Bench to bedside: targeting coagulation and fibrinolysis in acute lung injury

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The interplay between coagulation and inflammation has become an increasingly important area of focus in both experimental and clinical research in acute lung injury (13, 14, 23, 29, 33). At the Experimental Biology 2006 Meeting in San Francisco, California, a Symposium in the Translational Research Track was devoted to coagulation and fibrinolysis as potential therapeutic targets in acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS). Evidence summarized below suggests that activated coagulation and impaired fibrinolysis are important contributors to ALI and are regulated locally in the lung in addition to being influenced by systemic changes in coagulation. The presentations review the protective effects of coagulation blockade in animal models, as well as some negative studies. These apparently conflicting results demonstrate a need for continued careful dissection of coagulation pathways in a variety of models, where outcomes of coagulation activation may depend on the presence of cofactor-dependent signaling on cell surfaces as well as on the overall balance of fibrin generation and dissolution.

Role of protease-activated receptors in sepsis and ALI

Septicemia is commonly associated with disseminated intravascular coagulation (15). Anticoagulants reduce inflammation and improve outcome in animal models of severe sepsis, consistent with a causal role for the activation of coagulation in disease progression (11, 16, 25). Lack of correlation between inhibition of microvascular thrombosis and outcome in the same models may argue that coagulation factors contribute to death by directly enhancing the inflammatory response to endotoxin (18). As the main cellular mediators of potentially proinflammatory responses to coagulation proteases (5, 9, 10), protease-activated receptors (PARs) would be well positioned to drive coagulation-induced inflammation (Fig. 1).

To address the role of PARs in the systemic inflammatory response, we asked whether PAR deficiency would be protective in a mouse model of endotoxemia. To our surprise, neither single nor combined deficiencies in PARs offered protection from the inflammatory or lethal responses to endotoxin (3). Concordant with undiminished cytokine responses to endotoxin in PAR-deficient animals, intravenous infusion of thrombin or PAR agonist peptides did not elicit cytokine production in wild-type mice. Interestingly, PAR4 deficiency, although rendering mouse platelets insensitive to thrombin (22), did not significantly affect platelet consumption in this model. This raises the question of how platelets are consumed in response to endotoxin; intriguing new reports may suggest a direct role for platelet Toll-like receptors and perhaps neutrophils in this process (1). Platelets express functional Toll-like receptor-4 (2). Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-α production in vivo (2). Questioning any contribution of coagulation factors to disease progression in our model, we went on to show that transient thrombin inhibition and fibrinogen deficiency also did not improve survival. Platelet deficiency and antibody-induced thrombocytopenia rendered mice more sensitive to endotoxin, suggesting a net protective role for platelets during disease progression. In conclusion, our results from a mouse model of endotoxemia do not support a role for protease signaling or microvascular thrombosis in lethal responses to endotoxin.

Tissue factor blockade in baboon models of sepsis and ALI

An important characteristic of ALI is activation of coagulation and fibrin deposition in the lung (13, 14). Tissue factor (TF) binds to factor VIIa (FVIIa) and activates factor X (FX)
to FXa, forming a transient ternary complex, TF-FVIIa-FXa to activate coagulation. This complex also has an inflammatory function by presenting FVIIa and FXa to PARs on the cell surface. Cleavage of PARs initiates the inflammatory rather than coagulant functions of TF, offering discrete signaling opportunities, including upregulation of cytokine gene expression (4, 19–21, 33).

In a series of baboon studies, we hypothesized that TF-dependent initiation of coagulation is integral to the pathogenesis of ALI. The hypothesis was tested in baboons with Escherichia coli sepsis; in this model the baboons develop pulmonary and renal failure similar to humans with sepsis-induced ARDS (6, 31, 32). We blocked the TF complex at sequential steps in its assembly, using a competitive inhibitor of FVIIa binding (FVIIai), an antibody to the FX binding site on TF, and tissue factor pathway inhibitor (TFPI). TF blockade was protective when administered at the onset of sepsis and when given as rescue therapy, decreasing systemic inflammation, preventing fibrinogen depletion, and attenuating injury to lung, kidney, and other organs during E. coli sepsis. The data suggest that TF-dependent regulation of inflammation in the lung is unique and that discrete events in the pathogenesis of sepsis-induced organ failure are regulated at sequential levels of assembly of the TF complex. Although each strategy attenuated inflammation and improved pulmonary and renal function and histology (Fig. 2), study endpoints...
were not uniformly affected by different TF inhibition strategies and we found disparate effects on pulmonary and systemic vascular endpoints (6, 32). Possible explanations include incomplete or localized inhibition of downstream thrombin generation by the TF antibody or unrecognized FVIIa signaling through alternative PAR receptors in the lung.

One reason TF signaling may be especially important in lung injury is that a high level of TF expression relative to other organs may convey receptor specificity, localizing coagulation events (20, 21). The effects of PAR1 and PAR2 activation are complex and a requirement for a coreceptor, e.g., TF, is likely to be a key determinant of outcome (17). Our studies do not exclude a role for coagulation effects downstream of the TF complex, but because some downstream FXa-mediated events depend on binding rather than protease activity, pharmacologic strategies that prevent activation of FX may be more protective than those targeting the proteolytically active form, such as TFPI (33). Finally, our results suggest that organ protection correlates with degree of TF inhibition and therapeutic effect on coagulopathy. This has significant implications for translational coagulation strategies, where identifying surrogate measures to predict clinical efficacy is critical.

COAGULATION IN THE LUNG IN PNEUMONIA AND DURING MECHANICAL VENTILATION: LESSONS FROM ANIMALS, PATIENTS, AND HEALTHY VOLUNTEERS

Alveolar fibrin deposition is an important feature of ALI/ARDS (12) and pneumonia (12, 24). The mechanisms that contribute to disturbed alveolar fibrin turnover are increased fibrin production caused by localized TF-mediated thrombin generation and depression of bronchoalveolar urokinase plasminogen activator-mediated fibrinolysis caused by the increase of plasminogen activator inhibitors. Simultaneously, levels of endogenous inhibitors of coagulation such as activated protein C are low because of defective production and/or increased breakdown (7). These effects on pulmonary coagulation and fibrinolysis are regulated by various proinflammatory cytokines and are similar to those found in the intravascular spaces during severe systemic inflammation. Some studies also suggest that pulmonary coagulopathy is a feature of ventilator-

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3 Presented by Marcus Schultz.
associated lung injury (23). Indeed, animal studies from our laboratory of ventilator-induced lung injury showed attenuation of activation of coagulation and prevention of inhibition of fibrinolysis with lung protective settings of mechanical ventilation, depending on lower levels of plasminogen activator inhibitor type 1 (Fig. 3). In addition, noninjurious mechanical ventilator settings (with the use of lower tidal volumes) during general anesthesia for surgery largely prevented pulmonary coagulopathy compared with traditional ventilator settings (8). As yet, no information is available on changes in local levels of endogenous inhibitors of coagulation with mechanical ventilation.

Recent studies have demonstrated the beneficial effect of anticoagulant therapy in sepsis. Theoretical considerations suggest that anticoagulant therapy will benefit patients with primary lung pathology including ventilator-associated lung injury, but clinical studies are needed to examine this hypothesis before such therapy is to be advocated as a standard of care in critically ill patients. Presently, several trials are testing the hypothesis that anticoagulant therapy benefits patients with ALI: one study compares TFPI with placebo in patients with pneumonia. Two other studies compare recombinant human activated protein C with placebo in ALI/ARDS patients and patients with infectious ALI, respectively. No studies have been done yet on anticoagulant therapy to prevent ventilator-associated lung injury.

**THE PROTEIN C PATHWAY IN ALI: NEW THERAPEUTIC TARGET**

The role of the protein C system in modulating coagulation in the acutely injured lung is not well understood (29). To characterize the status of the protein C system in the intra-alveolar compartment in patients with clinical ALI/ARDS, we measured levels of protein C, thrombomodulin, and the endothelial protein C receptor (EPCR) in undiluted pulmonary edema fluid and plasma from patients with ALI/ARDS (30). Protein C levels were low in the plasma of patients with ALI/ARDS compared with normal controls regardless of the underlying etiology of lung injury (Fig. 4A), and lower levels were associated with adverse clinical outcomes. Intra-alveolar levels of protein C were slightly lower than simultaneous plasma levels. Thrombomodulin, the cell surface molecule that proteolytically activates protein C, was present in very high levels in the pulmonary edema fluid, much higher than simultaneous plasma levels, suggesting an intra-alveolar source (Fig. 4B). Levels of soluble EPCR were also higher in the edema fluid than the plasma, again suggesting an intra-alveolar source (28).

To further investigate the intra-alveolar source of thrombomodulin and EPCR in the acutely injured lung, we hypothesized that the alveolar epithelium can express thrombomodulin and EPCR and can activate protein C. We further hypothesized that both thrombomodulin and EPCR are shed from the alveolar epithelium in response to a proinflammatory stimulus and contribute to the high intra-alveolar levels of these proteins. In a series of in vitro experiments using both A549 cells and primary isolates of alveolar epithelial type II cells, we have shown that human alveolar epithelial cells can activate protein C; this ability to activate protein C is diminished in response to exposure to proinflammatory cytokines in concert with metalloproteolytic shedding of thrombomodulin and EPCR (27, 28).

Together, these findings suggest that the alveolar epithelium may play an important role in modulating intra-alveolar fibrin deposition through modulation of levels of the endogenous anticoagulant activated protein C. Inflammation or injury to the alveolar epithelium shifts its phenotype from anticoagulant (able to activate protein C) to procoagulant (unable to activate protein C). Therapies aimed at restoring normal intra-alveolar levels of protein C or activated protein C might be of therapeutic benefit in patients with clinical ALI/ARDS. Interestingly, in one study of the effects of intravenous recombinant activated protein C on the response to local instillation of endotoxin in the lungs of normal volunteers, intra-alveolar levels of activated protein C were very high even 2 h after discontinuation of the intravenous infusion (26). This finding suggests that systemic administration of recombinant activated protein C leads to high intra-alveolar levels and that activated protein C has a sustained half-life in the lung, a finding that may be of clinical value if exogenous administration of activated protein C is of therapeutic value in patients with ALI/ARDS.

**CONCLUSIONS**

In summary, the findings presented at this symposium, ranging from mouse to baboon, to human studies, indicate that alterations in coagulation and fibrinolysis may be of major pathogenetic importance in ALI/ARDS and other inflammatory conditions in the lung including pneumonia, sepsis, and ventilator-induced lung injury. The evolution of procoagulant and antifibrinolytic responses to infection in the lung and other organs may represent a protective response of the host that could have value in confining infection and its consequences to a single organ. However, in critically ill patients with severe lung injury, this response may become deleterious. Therefore, therapies targeted at activation of coagulation through the extrinsic coagulation cascade, including TF and modulation of coagulation through the protein C system, have the potential to favorably impact clinical ALI/ARDS. The results of ongoing clinical trials of these strategies will be of considerable interest.

**REFERENCES**


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*Presented by Lorraine B. Ware.*