The fragile architecture of the lung is constantly threatened by a barrage of insults originating from both air and blood interfaces. The necessity to protect our lungs has lead to the evolution of exquisite defence systems. These comprise a complex screen of antioxidants, a mucociliary escalator which is constantly clearing the respiratory tract and sophisticated immune defence mechanisms. When the insult involves vascular damage, the coagulation cascade is activated and is responsible for preventing blood loss at sites of injury by promoting the generation of a fibrin clot. This is vital for haemostasis, but chronic activation of this cascade with persistent fibrin deposition is frequently observed in respiratory diseases involving both the airways (e.g. asthma, chronic obstructive pulmonary disease) and the lung parenchyma (e.g. interstitial lung disease). It is often speculated that these events might be important in the pathogenesis of these diseases, but solid evidence for this has been scarce and, until quite recently, researchers have failed to provide data which could link the features of these diseases (i.e. chronic inflammation and tissue repair) to any known actions of coagulation cascade proteins.

In this article, we will review research developments which suggest an ever increasing range of cellular functions for elements of the coagulation cascade. These functions are such that many of the pro-inflammatory and fibroproliferative events which are commonly observed in respiratory diseases, are most commonly ascribed to elements of a cytokine network, could in fact be orchestrated by proteins of the coagulation cascade. This article will focus on the relevance of these proteins in interstitial lung diseases in which tissue repair and fibrosis are commonly observed.

The coagulation cascade

The coagulation cascade is usually only initiated upon damage to the endothelium. The main processes of this cascade have been reviewed recently [1], but are briefly discussed below and summarized in figure 1. The coagulation cascade comprises two pathways termed the extrinsic and the intrinsic pathways. The extrinsic pathway is triggered by endothelial cell expression of tissue factor (TF) at sites of vascular injury. TF acts as a receptor for factor VII which is converted to active VIIa. This complex then activates factor X which, together with factor Vα, is one of the main components of the prothrombinase complex, responsible for converting prothrombin to active thrombin. Thrombin in turn converts soluble fibrinogen into insoluble fibrin. The extrinsic pathway serves to initiate the co-agulation cascade, but it is the intrinsic pathway that is responsible for sustained blood coagulation. This pathway is maintained by the action of factor Xα and thrombin which are responsible for the activation of factors XII, XI and IX. These in turn lead to prolonged generation of the components of the prothrombinase complex and sustained generation of fibrin. The intrinsic pathway is also responsible for the formation of active factor XIII, which in turn stabilizes and cross-links the nascent fibrin clot.

The amount of fibrin present at sites of tissue injury is tightly regulated by the actions of initiators and inhibitors of the coagulation cascade, as well as the fibrinolytic pathway, responsible for the degradation of the clot, but which will not be discussed further here. Inhibitors include proteins such as lipoprotein-associated coagulation inhibitor and fibrinogen pathway inhibitor, responsible for the early shut-down of the extrinsic pathway; whereas antithrombin III is the major inhibitor of the intrinsic pathway due to its ability to block the activities of thrombin and factors IXα, Xα and XIα. Finally, activated protein C acts as another
major inhibitor of the intrinsic pathway by blocking the prothrombinase complex and further thrombin activation.

Involvement of coagulation cascade proteins in interstitial lung disease

The main function of the coagulation cascade is to limit blood loss and to provide a temporary matrix which will form the structural basis for subsequent repair processes. These processes include the migration and proliferation of inflammatory and mesenchymal cells, followed by the synthesis of extracellular matrix proteins. There is some evidence that persistent activation of the coagulation cascade may play a role in sustained inflammation in the lungs of patients with interstitial lung disease. For example, extensive alveolar fibrin deposition and hyaline membrane formation, as well as inflammatory cell infiltration, are commonly observed in patients with adult respiratory distress syndrome (ARDS) or infantile respiratory distress syndrome [2]. A significant proportion of these patients develop fibrotic lesions in the terminal stages of the disease, characterized by increased mesenchymal cell proliferation and collagen deposition. Supporting, but not providing direct evidence for a role for coagulation cascade proteins in these diseases, several studies have shown that there is also increased procoagulant activity in the lungs and bronchoalveolar lavage fluid from patients with these conditions [3, 4]. Similar findings have been reported for patients with chronic interstitial lung disease [5], including patients with idiopathic pulmonary fibrosis (IPF), as well as with pulmonary fibrosis associated with systemic sclerosis (SSc) [6, 7]. Consistent with these findings, we and others have shown that thrombin levels are increased in bronchoalveolar lavage (BAL) fluid obtained from patients with pulmonary fibrosis associated with SSc [8, 9], and that thrombin is a major fibroblast mitogen present in these fluids [9]. Similar findings have also been reported for animal models of fibrosis. For example, thrombin has been shown to be increased and act as a potent fibroblast mitogen in BAL fluid obtained from rats developing fibrosis following bleomycin administration [10]. More recently, TF expression was shown to be increased at sites characterized by persistent fibrin deposition and fibroproliferation in mice following a similar administration of bleomycin [11].

These studies suggest the involvement of coagulation proteins in these conditions but evidence that they may be playing a direct role in the pathogenesis of these diseases has been obtained in studies in which these products or their inhibitors were administered to experimental animals. For example, intravenous administration of fibrinogen [12] degradation products has been shown to lead to increases in endothelial permeability, extravasation of blood proteins, as well as interstitial and alveolar clot formation. Similar lung changes have also been observed following intravenous infusion of thrombin in sheep [13]; whilst thrombin inhibitors have been shown to be efficacious at reducing lung procoagulant activity and alveolar fibrin deposition in animal models of endotoxaemia in pigs [14], as well as reducing the severity of acute lung injury in newborn piglets [15], although the final outcome of the lesion obtained in this particular model was not reported. Taken together, these studies suggest an important role for these proteins in the pathophysiology of these conditions. The crucial question today, therefore, seems to be how are these proteins causing pulmonary inflammation and disease?

Role of coagulation cascade proteins in pulmonary inflammation and repair

Earlier studies proposed roles for fibrinogen, fibrin and fibrin(ogen) degradation products in chronic disease as a result of their effects on the pulmonary endothelium and the recruitment of inflammatory cells. Indeed, low molecular weight degradation products of fibrinogen, which are generated during the formation of fibrin clots by thrombin, have been demonstrated to increase vascular permeability [16] and to stimulate neutrophil chemotaxis [17]. In addition, thrombin itself can exert similar effects [18, 19]. Furthermore, the severity of pulmonary inflammation can be reduced by neutrophil depletion in animals infused with fibrinogen degradation products [20] and thrombin [21] supporting a direct role for coagulation proteins in the recruitment of these cells in lung inflammation. Thrombin is also a potent chemoattractant for macrophages [22], which accumulate in the lungs and BAL fluids of patients with interstitial lung disease [23] and are believed to play an important role in these conditions by producing pro-inflammatory cytokines and growth factors. More recently, Càss et al. [24] also provided convincing evidence that factor Xa has potent pro-inflammatory effects in vivo by demonstrating that subplantar injection of factor Xa results in an inflammatory reaction characterized by oedema formation and mast cell infiltration. Although this study was not carried out in the lung, factor Xa may exert similar effects there, and may be particularly important in conditions associated with mast cell recruitment and activation.

Coagulation proteins are also known to affect mesenchymal cells and tissue repair processes (for a summary of these cellular effects see figure 2). Thrombin is a potent chemoattractant and mitogen, both for fibroblasts [25] and smooth muscle cells [26] and stimulates them to produce cytokines and growth factors, including platelet-derived growth factor (PDGF) [8], interleukin (IL)-6 [27], basic fibroblast growth factor (bFGF) [28] and transforming growth factor-β1 (TGF-β1) [29]. Furthermore, we have

Fig. 2. – Pluripotent biological effects of products of the coagulation cascade.
recently shown that thrombin is a potent stimulator of smooth muscle cell [30] and fibroblast (Chambers et al., unpublished data) procollagen production and gene expression. These cellular effects of thrombin may play an important role in sustaining inflammation at sites of lung injury and may also be important in the development of fibrotic lesions, which are commonly observed in patients with interstitial lung disease.

Factor Xa, fibrin, and fibrinogen degradation products have similarly been shown to exert a number of cellular effects. For example, factor Xa is a smooth muscle cell mitogen [31] and stimulates growth factor production [32]. Fibrinogen degradation products stimulate fibroblast proliferation [33], whereas fibrin stimulates macrophage to secrete IL-1 [34]. Conversely, inflammatory cytokines, such as IL-6 have been shown to induce the secretion of fibrinogen by alveolar epithelial cells [35]. In addition, type II epithelial cells have been shown to be major sources of TF. Cells expressing tissue factor have been located close to areas associated with fibrin deposition and fibroblastic foci in the lungs of patients with pulmonary fibrosis. These findings raise the possibility that locally produced tissue factor can cause activation of the extrinsic coagulation cascade and sustained alveolar clot formation in the absence of vascular injury. Taken together these data strongly suggest that these coagulation proteins can play direct roles at all stages of the development of interstitial lung disease, from the initial vascular leakage to the development of the final fibrotic lesion.

Conclusions and future directions

It is now clear that products of the coagulation cascade exert a wide range of biological activities. In this article, we propose that higher organisms have developed an integrated network in which coagulation cascade proteins, as well as cytokines, regulate the key process of inflammation and repair. However, the precise relationship between these different polypeptide mediators continues to be investigated, and it is likely that coagulation proteins are present at the very earliest stages of disease and may perpetuate inflammation and repair processes by inducing the recruitment, as well as, the proliferation of inflammatory and mesenchymal cells in chronic lung disease. In addition, they can also induce the release of pro-inflammatory and profibrotic cytokines, which in turn contribute to sustaining inflammation and repair processes. Coagulation cascade proteins and their receptors may, therefore, represent potential targets for therapeutic intervention in interstitial lung diseases. Current research efforts are focused on developing agents that are capable of blocking the cellular effects of these proteins with minimal haemostatic complications. Their usefulness as therapeutic agents awaits confirmation, but this information should be forthcoming as we continue to unravel the function of coagulation proteins in disease processes and the receptor systems through which they act.

References


