell u-PA revealed the following: (1) phorbol myristate acetate (PMA) but not the inactive structural analogue 4α-PMA upregulated u-PA activity, putatively via the protein kinase C pathway; (2) PMA induction of u-PA activity was substantially inhibited by dexamethasone and completely inhibited by cycloheximide; (3) early day unstimulated alveolar epithelial cells showed no detectable u-PA mRNA and no constitutive synthesis of u-PA, whereas PMA led to accumulation of a 2.5-kilobase u-PA mRNA and synthesis of u-PA; and (4) cycloheximide did not abolish the PMA induction of u-PA mRNA, which suggested that intermediate protein synthesis was not necessary for activation of gene transcription.

We now report that rat alveolar epithelial cells also express a substantial amount of procoagulant activity as assessed by both recalcification and amidolytic assays. In the recalcification assay, clotting times were measured with a fibrometer after the addition of 100 μl of pooled normal plasma and 100 μl of 25 mM CaCl2 to 100 μl of cell lysates harvested in Tris-buffered saline. The clotting times were prolonged in plasma deficient in factor VII or factor X, but not factor VIII, indicating that tissue factor was the procoagulant (Table 1). Activity was quantitated by reference to a standard tissue factor preparation and normalized to the amount of protein in the sample. A comparable amount of tissue factor activity was found by amidolytic assay.

The binding of factor VII to the membrane glycoprotein tissue factor initiates the extrinsic pathway of coagulation. Evidence suggests that in extravascular tissues, the extrinsic pathway is of prime importance. The immunohistochemical distribution of tissue factor in normal human tissues suggests that it serves as a protective "envelope" ready to initiate coagulation if vascular integrity is compromised. In the lung, alveolar epithelial cells were one site with significant staining for the tissue factor antigen. This observation, coupled with the finding of substantial tissue factor activity in these cells, suggests that tissue factor is a physiologically relevant product of the alveolar epithelium.

The regulation of tissue factor by intravascular cells such as monocytes and endothelial cells has received considerable attention. However, little is known about regulation of epithelial cell tissue factor activity. We observed that PMA treatment induces downregulation of alveolar epithelial cell tissue factor activity (Fig 1). Current studies are aimed at elucidating the mechanisms of this regulation. We speculate that downregulation of epithelial cell tissue factor activity may be an important component of the repair process in that it alters the procoagulant/fibrinolytic balance to favor...

**Table 1—Alveolar Epithelial Cell Procoagulant Activity**

<table>
<thead>
<tr>
<th>Source of Clotting Factors</th>
<th>Procoagulant Activity (mU/μg Protein)</th>
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<tbody>
<tr>
<td>Pooled normal plasma</td>
<td>729</td>
</tr>
<tr>
<td>Factor VIII-deficient plasma</td>
<td>817</td>
</tr>
<tr>
<td>Factor VII-deficient plasma</td>
<td>40</td>
</tr>
<tr>
<td>Factor X-deficient plasma</td>
<td>&lt;30</td>
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</tbody>
</table>

*Alveolar epithelial cell lysates were incubated with either pooled normal plasma or factor-deficient plasma and CaCl2, and recalcification clotting times were measured. Clotting times were converted to tissue factor activity, with data expressed per μg protein. Each value represents the mean of 3 assays.*
fibrin resorption.

The alveolar epithelium should be considered an important contributor to alveolar fibrin deposition and subsequent resorption that typifies acute lung injury. Alveolar epithelial cells express key components of both the coagulation and fibrinolytic cascade: tissue factor and u-PA, respectively. Further, with respect to PMA, these components appear to be coordinately regulated. Further investigation of the modulation of these alveolar epithelial cell products may provide new insights into the alveolar cell response to acute lung injury and suggest potential strategies to favorably manipulate this response.

REFERENCES


Hypertrophic Alveolar Type II Cells Isolated after Silica-Induced Lung Injury Are Progressing Through the Cell Cycle and Maintain a Commitment to DNA Synthesis in Primary Culture*

Ralph J. Penos, M.D.; and Robert J. Mason, M.D.

A cute lung injury frequency results in damage to type I cells and disruption of the alveolar epithelial lining. Epithelial repair occurs when alveolar type II cells proliferate and differentiate into type I cells, restoring the integrity of the alveolar epithelium. Alveolar type II cell hyperplasia and hypertrophy occur after silica-induced lung injury and are a common response in the course of alveolar epithelial repair. We performed cell cycle analysis of elutriated type II cells isolated after silica instillation in the rat to determine if hypertrophic type II cells were progressing through the cell cycle and were proliferating cells.

Alveolar type II cells were isolated from rats 14 d after intratracheal instillation of silica (100 mg/kg) and separated into groups of increasing cell size by centrifugal elutriation. The cells were fixed in 70% ethanol and incubated with propidium iodide and RNase prior to analysis on an Epect flow cytometer. The percentage of cells in the G2/M (proliferative) phase of the cell cycle increased with increasing type II cell size: 3.3±0.7% in the smallest cells and 24.5±5.2% in the largest cells.

To determine the proliferative potential of silica type II cells in primary culture and to correlate alveolar type II cell size with the level of in vitro DNA synthesis, we studied DNA synthesis in type II cells isolated after silica instillation. Alveolar type II cells were isolated from rats 1, 2, 3, and 4 wk after intratracheal instillation of silica (100 mg/kg), cultured in Dulbecco’s modified eagle medium supplemented with 10% fetal bovine serum, and labeled with tritiated thymidine from 1 d to 3 d in culture. DNA synthesis was determined by (3H) thymidine incorporation and autoradiographic labeling index. The level of thymidine incorporation increased progressively from 22.3±5.4 × 10³ dpm/well 7 d after silica instillation to 34.4±5.0 × 10³ dpm/well at 28 d. The plating efficiency and type II cell purity were the same at all time points after silica instillation.

To determine if there were differences in the proliferative potential of normotrophic and hypertrophic silica type II cells, type II cells isolated 14 d after silica instillation were separated into groups of increasing cell size by centrifugal elutriation. The plating efficiency and alveolar type II cell purity (<88%) were the same in all groups of elutriated cells. The hypertrophic type II cells had a higher level of thymidine incorporation (22.0±2.8 × 10³ dpm/well) than the normotrophic type II cells (11.1±0.7 × 10³ dpm/well, p<0.01). Each group of elutriated type II cells was capable of responding to the same level of stimulated DNA synthesis in the presence of insulin, epidermal growth factor, and cholera toxin. The autoradiographic labeling index increased.

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