REVIEW

Coagulation and fibrinolysis in human acute lung injury – New therapeutic targets?

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Abstract. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common, life-threatening causes of acute respiratory failure that arise from a variety of local and systemic insults. The need for new specific therapies has led a number of investigators to examine the role of altered coagulation and fibrinolysis in the pathogenesis of ALI/ARDS. This review summarizes our current understanding of coagulation and fibrinolysis in human ALI/ARDS with an emphasis on pathways that could be potential therapeutic targets including the tissue factor pathway, the protein C pathway and modulation of fibrinolysis via plasminogen activator inhibitor-1. The available data suggest that clinical ALI and ARDS are characterized by profound alterations in both systemic and intra-alveolar coagulation and fibrinolysis. Fibrin deposition in the airspaces and lung microvasculature likely results from both activation of the coagulation cascade and impaired fibrinolysis, triggered by inflammation. Modulation of fibrin deposition in the lung through targeting activation and modulation of coagulation as well as fibrinolysis may be an important therapeutic target in clinical ALI/ARDS that deserves further exploration. (Keio J Med 54 (3): 142–149, September 2005)

Key words: fibrin deposition, thrombosis, activated protein C, tissue factor, plasminogen activator inhibitor

Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common, life-threatening causes of acute respiratory failure that arise from a variety of local and systemic insults.[1] Despite some improvement in mortality with a lung-protective ventilator strategy,[2] both morbidity[3] and mortality remain high.[4] The need for new specific therapies has led a number of investigators to examine the role of altered coagulation and fibrinolysis in the pathogenesis of ALI/ARDS. Although the presence of intra-alveolar fibrin lining the denuded alveolar surface has long been recognized as a pathological hallmark of ALI/ARDS,[5] the mechanisms that govern fibrin deposition in the acutely injured lung are poorly understood. This review will summarize our current understanding of coagulation and fibrinolysis in human ALI/ARDS with an emphasis on pathways that could be potential therapeutic targets. These pathways are summarized in Fig. 1 and include the tissue factor pathway, the protein C pathway and modulation of fibrinolysis via plasminogen activator inhibitor-1 (PAI-1).

Tissue Factor and Tissue Factor Pathway Inhibitor

Initiation of coagulation through the extrinsic pathway is likely the predominant mechanism of activation of coagulation in the lung in ALI/ARDS. Tissue factor (TF) is a potent initiator of the extrinsic coagulation cascade. TF is a membrane bound protein that when complexed to factor VIIa converts factor X to Xa leading to the downstream generation of thrombin and conversion of fibrinogen to insoluble fibrin (Fig. 1). In the normal human lung, both alveolar macrophages and alveolar epithelial cells stain positive for TF[6] and in culture, both alveolar macrophages and alveolar epithelial cells have TF activity.[7] Initiation of coagulation through the TF pathway is modulated by the balance between TF and tissue factor pathway inhibitor (TFPI),

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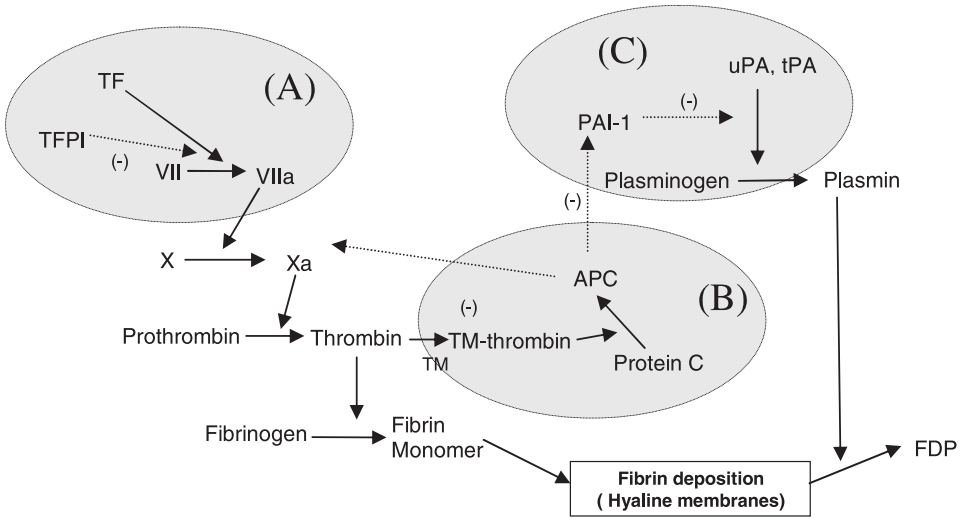

Fig. 1 Summary of coagulation and fibrinolytic pathways that may be therapeutic targets in clinical ALI/ARDS. A. Activation of coagulation by Tissue Factor (TF). TF initiates the extrinsic pathway of coagulation by acting as a cofactor for the activation of factor VII to VIIa. The factor VIIa-TF complex activates factor X to Xa which catalyzes the conversion of prothrombin to thrombin, leading to fibrin deposition. Tissue factor pathway inhibitor (TFPI) can bind to the TF:VIIa:Xa complex thus inhibiting downstream thrombin formation and fibrin deposition. **B. The protein C pathway.** The protein C pathway is a major endogenous inhibitor of coagulation. Once generated, thrombin binds to the cell surface receptor thrombomodulin (TM), activating the zymogen protein C to the activated protein C (APC). APC (along with protein S) inhibits Xa and thrombin formation through the inactivation of factors V and VIII (not shown). **C. Fibrinolysis.** Fibrinolysis is initiated by urokinase (uPA) and tissue plasminogen activator (tPA) both of which cleave plasmin from plasminogen. Plasmin degrades fibrin into fibrin degradation products (FDP). Plasminogen activator inhibitor-1 (PAI-1) depresses fibrinolysis by inhibiting uPA and tPA. PAI-1 can be neutralized by APC. The net result of upregulated coagulation and downregulated fibrinolysis in the acutely injured lung is fibrin deposition.

an endogenous inhibitor of TF. TFPI is secreted by the vascular endothelium and circulates in the plasma and bound to the surface of platelets.[8] TFPI can prevent the activation of X to Xa through binding to the TF:VIIa:X complex thereby inhibiting downstream formation of thrombin and deposition of fibrin. Thus, the balance between TF and TFPI levels in the lung may be an important determinant of intra-alveolar fibrin deposition.

An important role for TF in initiating intra-alveolar coagulation in clinical ALI/ARDS was first reported by Idell.[9] In serial bronchoalveolar lavage (BAL) samples from patients with ARDS, procoagulant activity was upregulated by three days after the onset of ARDS and resolved by seven days.[9] TF activity was primarily responsible for the procoagulant activity in the BAL since almost all of the procoagulant activity was blocked by an antibody to TF. TF-dependent procoagulant activity in the alveolar compartment has also been reported in patients at risk for ALI/ARDS.[10] Upregulation of TF-dependent procoagulant activity is not completely specific for ALI/ARDS, however. Gunther *et al.* found increases in TF-dependent procoagulant activity in the BAL of patients with pneumonia even in the absence of respiratory failure or ARDS.[11] Upregulation of the TF pathway also occurs systemically in clinical ALI/ARDS. Circulating levels of TF were increased in patients with ARDS but not in patients at risk for ARDS or without ARDS.[12]

Levels of TFPI, the endogenous TF inhibitor, have also been measured in the plasma and BAL of patients with ALI/ARDS. Sabharwal *et al.*[13] reported a modest increase in levels of TFPI compared to normals in the plasma of patients at risk (1.3-fold) and those with established ARDS (1.8-fold) while Gando *et al.*[14] found no difference in TFPI levels between control, at risk patients or patients with ARDS. By contrast, substantial increases in TFPI were seen in the BAL in those at risk (7-fold) and with established ARDS (20-fold) compared to normals.[13] TF levels were not measured in that study. Thus, changes in TFPI levels appear to be more pronounced in the alveolar compartment in ALI/ARDS than in the plasma. The effect of the balance of TF and TFPI on overall procoagulant activity has not been studied in ALI/ARDS but has been reported in other lung diseases. The procoagulant activity of BAL was increased in patients with bacterial or *Pneumocystis carinii* pneumonia or interstitial lung disease compared to controls but TFPI levels were similar to controls[15] suggesting that increases in TF activity were not balanced by comparable increases in TFPI. In patients with idiopathic pulmonary fibrosis, both TF and TFPI levels were increased in the BAL and correlated with severity of disease; however, the procoagulant activity

of the BAL was increased compared to controls, again demonstrating that the increases in TF were not balanced by increases in TFPI.[16]

The fundamental role of TF in initiation of coagulation combined with the systemic and intra-pulmonary abnormalities of TF and TFPI in clinical ALI/ARDS makes the TF pathway an attractive target for therapeutic intervention. Blockade of TF activity either prior to or in the early stages of ALI with TFPI is protective in several animal models of sepsis and ALI.[17,18] However, human studies to date have been disappointing. A recent phase III trial of recombinant TFPI in sepsis, the most common cause of ALI/ARDS, had no effect on mortality[19] despite a prior encouraging phase II study.[20] A site inactivated form of factor VIIa (factor VIIai) that binds to and inhibits TF activity has been developed as an anticoagulant. In a baboon model of sepsis factor VIIai was protective.[21–23] A clinical trial in patients with ALI/ARDS is currently underway.

Protein C and Thrombomodulin

The protein C pathway is an important endogenous regulator of coagulation and fibrinolysis. Synthesized by the liver, protein C is a vitamin K-dependent glycoprotein that circulates as an inactive zymogen.[24] It is transformed to its active form on the cell surface by the TM-thrombin complex. Another cell surface protein, the endothelial cell protein C receptor (EPCR) can further enhance protein C activation by binding to the TM-thrombin complex.[25] Activated protein C proteolytically destroys coagulation factors Va and VIIIa, suppressing further thrombin formation (Fig. 1).[26,27] Activated protein C has anti-inflammatory and anti-apoptotic effects in addition to its anti-coagulant properties that include suppression of production of pro-inflammatory cytokines, inhibition of leukocyte attachment to the endothelium,[28,29] and inhibition of p53 mediated apoptosis.[30] Activated protein C also can neutralize PAI-1, thereby promoting fibrinolysis.[31] Thus, modulation of the protein C pathway could have key regulatory effects on coagulation, fibrinolysis and inflammation in ALI/ARDS.

The majority of studies of the protein C pathway have focused on the endothelium as the primary site for activation of protein C. However, recent studies suggest that the lung epithelium can actively modulate the protein C pathway. Cultured human airway epithelial cells express protein C, EPCR and thrombomodulin and protein C was activated by these cells in the presence of thrombin.[32] Exposure to inflammatory cytokines downregulated the activation of protein C.[32] Alveolar epithelial cells also may modulate the protein C pathway. We reported that both a human alveolar epithelial cell line (A549) and primary isolates of human alveolar epithelial type II cells release thrombomodulin in response to stimulation by a mixture of pro-inflammatory cytokines or hydrogen peroxide (Fig. 2).[33] Thus, the alveolar epithelium may modulate intra-alveolar coagulation and inflammation through the protein C pathway similar to the well-described role of the vascular endothelium in modulating this pathway.

In humans, most of the clinical studies of the protein C pathway have been in patients with sepsis. In 70 patients with severe sepsis[34] plasma protein C levels were markedly reduced compared to normal levels and lower levels were associated with higher mortality, ventilator dependency and a higher prevalence of ARDS. Patients with severe sepsis also vary in their ability to generate activated protein C.[35] In a study of 32 patients with severe sepsis, activated protein C levels were higher in survivors although in that study there was no difference in protein C levels between survivors and nonsurvivors. Thrombomodulin levels in the plasma are elevated in patients with sepsis, and high levels correlate with the development of multiple organ failure.[36,37] Levels of soluble EPCR are also high in patients with sepsis.[38] However, there was no correlation between the soluble EPCR levels and clinical outcomes including multiple organ failure score or survival.[38]

There have been relatively few studies of the protein C pathway in patients with ALI/ARDS. In a single center study, plasma protein C levels were lower in ALI/ARDS compared with normal controls even in the absence of sepsis.[33] Levels of protein C in the alveolar compartment were even lower than plasma levels and lower levels of both plasma and edema fluid protein C were associated with worse clinical outcomes.[33] Like patients with sepsis, patients with ALI/ARDS have high circulating levels of thrombomodulin. In addition, thrombomodulin levels in the alveolar compartment in ALI/ARDS were 2-fold higher than simultaneous plasma samples, suggesting an intra-alveolar source (Fig. 3). High thrombomodulin levels in the plasma or alveolar compartment were associated with worse clinical outcomes.[33] Thus, abnormalities of the protein C pathway in ALI/ARDS are similar to abnormalities in sepsis and are strongly associated with adverse outcomes.

The observation that protein C and activated protein C levels are low in patients with severe sepsis led to a phase III randomized, multicenter clinical trial of recombinant human activated protein C (drotecogin-alfa activated) in patients with severe sepsis and at least one organ failure. In this landmark trial, administration of recombinant human activated protein C significantly improved survival compared to placebo. The absolute risk reduction in mortality at 28 days was 6.1%.[39] This study was not designed to specifically evaluate the role

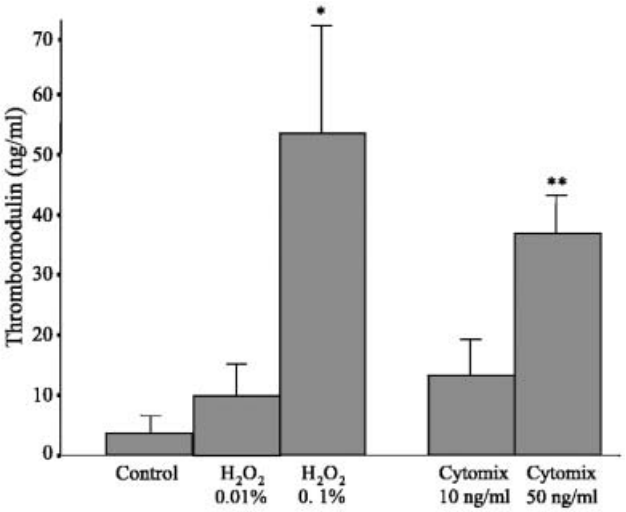

Fig. 2 Alveolar epithelial cells release soluble thrombomodulin in response to injurious stimuli. Confluent monolayers of human alveolar epithelial type II-like cells (A549 cells) were exposed for 6 hours to varying concentrations of H_2O_2 or cytomix (equal amounts of TNF-α, IL-1β, IFN-γ), and conditioned media was collected for assay of thrombomodulin by ELISA. *$p < 0.01$ compared with 0.01% H_2O_2 or control. **$p < 0.01$ compared with 10 ng/ml cytomix or control. Reproduced with permission from *American Journal of Physiology Lung Cellular and Molecular Physiology.*[33]

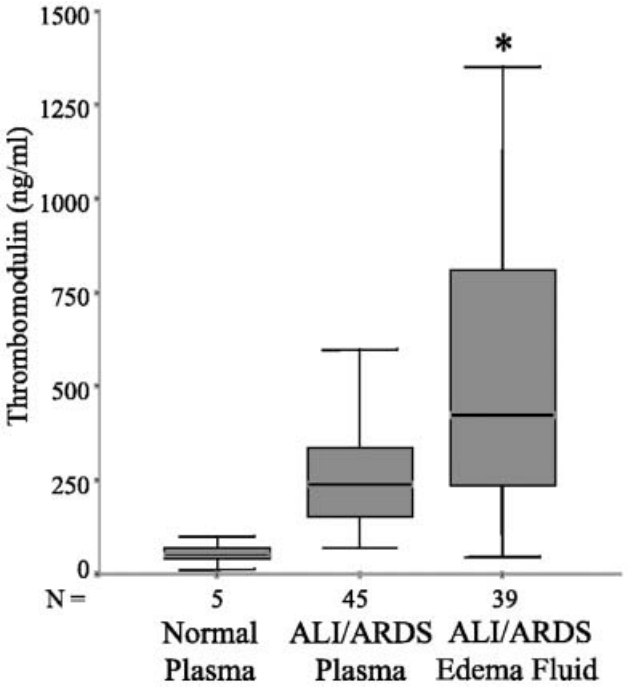

Fig. 3 Boxplot summary of thrombomodulin levels in normal plasma and pulmonary edema fluid from 45 patients with ALI or ARDS. Box encompasses 25th to 75th percentile, error bars encompass 10th to 90th percentile, and horizontal bar shows the median. *$p = 0.001$ compared with normal plasma or ALI/ARDS plasma. Reproduced with permission from *American Journal of Physiology Lung Cellular and Molecular Physiology.*[33]

of activated protein C in ALI/ARDS. However, a retrospective analysis showed that in the group of patients with respiratory system dysfunction, many of whom likely had ALI/ARDS, the time to resolution of respiratory dysfunction was significantly shorter in the treated group compared to the placebo group.[40] A multicenter phase II trial of recombinant activated protein C in ALI and ARDS is currently underway. Interestingly, in a human model of pulmonary inflammation produced by instillation of endotoxin into the distal airspaces, intravenous administration of recombinant activated protein C produce sustained elevations in BAL levels of activated protein C[41] that were accompanied by reduced leukocyte accumulation in the airspaces and reductions in neutrophil chemotaxis, independent of pulmonary cytokine or chemokine release.[42] These findings suggest that in addition to its anticoagulant effects, systemically administered activated protein C may have potent anti-inflammatory effects in the distal airspaces that are mediated through modulation of neutrophil function.

Plasminogen Activators and Inhibitors

The balance between activation of coagulation and activation of fibrinolysis is likely an important determinant of the amount and duration of fibrin deposition in the injured lung. Plasminogen activator (PA) and plasminogen activator inhibitor-1 (PAI-1) regulate fibrinolysis, the dissolution of fibrin clots, through modulation of the conversion of plasminogen to plasmin, a major fibrinolytic enzyme (Fig. 1). There are two forms of plasminogen activator (PA), urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA). uPA is a cell surface protein that is responsible for activating fibrinolysis at the tissue level[43] while tPA is a soluble protein that activates intravascular fibrinolysis.[44] Plasminogen activator inhibitor-1 (PAI-1) is a major endogenous inhibitor of both uPA and tPA.

There are multiple cellular sources of PA and PAI-1 that may be relevant to human ALI/ARDS. Unstimulated alveolar macrophages are pro-fibrinolytic. Primary isolates of human alveolar macrophages have PA activity and degrade a fibrin matrix in the presence of plasminogen.[45] By contrast, stimulated alveolar macrophages are anti-fibrinolytic. Human alveolar macrophages exposed to endotoxin inhibit fibrinolysis through an increase in PAI-1 activity.[46,47] Increased PAI-1 activity has also been observed in macrophages isolated from patients with lung disease. For example, alveolar macrophages from patients with idiopathic pulmonary fibrosis (IPF) have increased PAI-1 staining compared to normal controls[48] and alveolar macrophages from

patients with ARDS have increased PAI-1 mRNA as demonstrated by in situ hybridization.[47] Overall, these findings suggest that when the lung is exposed to injurious stimuli there is a decrease in the fibrinolytic activity of alveolar macrophages through decreased PA activity and increased PAI-1 activity.

Like macrophages, human lung microvascular endothelial cells can secrete both PA[49] and PAI-1.[50] Proinflammatory stimuli increase PA secretion several-fold[49,50] but the net effect on fibrinolysis is unclear. In one study of human lung microvascular endothelial cells isolated from patients with ARDS, the cells from ARDS patients constitutively expressed more PAI-1 than controls and had a lower fibrinolytic potential as measured by the PA/PAI-1 ratio.[51] Although the data are not completely concordant, the studies from ARDS patients suggest that similar to alveolar macrophages, the pulmonary microvascular endothelium in its basal state favors fibrinolysis. Injurious stimuli shifts the balance away from fibrinolysis.

The lung epithelium can also express both plasminogen activators and inhibitors and is thus capable of regulating intra-alveolar fibrinolysis. However, there are relatively few studies of human lung epithelial cells. Primary isolates of rat alveolar epithelial cells express both PA and PAI-1; levels increase with time in culture as the cells differentiate from a type II cell phenotype to a type I phenotype.[52,53] Exposure of the cells to lipopolysaccharide or TNF-α, increased PAI-1 and uPA suggesting that inflammatory mediators modulate the fibrinolytic capacity of alveolar epithelial cells. A human alveolar epithelial type II cell line (A549) had increases in uPA mRNA, protein, and activity following exposure to IL-1β and TNF-α, but unlike rat alveolar epithelial cells, there was no change in PAI-1 expression.[54] By contrast, freshly isolated alveolar type I cells do express PAI-1 mRNA.[55] Thus, PAI-1 expression may be a phenotype of alveolar type I cells and not type II cells, similar to the findings with rat alveolar epithelial cells. Taken together, the *in vitro* data suggests that the lung epithelium is probably capable of modulating intra-alveolar fibrinolysis through expression of PA, and PAI-1.

There are only a few studies that address fibrinolysis in patients with ALI/ARDS. In a study comparing normal controls to four groups of patients (patients with pneumonia who were spontaneously breathing, patients with pneumonia requiring mechanical ventilation, patients with ARDS without pneumonia and patients with ARDS with pneumonia) there was reduced fibrinolytic capacity and an increase in uPA in all groups compared to control as well as an increase in PAI-1 activity in all groups except those with pneumonia who were spontaneously breathing.[11] Prabhakaran *et al.* measured PAI-1 antigen levels in plasma and simultaneous undiluted

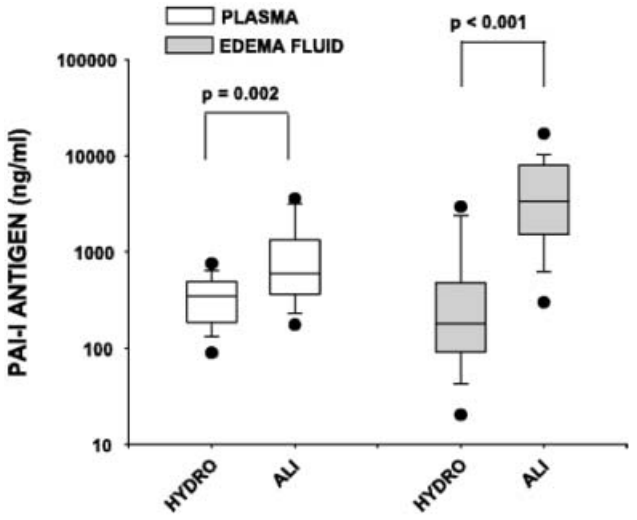

Fig. 4 Plasma and edema fluid PAI-1 antigen in patients with acute lung injury (ALI) and hydrostatic pulmonary edema (HYDRO). PAI-1 values were measured in simultaneously drawn plasma (open boxes) and edema fluid (filled boxes) by ELISA from patients with clinical ALI or hydrostatic edema. Data are plotted in box plot format (median, 25–75%) on a log scale and compared using Mann-Whitney's *U*-test. P values are as indicated. Reproduced with permission from *American Journal of Physiology Lung Cellular and Molecular Physiology.*[56]

pulmonary edema fluid from patients with ALI/ARDS and compared them to control patients with pulmonary edema due to hydrostatic causes.[56] Levels of PAI-1 were higher in both the edema fluid and plasma of patients with ALI/ARDS compared to the control patients (Fig. 4) and correlated with mortality in patients with ALI/ARDS. In patients with ALI/ARDS, PAI-1 levels were substantially higher in the edema fluid than the plasma suggesting an intra-alveolar source of PAI-1. In other studies that only examined plasma levels, patients with ARDS and those at risk for ARDS had higher circulating levels of PAI-1 than controls.[57,58] However, plasma levels did not correlate with lung injury score, pulmonary microvascular permeability, or clinical outcome.[58] Taken together, these findings suggest that alterations in the intra-alveolar fibrinolytic environment may have more impact on outcomes in ALI/ARDS than alterations in systemic fibrinolytic pathways. Although there are no studies to date in patients with ALI/ARDS, polymorphisms in the promoter region of PAI-1 have been studied in two populations of patients at risk for ALI/ARDS, patients with meningococcal septicemia and patients with severe trauma at risk for ALI/ARDS.[59] Deletions in the promoter region lead to impaired fibrinolytic ability and are associated with disease severity and outcome in meningococcal disease[60–62] and with susceptibility to sepsis and multi-organ failure in patients with severe trauma.[63]

To summarize, there is substantial evidence that the fibrinolytic system is profoundly altered in patients with ALI/ARDS, both systemically and in the alveolar compartment. Upregulation of PAI-1, the major inhibitor of fibrinolysis, appears to play a primary role in the shift from pro-fibrinolytic to anti-fibrinolytic phenotypes in a variety of cell types including endothelium, lung epithelium and alveolar macrophages. A variety of strategies are being explored to develop inhibitors of PAI-1[64] that might be of therapeutic use in ALI/ARDS or other diseases associated with high levels of PAI-1 such as cardiovascular disease. However, no inhibitor of PAI-1 is currently clinically available.

Conclusions

Clinical ALI and ARDS are characterized by profound alterations in both systemic and intra-alveolar coagulation and fibrinolysis. Fibrin deposition in the airspaces and the lung microvasculature likely results from inflammation-induced activation of the coagulation cascade and impairment of fibrinolysis. Activation of coagulation with resultant fibrin deposition also has proinflammatory consequences, serving to further amplify the inflammatory cascade. Current evidence from human studies suggests that in the acutely injured lung there is activation of tissue factor, downregulation of protein C activation, and increased production of PAI-1. Together these abnormalities shift the intra-alveolar environment from anticoagulant and profibrinolytic to procoagulant and antifibrinolytic. Modulation of fibrin deposition in the lung through targeting coagulation and fibrinolysis may be an important therapeutic target in clinical ALI/ARDS. Because the lung epithelium appears to be an important site for modulation of intra-alveolar fibrin deposition, it represents a unique therapeutic target. Targeting the lung epithelium with inhaled therapies to modulate intra-alveolar fibrin deposition might avoid the deleterious side effects of systemic therapies and deserves further study.

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