Current view on alveolar coagulation and fibrinolysis in acute inflammatory and chronic interstitial lung diseases

Malgorzata Wygrecka, Ewa Jablonska, Andreas Guenther, Klaus T. Preissner, Philipp Markart

Departments of 1 Biochemistry, and 2 Internal Medicine, Faculty of Medicine, University of Giessen Lung Center, Giessen, Germany

Summary

Acute inflammatory and chronic interstitial lung diseases are characterized by excessive and persistent fibrin deposition in the lung. Intraalveolar fibrin accumulation, observed under these conditions, arises from a leakage of plasma proteins (including fibrinogen) into the alveolar space in combination with a disbalance of alveolar haemostasis. Tissue factor in association with factor VIIa and inhibition of urokinase by plasminogen activator inhibitor-1 are major factors that are responsible for the procoagulant and antifibrinolytic state. In addition, in acute respiratory distress syndrome (ARDS) patients, factor VII-activating protease and extracellular RNA, which may be released into the extracellular milieu from damaged cells during lung injury, may contribute to fibrin formation as well. Fibrin itself can increase vascular permeability, influence the expression of inflammatory mediators and alter the migration and proliferation of various cell types. Additionally, fibrin may inactivate pulmonary surfactant and provide a matrix on which fibroblasts can migrate and produce collagen. Furthermore, cellular activities of haemostatic proteases may also contribute to proinflammatory and fibrotic processes in the lung. The application of coagulation inhibitors, like tissue factor pathway inhibitor, active site-inactivated factor VIIa, activated protein C, antithrombin, heparin or hirudin turned out to be beneficial in experimental models of acute and chronic lung injury. However, the ability of anticoagulant and profibrinolytic agents to improve clinical outcome remains to be elucidated. In the current article, the role of the alveolar coagulation and fibrinolysis systems in acute inflammatory and chronic interstitial lung diseases is discussed with regard to pathomechanisms and modalities of intervention.

Keywords

Coagulation factors, coagulation inhibitors, fibrinogen / fibrin, haemostasis, lung

Introduction

Alterations of the alveolar haemostatic balance and excessive deposition of intraalveolar fibrin occur in acute inflammatory lung diseases, such as acute respiratory distress syndrome (ARDS [1, 2]). ARDS is initiated by direct injury of the lung (e.g. pneumonia, inhalation of toxic gases, aspiration of gastric contents) or by systemic inflammatory processes (e.g. sepsis). The early exudative phase of ARDS is characterized by i) pulmonary edema formation due to increase in capillary and alveolar epithelial permeability, ii) increased pulmonary vascular resistance due to microembolism / microthrombosis and release of vasoconstrictive mediators with consecutive maldistribution of pulmonary perfusion, iii) surfactant inactivation with consecutive atelectasis formation and maldistribution of pulmonary ventilation, iv) ventilation perfusion mismatch with intrapulmonary shunt flow and severely impaired gas exchange, v) loss of pulmonary compliance. The prognosis of ARDS is worsened by secondary (nosocomial) pneumonia, by the development of sepsis with multiple organ failure, and by the evidence of lung organization and fibrosis. The mortality of ARDS is high despite therapeutic advances: mortality rates range from 30 to 60% in different studies (3, 4).

Similar alterations of the alveolar haemostatic balance favouring extravascular pulmonary fibrin accumulation have been observed in chronic fibrosing interstitial lung diseases with considerable inflammation of the lung parenchyma such as sarcoidosis and hypersensitivity pneumonitis (5–7). Of note, qualitatively and quantitatively comparable changes of alveolar procoagulant and (anti)-fibrinolytic activities with excessive deposition of intraalveolar fibrin have been demonstrated in idiopathic pulmonary fibrosis (IPF [5, 7–10]). IPF represents a spe-
cific form of chronic fibrosing idiopathic interstitial pneumonia (11) and is typically characterized by a progressive and usually fatal course with a medium survival of 2–3 years (12, 13). In contrast to other forms of chronic interstitial lung diseases such as sarcoidosis and hypersensitivity pneumonitis, IPF is characterized by only limited inflammation, and it has been suggested that IPF is mainly a disorder of alveolar epithelial injury, abnormal alveolar wound repair and remodelling (14, 15). These differences between inflammatory and non-inflammatory triggered forms of chronic fibrosing interstitial lung diseases are also reflected by different whole genome expression profiles (16). However, alterations of the alveolar haemostatic balance with subsequent deposition of fibrin seem to be independent of the driving mechanisms (inflammation versus alveolar epithelial injury/abnormal wound repair) and appear to reflect a rather uniform response pattern to acute and chronic lung injury.

Fibrin, haemostatic proteases, and acute inflammatory and chronic interstitial lung diseases

Intraalveolar fibrin accumulation, observed under these conditions, arises from the imbalance between locally produced pro- and anti-coagulant factors, in combination with leakage of plasma proteins (including fibrinogen) into the alveolar space. Increased procoagulant activity in bronchoalveolar lavage (BAL) fluids of patients with acute inflammatory and chronic interstitial lung diseases is accompanied by a decreased fibrinolytic activity (1, 2, 5–10). Identical alterations of the haemostatic balance in the alveolar space have been observed in a wide range of experimental animal models of acute lung injury and pulmonary fibrosis (17–19). In clinical and experimental acute lung injury and lung fibrosis the procoagulant response is mainly attributable to tissue factor (TF) associated with factor VIIa, whereas the decreased fibrinolytic activity is ascribed to inhibition of urokinase type (u-PA) and tissue type (t-PA) plasminogen activators by plasminogen activator inhibitor (PAI)-1 as well as blockage of plasmin by a2-plasmin inhibitor (1, 2, 5–10, 17–19).

Although fibrin is required for reparative processes and normal wound healing, persistent and excessive deposition of extravascular fibrin is thought to contribute to the pathomechanisms of acute inflammatory and chronic interstitial lung diseases in several ways. Fibrin may serve as a reservoir of profibrotic growth factors (20). It incorporates and inactivates pulmonary surfactant, the lung lipoprotein complex critical for maintaining low alveolar surface tension (21). Surfactant dysfunction leads to atelectasis and loss of lung compliance. Moreover, inactivation of the surfactant system, in conjunction with “glueing” of the adjacent alveolar walls by fibrin, is thought to provide a provisional matrix on which fibroblasts proliferate and produce collagen (22). Furthermore, fibrin and fibrin degradation products are potent chemotactic proteins that enhance neutrophil recruitment in the lung (23), increase vascular permeability (24), and influence migration and proliferation of inflammatory cells (25).

In addition to fibrin deposition, the altered alveolar haemostatic system may contribute to the pathogenesis of acute inflammatory and chronic interstitial lung diseases by other mechanisms. For example, plasmin may be involved in proteolytic activation of growth factors, cytokines and matrix metalloproteases as well as in the degradation of matrix glycoproteins. In addition, components of the plasminogen activation system may have biological roles aside from controlling plasmin-mediated proteolysis. In this regard, the u-PA/PAI-1 system may influence cell migration, cell adhesion and cell proliferation (26–30). Furthermore, various coagulation proteases such as thrombin, factor Xa and the TF-factor VIIa complex exhibit cellular activities that may also contribute to fibrotic and inflammatory processes in the lung. Most of these functions are mediated via proteolytic activation of protease activated receptors (PARs). For instance, thrombin and factor Xa stimulate fibroblast proliferation and procollagen production in a PAR-1-dependent manner (31, 32). Additionally, thrombin induces differentiation of normal lung fibroblasts to myofibroblasts via PAR-1 activation (33). Furthermore, activation of PAR-1 by thrombin, factor Xa and by the TF-factor VIIa complex can increase the expression of profibrotic and proinflammatory cytokines (34 –37). A potential role for PAR-1 in pulmonary fibrosis is underscored by the recent finding that PAR-1-deficient mice are protected against bleomycin-induced lung fibrosis (38). Additional evidence for fibrin-independent mechanisms of the altered alveolar haemostatic system involved in inflammatory and fibrotic processes of the lung arise from the recent observation that lung injury and pulmonary fibrosis still develop in fibrinogen-null mice after bleomycin administration (39).

Figure 1 depicts how alterations in the alveolar haemostatic balance may contribute to inflammatory and fibrotic processes of the lung.

The TF-factor VIIa complex and alveolar procoagulant activity in acute inflammatory and chronic interstitial lung diseases

BAL fluid procoagulant activity in patients with acute inflammatory lung diseases such as ARDS and chronic interstitial lung diseases including sarcoidosis, hypersensitivity pneumonitis and IPF is predominantly attributable to TF (1, 5–10). Different inflammatory cytokines and profibrotic growth factors are known to stimulate the expression of TF on several cell types leading to extrinsic coagulation pathway activation outside the bloodstream (40–42). Moreover, animal studies employing inhalative lipopolysacharide (LPS) or bleomycin administration demonstrated that alveolar macrophages and – to a lesser extent – alveolar type II cells are the main sources of locally produced TF in response to lung injury (43, 44). A critical role for TF-initiated activation of the alveolar coagulation cascade in IPF patients is supported by the observation that TF-BAL fluid levels correlate with the deterioration of lung compliance (5). Similarly, in humans who develop ARDS after trauma or sepsis, plasma TF levels correlate with lung injury score, reduction in platelet count and disseminated intravascular coagulation (DIC) score (45). As stated above, in addition to its procoagulant effects, proteases of the TF-initiated coagulation pathway exert cellular functions through PARs that may also contribute to inflammation and fibrosis following tissue injury.
Factor VII-activating protease (FSAP) contributes to increased alveolar procoagulant activity in ARDS patients

Recently published data indicate that a part of the extrinsic coagulation pathway activation observed in the alveolar space of ARDS patients may be triggered by FSAP (46). FSAP circulates as a single-chain zymogen in plasma (47, 48). The proenzyme is converted into the enzymatically active two-chain form by autoactivation, particularly in the presence of polyanions, such as heparin, dextran sulphate, or extracellular RNA (49–52). The precise role of FSAP in different physiological and pathophysiological states is presently not fully understood; however, a dual role of FSAP in haemostasis has been proposed: FSAP is a potent activator of factor VII in vitro (48), but also activates prourokinase and may contribute to plasin formation as well (53, 54). Recently, we detected markedly increased FSAP protein level and activity in plasma and BAL fluids of ARDS subjects. FSAP was found to contribute to factor VIIa generation and to
elevated procoagulant activity in the BAL fluids of these patients (46). Thus, increase in FSAP procoagulant activity may represent a novel pathological mechanism contributing to alterations of the alveolar haemostasis and to extravascular fibrin deposition in ARDS-lungs. Moreover, FSAP expresses activities related to cell migration and proliferation (54, 55). These activities are mediated by the interference with different growth factors and/or FSAP's ability to activate pro-u-PA. Therefore, it is possible that increased levels of FSAP may modulate inflammatory processes in the lungs of ARDS patients via these haemostasis-independent cellular properties. We also detected elevated levels of extracellular RNA in ARDS BAL fluids (56), an important FSAP cofactor, which may be released from damaged cells during lung injury. Thus, auto-activation of FSAP and potentiation of its role in intraalveolar fibrin formation and inflammation may be expected under these conditions. Additionally, extracellular RNA was found to augment auto-activation of proteases of the contact phase pathway of blood coagulation, such as factors XII and XI (57), and our recent observations indicate indeed an important role of the contact phase proteases in the pathomechanisms of acute inflammatory and chronic interstitial lung diseases (58).

**Insufficient counterbalance of increased procoagulant activity by natural inhibitors**

In patients with acute inflammatory and chronic interstitial lung diseases and in respective animal models increased intraalveolar activation of the coagulation system is not sufficiently counterbalanced by natural inhibitors such as antithrombin (AT), tissue factor pathway inhibitor (TFPI) or activated protein C (APC) (9, 43, 45, 59–65). These inhibitors interfere with the TF-factor VIIa-induced activation of the coagulation cascade on different levels: AT neutralizes thrombin and several other proteinases of the coagulation cascade, TFPI predominantly inhibits the TF-factors VIIa-Xa complex, and APC inactivates the coagulation factors Va and VIIIa to limit thrombin formation and fibrin generation. APC is generated from the respective zymogen by the thrombin-thrombomodulin complex, whereby increased oxidation and shedding of thrombomodulin from the cell surface may be involved in the observed reduction of APC in acute inflammatory and chronic interstitial lung diseases. In line with these considerations, in acute lung injury and ARDS patients elevated levels of soluble thrombomodulin have been observed in pulmonary edema fluid and correlate with a worse clinical outcome (62).

Next to impaired inhibition of the coagulation cascade, pulmonary APC deficiency may contribute to increased fibrin deposition in the acutely and chronically injured lung by influencing the fibrinolytic system: APC is known to indirectly stimulate fibrinolysis by binding to and inhibiting PAI-1 (66). Besides its function in the regulation of coagulation and fibrinolysis, anti-inflammatory properties of APC have been described: APC inhibits the production of inflammatory cytokines, attenuates the activation and extravasation of leukocytes and the expression of adhesion molecules on endothelial cell surfaces (67). Furthermore, antifibrotic activities of APC have been noted: APC inhibits the expression of platelet-derived growth factor (PDGF), a potent mitogenic factor for fibroblasts, in different cell types (68). Therefore, it is well imaginable that APC deficiency may contribute to the pathomechanisms of acute inflammatory and chronic interstitial lung diseases not only via the loss of its antithrombotic and profibrinolytic functions but also of its direct antiinflammatory and antifibrotic properties.

**Fibrin deposition in the alveolar compartment due to increased anti-fibrinolytic activity**

A marked reduction of the fibrinolytic activity in the alveolar compartment of experimental models and patients with acute inflammatory and chronic interstitial lung diseases has been constantly observed. Decreased fibrinolytic activity results from the disbalance of inhibition of plasmin and/or u-PA/t-PA by increased expression of α2-plasmin inhibitor and PAI-1, respectively (1, 2, 5–8, 17, 18). Alveolar macrophages, alveolar type II cells but also fibroblasts appear to be the main sources of locally produced PAI-1 in these disorders (18, 43, 44). The importance of alterations in the u-PA/PAI-1 system for the pathomechanisms of acute inflammatory lung diseases and pulmonary fibrosis is further underscored by studies in genetically altered mice: Mice deficient in PAI-1 showed an enhanced fibrinolytic activity and an attenuated fibrinolytic response to bleomycin challenge, whereas in PAI-1 overexpressing mice the fibroproliferative response was found to be potentiated (69). Similarly, inducible expression of u-PA in the alveolar compartment ameliorated lung injury after bleomycin application in mice (70). Furthermore, transgenic mice expressing a u-PA-surfactant protein B fusion protein were protected from acute lung injury after inhalative LPS challenge and showed a markedly attenuated fibrotic response and an improved survival after bleomycin application (71).

It was recently suggested that elevated levels of the thrombin-activatable fibrinolysis inhibitor (TAFI) may also contribute to the reduction of fibrinolytic activity in the alveolar space of patients with chronic interstitial lung diseases due to its ability to remove binding sites for plasminogen on fibrin (72). No data are currently available regarding TAFI levels in the alveolar compartment of patients with acute inflammatory lung diseases like ARDS.

**Therapeutic approaches**

Based on the previous considerations, anticoagulant and profibrinolytic therapeutic interventions may provide protection during acute inflammatory and chronic interstitial lung diseases. In the following paragraph, we will present selected data regarding the effectiveness of different anticoagulant and profibrinolytic agents in animal models and human subjects. Some of these agents have been investigated in sepsis models and in septic patients rather than in acute lung injury or ARDS per se. However, sepsis represents the major risk situation for acute lung injury and ARDS and the coagulation abnormalities observed in these disorders in principle do not differ from those in sepsis.

Overall, the results from experimental interventional trials using anticoagulants and profibrinolytic molecules for the treatment of acute inflammatory and fibrotic lung diseases are promising. Different models have been utilized including that of bleomycin-induced lung injury. The bleomycin model of lung fi-
brosis has several limitations, in particular, it represents a model that is primarily driven by inflammation. Unfortunately, animal models of lung fibrosis with limited inflammation that may be more representative of IPF (e.g. surfactant protein C-knockout mice, Hermansky-Pudlak-Syndrome mice, amiodarone-induced lung fibrosis) have not yet been investigated in the context of alveolar coagulation and fibrinolysis.

In contrast to the encouraging results from preclinical studies, the ability of anticoagulant and profibrinolytic agents to improve clinical outcome in acute inflammatory lung diseases such as ARDS and in chronic interstitial lung diseases, particularly in IPF, is currently still uncertain and remains to be elucidated.

**Active site-inactivated factor VIIa and TFPI**

In several experimental models of sepsis and acute lung injury application of active site-inactivated factor VIIa (FVIIia) or TFPI provided lung protection. FVIIia- or TFPI-treated animals had less severe lung injury, as evidenced by improved lung histology, restored lung compliance, preserved gas exchange, reduced lung edema formation, and a prolonged survival time (73, 74). Lung protection appeared to be due to anti-coagulant as well as anti-inflammatory properties of the interventional agents, since both fibrin deposition and alveolar inflammation were reduced in response to the treatment. In human beings, a phase II randomized placebo-controlled study in patients with severe sepsis demonstrated improved lung function and a trend towards reduction in 28-day all-cause mortality in response to administration of recombinant TFPI (75). However, the promising results from this phase II study were not confirmed in a subsequent phase III study including 1,754 patients: Treatment with recombinant TFPI had no effect on all-cause mortality in patients with severe sepsis and was associated with an increased risk in bleeding (76).

**Hirudin, antithrombin, and heparin**

Exogenous delivery of the highly specific direct thrombin inhibitors hirudin or AT was protective in several animal models of acute lung injury (e.g. after LPS administration and in ischemia reperfusion models of lung transplantation). Administration of these agents provided lung protection by reducing vascular injury, leukocyte infiltration, and vascular permeability (77–79). In addition, heparin was beneficial in some experimental models of acute lung injury preventing fibrin deposition, improving gas exchange, and reducing pulmonary edema formation (80). However, the possible effectiveness of heparin was not confirmed in other experimental studies of acute lung injury (81).

Regarding pulmonary fibrosis, beneficial effects of heparin administration were observed in the bleomycin model of lung fibrosis. Inhalative heparin administration in bleomycin-treated spontaneously breathing rabbits improved lung compliance, suppressed the accumulation of collagen in the lung tissue and virtually abrogated the histological signs of fibrosis (82). Furthermore, administration of a direct thrombin inhibitor (UK-156406) was recently shown to afford protection from lung fibrosis in the same model (83).

On the clinical side, AT treatment has been investigated in patients with sepsis and septic shock (84–87). In a small, prospective, randomized, placebo-controlled phase II study including 42 patients a non-significant reduction in 30-day all-cause mortality was observed in response to AT treatment (85). However, in the large KyberSept trial, which included a total of 2,314 patients, overall mortality at 28 days did not differ between the AT and placebo groups (86). In patients receiving AT and concomitant heparin, a significantly increased incidence of bleeding complications was noted as compared to AT alone and no effect of AT on mortality was seen. In contrast, there was some evidence to suggest a treatment benefit of AT in the subgroup of patients not receiving concomitant heparin: The 28-day mortality was non-significantly lower in the AT group than in the placebo group and this trend became significant after 90 days. Furthermore, AT treatment without heparin did not increase the incidence of serious thromboembolic events (86, 87). In conclusion, it is currently still uncertain whether AT has a beneficial role in the treatment of patients with sepsis. In particular, the potential benefits of AT treatment in sepsis in the absence of heparin need to be evaluated in future clinical trials. Additionally, the influence of AT on the clinical course of acute lung injury or ARDS remains to be tested.

Currently, there is only one study available that examined the potential effectiveness of anticoagulant treatment in IPF patients (88). In this study, the overall mortality as well as the mortality associated with acute exacerbations of IPF were significantly reduced in response to systemic administration of low molecular weight heparin (or warfarin) in addition to standard steroid treatment (88). However, this study has several limitations: Only a low number of patients has been investigated and the exact mechanisms of the beneficial effect of anticoagulation therapy have not been characterized. Future studies will clarify whether anticoagulants will eventually prove effective in the clinical treatment of IPF patients.

**Activated protein C (APC)**

Beneficial effects of APC administration were observed in LPS-induced acute lung injury in rats as well as in bleomycin-induced lung fibrosis in mice. The protection was mediated by the anticoagulant, profibrinolytic, antiinflammatory, and antifibrotic effects of APC: Activation of the coagulation cascade was suppressed, fibrinolysis was promoted, and the secretion of inflammatory cytokines and PDGF was decreased (68, 89, 90). In humans, in the large, randomized, placebo-controlled PROWESS study, which included patients with severe sepsis and at least one organ dysfunction, administration of recombinant human APC (drotrecogin alpha [activated]) was associated with increased ventilator-free days and improvement in survival (91). As stated above, APC may exert its beneficial effects by acting as an anti-thrombotic and profibrinolytic agent, thereby reducing inflammation-induced fibrin deposition, and by acting as an anti-inflammatory agent to attenuate the severity of lung tissue injury and remodeling. In the PROWESS trial, some of the patients had ARDS, but no subgroup analysis was performed for ARDS patients. Thus, the potential clinical efficacy of recombinant human APC in ARDS remains to be addressed in future studies. The absence of a beneficial treatment effect, together with an increased incidence of serious bleeding complications, indicates that APC should not be used in patients with severe sepsis who are at low risk for death, such as those with single-organ failure.
or an Acute Physiology and Chronic Health Evaluation (APACHE) II score less than 25 (92).

**Soluble thrombomodulin**

A beneficial effect of recombinant human soluble thrombomodulin was reported in endotoxin-challenged mice and in rats receiving LPS. The improvement of lung function was associated with reduced leukocyte accumulation and with the reduction of vascular permeability and interstitial edema. In these studies, it was also shown that the protective effect is most likely mediated by soluble thrombomodulin-induced generation of APC (93, 94). Currently, there are no results from clinical studies available that investigated a potential role for soluble thrombomodulin application in acute inflammatory or chronic interstitial lung diseases.

**Plasminogen activators**

An alternative possibility to treat acute inflammatory and/or chronic interstitial lung diseases is to correct the fibrinolytic pathway. However, experimental as well as clinical studies are rare. Exogenous delivery of u-PA or t-PA was protective in a pig model of acute lung injury: u-PA- or t-PA-treated animals showed less severe lung injury, preserved gas exchange and improved survival (95). Attenuation of experimental bleomycin-induced lung fibrosis was observed after inhalative, intratracheal or systemic administration of u-PA (82, 96, 97). On the clinical side, a phase I study documented an improvement in partial pressure of oxygen in arterial blood in 20 ARDS patients in response to treatment with u-PA and t-PA (98). However, controlled clinical studies that investigated the application of plasminogen activators in acute lung injury/ARDS or chronic interstitial lung diseases are currently not available.

**Conclusions**

Together, there is clear evidence that alterations in the alveolar coagulation and fibrinolytic systems and consecutive deposition of extravascular fibrin are involved in the pathogenesis of acute inflammatory and chronic interstitial lung diseases. In diverse forms of these disorders upregulation of procoagulant activity and depression of fibrinolysis in the alveolar space has been demonstrated. Moreover, in animal studies a consistent reduction in lung injury was observed upon administration of anticoagulants, such as TFPI, FVIIa, AT, hirudin, soluble thrombomodulin, or APC.

At this point, however, many open questions remain. For instance, the proportion of the procoagulant response to lung injury that is harmful versus the one that is required for proper healing and repair has to be defined. The exact mechanisms by which anticoagulant and fibrinolytic molecules protect the lung against tissue injury need to be characterized. Particularly, the relative importance of anticoagulant and fibrinolytic treatments as opposed to anti-inflammatory and antifibrotic modalities to achieve beneficial effects has to be determined. Furthermore, it remains to be established whether anticoagulants and fibrinolytic drugs are safe and useful for the treatment of patients with acute inflammatory and chronic interstitial lung diseases. Moreover, it is still uncertain, whether these therapeutic strategies – even if they protect the lung from septic conditions – are beneficial in non-septic acute lung injury or ARDS. Eventually, the function of “new” haemostatic factors, such as extracellular RNA and FSAP, and their potential role as target molecules for therapeutic interventions in these disorders needs to be further explored. A better understanding of the complex interplay between coagulation, inflammation and remodelling in the lung in response to tissue injury may help to answer some of these questions in the future.

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