Mechanisms of Alveolar Fibrosis Following Acute Lung Injury*

Presence of Angiogenesis Bioactivity in the Lower Respiratory Tract

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Survival after acute lung injury depends on prompt alveolar repair, a process frequently subverted by acute intraalveolar fibrosis in which exuberant growth of granulation tissue obliterates the alveolar air space. To gain insight into this process, we used immunohistochemical methods to examine the cellular populations present in the alveolus of lung specimens from 6 patients with acute lung injury. In addition to myofibroblasts, capillaries (factor VIII®) were present in the developing granulation tissue, indicating that angiogenesis was part of the pathologic process. We therefore hypothesized that angiogenesis factors would be present on the alveolar epithelial surface of the lung after acute lung injury, providing a pathogenic link to the new vessel growth observed.

To obtain samples from the alveolar epithelial surface, bronchoalveolar lavage (BAL) was performed in 24 patients with acute lung injury. Recovered fluid was examined for its ability to induce endothelial cell chemotaxis, an indication of angiogenesis bioactivity. With both large-vessel and microvascular endothelial cells examined, BAL fluid from 16 of the 24 patients had chemotactic bioactivity, while BAL fluid from all 8 control subjects was negative (p<0.05). To assess the formation of capillary networks in vitro, concentrated BAL fluid from the 2 patients with the greatest amount of in vitro bioactivity was implanted into the avascular cornea of rabbits. Both samples induced capillary growth comparable to that induced by the positive control agent, basic fibroblast growth factor (bFGF). Partial characterization of the angiogenesis bioactivity showed that it was trypsin sensitive, acid unstable (pH=3), and lipid inextractable—all observations suggesting that it was a peptide. Heparin-Sepharose chromatography disclosed that 30% of the activity was bound (0.05 M NaCl, pH=7.40; immunoblot © for bFGF), while 70% was nonbinding and thus distinct from FGF. Anion exchange chromatography (DEAE-S) of the active portion not binding to heparin resolved it into two fractions: 50% nonbinding and 50% binding (0.05 M NaCl, pH=7.40). Further anion exchange analysis of the DEAE-S binding activity using Mono Q and a linear salt gradient (0.125-0.300 M NaCl, 0.02 M Tris, pH=7.40) revealed a single peak of bioactivity at 0.260 M NaCl.

Thus, the fibrotic alveolar air space of patients with severe acute lung injury contains bFGF reactive material as well as a peptidelike angiogenesis bioactivity. Angiogenesis factors within the lower respiratory tract of patients with acute lung injury may provide signals for capillary migration and replication into the air space, furnishing a nutrient blood supply for the rapid ingrowth and replication of myofibroblasts as they ablate the gas exchange surface.

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