To function effectively as a gas exchange organ, the alveolar architecture of the lung has evolved as an exceedingly thin membrane that allows inspired air to come into close proximity to capillary blood. Much of this membrane is composed of a single layer of capillary endothelial cells that are separated from a single layer of type I alveolar epithelial cells by a shared basement membrane (1). Though well adapted for its gas exchange function, the delicate nature of this structure places the capillary-alveolar wall at considerable risk for damage. Because the adult human lung is exposed to over 10,000 liters of air per day, there is ample opportunity for inhaled toxins and microbes to gain entry and cause injury to the alveolar epithelium. In addition, the capillaries within the alveolar walls are the first microvascular network that blood reaches after returning from most organs of the body. In this location, the capillary endothelium is exposed to any infectious or toxic agent that gains access to the blood stream at sites distant from the lung. To contend with the competing requirements for efficient gas exchange and preservation of structure, the lung has developed a complex set of strategies to limit injury and promote repair. Important components of these processes are the coagulation and fibrinolytic systems. In this issue of *Thrombosis and Haemostasis*, Wygrecka and colleagues (2) have provided detailed information about how these systems are modulated within the lung.

The coagulation and plasminogen activation systems are known to play a variety of complex and interconnected roles during lung injury and repair. Tissue factor produced by both alveolar epithelial cells (3) and macrophages (4, 5) triggers the coagulation cascade that limits the extravasation of plasma into the alveolar compartment from damaged blood vessels (6-8). The formation of a fibrin matrix within the alveolar space, although potentially beneficial in achieving haemostasis, also can have a deleterious effect by sequestering surfactant, the alveolar lining material that is responsible for lowering air-fluid surface tension and stabilizing alveolar size (9). The role of the plasminogen activation system in repair processes is multifaceted. Effective restoration of lung tissue requires timely removal of the provisional fibrin-rich matrix to prevent fibroblasts from invading and forming collagenous scars that permanently obliterate the airspaces (10). Of the plasminogen activators, urokinase-type plasminogen activator (uPA) is thought to play a more central role in this activity compared to tissue-type plasminogen activator (6, 11-14). In addition to initiating fibrin degradation, the plasminogen activation system can potentially assist in lung repair by degrading non-fibrin provisional matrix proteins, activating other proteases including matrix metalloproteinases, activating growth factors, releasing growth factors from cellular surfaces or extracellular sites, modulating cellular adhesion and movement, and initiating intracellular signaling processes (10). As with all protease cascades, these enzymes are tightly regulated by inhibitors including plasminogen activator inhibitor-1 and -2 (PAI-1, PAI-2) and α2-antiplasmin.

The balance of factors that control coagulation and fibrinolysis are perturbed in many diseases of the lung. Bronchoalveolar lavage fluid obtained from normal human or animal lungs has both procoagulant activity from tissue factor and fibrinolytic activity from the presence of uPA in excess of its inhibitors (6, 13, 15). In both acute and chronic human lung disease such as the acute respiratory distress syndrome, idiopathic pulmonary fibrosis, and sarcoidosis, the procoagulant activity is increased and the fibrinolytic activity is suppressed (6, 13, 15-20). A similar pattern is seen in animal models of fibrotic lung disease such as that induced by bleomycin administration (12, 18). All of these conditions manifest a procoagulant state due to increased cell specific control of coagulation and fibrinolysis within the lung

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Editorial Focus

Cell specific control of coagulation and fibrinolysis within the lung

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The coagulation and plasminogen activation systems are known to play a variety of complex and interconnected roles during lung injury and repair. Tissue factor produced by both alveolar epithelial cells (3) and macrophages (4, 5) triggers the coagulation cascade that limits the extravasation of plasma into the alveolar compartment from damaged blood vessels (6-8). The formation of a fibrin matrix within the alveolar space, although potentially beneficial in achieving haemostasis, also can have a deleterious effect by sequestering surfactant, the alveolar lining material that is responsible for lowering air-fluid surface tension and stabilizing alveolar size (9). The role of the plasminogen activation system in repair processes is multifaceted. Effective restoration of lung tissue requires timely removal of the provisional fibrin-rich matrix to prevent fibroblasts from invading and forming collagenous scars that permanently obliterate the airspaces (10). Of the plasminogen activators, urokinase-type plasminogen activator (uPA) is thought to play a more central role in this activity compared to tissue-type plasminogen activator (6, 11-14). In addition to initiating fibrin degradation, the plasminogen activation system can potentially assist in lung repair by degrading non-fibrin provisional matrix proteins, activating other proteases including matrix metalloproteinases, activating growth factors, releasing growth factors from cellular surfaces or extracellular sites, modulating cellular adhesion and movement, and initiating intracellular signaling processes (10). As with all protease cascades, these enzymes are tightly regulated by inhibitors including plasminogen activator inhibitor-1 and -2 (PAI-1, PAI-2) and α2-antiplasmin.

The balance of factors that control coagulation and fibrinolysis are perturbed in many diseases of the lung. Bronchoalveolar lavage fluid obtained from normal human or animal lungs has both procoagulant activity from tissue factor and fibrinolytic activity from the presence of uPA in excess of its inhibitors (6, 13, 15). In both acute and chronic human lung disease such as the acute respiratory distress syndrome, idiopathic pulmonary fibrosis, and sarcoidosis, the procoagulant activity is increased and the fibrinolytic activity is suppressed (6, 13, 15-20). A similar pattern is seen in animal models of fibrotic lung disease such as that induced by bleomycin administration (12, 18). All of these conditions manifest a procoagulant state due to increased
levels of tissue factor and an anti-fibrinolytic state due to increased levels of PAI-1 and antiplasmins. Unfortunately, these changes appear to accentuate the severity of tissue injury and fibrosis. Interventions designed to block coagulation and promote plasminogen activation have been shown to have beneficial effects in animal models of lung injury. For example, preventing tissue factor from initiating coagulation reduces lung inflammation and injury induced by endotoxin (21) or bleomycin (14). Enhancing plasminogen activation reduces the extent of pulmonary fibrosis induced by bleomycin (14, 22-27). Conversely, suppression of plasminogen activation worsens collagen accumulation (24, 28).

Given these effects of the coagulation and fibrinolytic systems, it is important to have a detailed understanding of how their activities are altered by different challenges. The study published by Wygrecka and colleagues in this issue of Thrombosis and Haemostasis extends considerably what is known about the response of the lung to endotoxin (2). The investigators’ decision to study the effects of endotoxin is quite relevant. In addition to being an important trigger of inflammatory cascades during Gram negative bacterial pneumonias, endotoxin has a similar role when it reaches the lung by the vascular route from sites of distant infection and participates in initiating the severe lung injury seen in the acute respiratory distress syndrome (29, 30).

Endotoxin is also thought to contribute to asthma as well as various occupational lung diseases such as those that occur in cotton handlers and grain processors (31). Furthermore, previous work has shown that endotoxin administration increases tissue factor (32) and plasminogen activator inhibitor-1 (33) within the lung.

Immunohistochemistry has been used to localize coagulation and fibrinolytic proteins within the lung (17, 34-39). However, determining where antigens are located does not necessarily reveal which cells are making them but rather where they are accumulating. In situ hybridization has been used to localize relevant mRNAs to individual or groups of cells (18, 38) but the technique is too imprecise to provide an accurate measure of changes in message level that occur in various pathological states. The technique employed by Wygrecka and colleagues involving real-time RT-PCR performed on cells harvested by laser-assisted microdissection represents a major advance. It allows the investigators to unequivocally determine mRNA levels within the cells of interest.

Using these advanced methods, the investigators found that endotoxin induces changes in mRNA levels of tissue factor and components of the plasminogen activation system, and that levels vary considerably between different cell types in the lung. Some of the more impressive changes include a marked increase in tissue factor mRNA in macrophages and PAI-1 mRNA in type II alveolar epithelial cells and endothelial cells. These findings help determine which cell types are responsible for the observed changes in coagulant and fibrinolytic activities that have been observed in studies of lung tissue or bronchoalveolar lavage fluid. Furthermore, the route of administration of endotoxin affects the response. Airway exposure stimulates tissue factor induction in alveolar macrophage greater than does an intravenous challenge. Conversely, intravenous delivery of endotoxin affects blood leukocytes more than when administered via the airway.

Although the investigators’ microdissection and RT-PCR techniques provide a high level of precision and quantification, there are some limitations. First, two important cell types within the lung, namely type I epithelial cells and microvascular endothelial cells, are not accessible by this approach because of their locations and very thin profiles. Because these cells are responsible for covering over 90% of the alveolar air surface and 100% of the capillary surface, respectively, their response to endotoxin would be very interesting. To try to compensate for this limitation, Wygrecka and colleagues have captured and studied pulmonary artery endothelial cells, which can be isolated by their techniques. Although this cell is certainly of interest, its response to endotoxin may not duplicate the properties of pulmonary capillary endothelial cells (40). A second limitation of the experimental approach is that it provides information only about mRNA levels. Although message abundance is an important modulator of the levels of coagulation and fibrinolytic proteins, there are numerous events downstream of mRNA that control whether coagulation and fibrinolysis occur. Many complex factors determine if a protease cleaves its target including whether the enzyme is activated, bound to its inhibitor, and/or bound to receptors on cellular surfaces, extracellular matrices, or its substrate.

A tangential, but intriguing observation that was reported by Wygrecka and colleagues is the difference in response of alveolar macrophages to endotoxin depending on whether the macrophages were harvested by bronchoalveolar lavage or by microdissection from tissue (2). Most striking is the change from baseline in PAI-2 mRNA levels where endotoxin induces a 230-fold increase in microdissected macrophages, while only an 8-fold increase in lavage-obtained macrophages. This observation provides an important lesson for investigators interested in alveolar macrophage biology. Macrophages retrieved by bronchoalveolar lavage may not be representative of those that remain adherent to the alveolar epithelium. Such differences may be explainable by the known relationships between integrin expression, cell adherence, and macrophage phenotype (41).

In summary, the study of Wygrecka and colleagues demonstrates the exquisite control of procoagulant and plasminogen activator activities within the alveolar compartment of the lung. With the increasing realization that imbalances in these systems contribute to adverse outcomes in disease states, a better understanding of which cells in the lung are responsible for the observed effects will help focus future investigations and promote the development of targeted interventions to reduce lung injury, promote repair, and limit fibrosis.
References


