Eur Respir J 2003; 22: Suppl. 44, 33s–35s
DOI: 10.1183/09031936.03.00001003

European Respiratory Journal
ISSN 0904-1850

Role of coagulation cascade proteases in lung repair and fibrosis

R.C. Chambers

The specific aims of this presentation are: 1) to review the evidence that activation of the coagulation cascade is a common feature of fibrotic lung disease and other respiratory conditions associated with excessive deposition of extracellular matrix; 2) to describe the profibrotic effects of coagulation proteases and the signalling receptors involved; and 3) to provide evidence that coagulation proteases and their signalling receptors contribute to experimentally induced lung fibrosis.

Introduction

The fragile architecture of the lung is constantly under threat from both external and internal insults. When these insults lead to damage of the extensive vascular network of the lung, activation of the coagulation cascade (fig. 1) ensures that there is minimal blood loss by temporarily plugging damaged vessels with a stable clot consisting of aggregated platelets enmeshed in fibrin. However, excessive or smouldering activation of the coagulation cascade has been implicated in promoting lung inflammation and subsequent interstitial and alveolar fibrosis [1, 2]. Intra-alveolar accumulation of fibrin occurs in the lungs of patients with pulmonary fibrosis [3], in acute lung injury and in the acute respiratory distress syndrome (ARDS) [4], in which rapid fibroproliferation and matrix synthesis can lead to the development of extensive fibrotic lesions. Bronchoalveolar lavage fluid from patients with ARDS has also been reported to contain tissue factor-factor VII/VIIa complexes that can trigger activation of the extrinsic coagulation cascade [4]. Levels of active thrombin have been shown to be increased in the lungs of patients with pulmonary fibrosis associated with systemic sclerosis [5], in pulmonary fibrosis associated with chronic lung disease of prematurity [6] and in asthma [7]. Several procoagulant factors (fibrinogen, factors VII and X) have also been identified in patients with intra-alveolar fibrosis associated with idiopathic bronchiolitis obliterans-organising pneumonia [8].

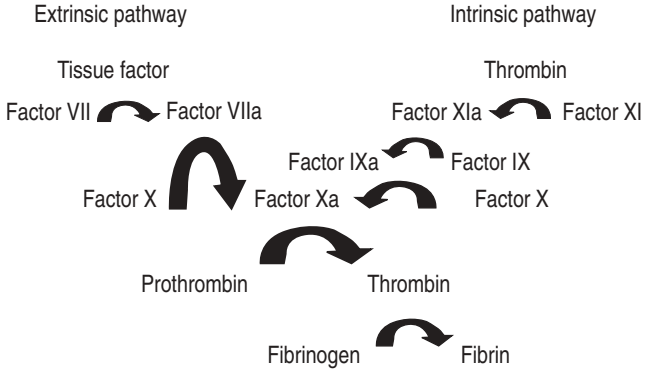

Fig. 1. – The coagulation cascade. The figure shows the stepwise activation of coagulation proteases leading to the generation of thrombin *via* both the extrinsic and intrinsic coagulation pathways. Thrombin converts soluble fibrinogen to insoluble fibrin and exerts numerous cellular effects, including promoting platelet aggregation, *via* activation of protease-activated receptors.

Correspondence: R.C. Chambers, Centre for Respiratory Research, University College London, Rayne Institute, 5 University Street, London WC1E 6JJ, UK. Fax: 44 2076796973. E-mail: R.Chambers@ucl.ac.uk

The importance of excessive and persistent fibrin deposition in lung repair and fibrosis has been demonstrated by extensive studies performed in experimental models using genetically modified mice in which fibrinolytic capacity in the lung was either up- or downregulated. Bleomycin-induced pulmonary fibrosis is increased in mice overexpressing plasminogen activator inhibitor (PAI)-1 (favouring fibrin persistence) and is decreased in PAI-1-knockout mice (favouring fibrin clearance) [9]. Fibrin is thought to influence the fibrotic response by acting as a provisional matrix and reservoir of growth factors for fibroblasts and inflammatory cells. However, fibrinogen-knockout mice were not protected in this model [10, 11], so that fibrin would seem to promote, but is not required for fibrosis.

Protease-activated receptors: sensors for lung injury and initiators of repair and fibrosis

In addition to their critical role in blood coagulation, thrombin and immediate upstream coagulation proteases of the extrinsic coagulation cascade exert a number of cellular effects by activating a novel family of seven transmembrane G-protein coupled receptors, termed protease-activated receptors (PARs) [12]. These receptors display a unique mechanism of activation involving the unmasking of a tethered ligand by limited proteolysis (fig. 2). Conformational changes induced following interaction of the tethered ligand with the second extracellular loop initiates cell signalling *via* heterotrimeric

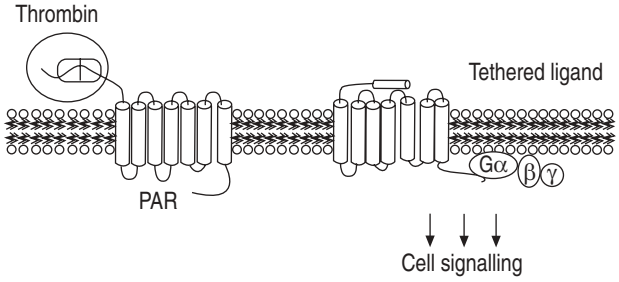

Fig. 2. – Activation of protease-activated receptors (PARs). PARs are activated by limited proteolysis and the unmasking of a tethered ligand. Interaction of this ligand with the second extracellular loop of the receptor initiates cell signalling *via* interaction with heterotrimeric G-proteins.

G-proteins. To date, four such receptors have been characterised, of which three (PAR-1, -3 and -4) are activated by the main coagulation protease, thrombin, whereas factor Xa and tissue factor-factor VIIa-factor Xa complexes activate both PAR-1 and PAR-2 [2]. There is increasing evidence that coagulation protease signalling is mechanistically coupled and thus an integrated part of the tissue factor-VIIa-initiated coagulation pathway. This has led to the suggestion that PARs may act as cellular sensors of tissue injury. In addition, the cellular responses initiated upon activation of PARs are consistent with the notion that these receptors play a critical role in orchestrating subsequent inflammatory and repair responses, as part of the normal response to tissue injury.

Profibrotic effects of coagulation proteases *in vitro*

Research performed in the author's laboratory has concentrated on elucidating the effects of thrombin and its major cellular receptor PAR-1 on fibroblast function *in vitro* and lung fibrosis *in vivo*. It has been shown that thrombin is a potent promoter of fibroblast proliferation [13] and extracellular matrix production [14]. Thrombin has also been shown to promote the transformation of fibroblasts into smooth muscle α-actin-positive contractile myofibroblasts [15]. This is the predominant fibroblast phenotype present in active fibrotic lesions and responsible for excessive deposition of matrix proteins. Although lung fibroblasts express all four PARs, current evidence suggests that PAR-1 is the main receptor responsible for mediating thrombin's effects on fibroblast function [2]. More recent unpublished data from the author's laboratory has also shown that the profibrotic effects of factor Xa, including its effects on fibroblast proliferation and procollagen production, are mediated *via* activation of PAR-1 rather than PAR-2. Current *in vitro* evidence has further shown that these coagulation proteases do not influence fibroblast function following PAR-1 activation directly but act *via* the induction of a host of secondary mediators, including platelet-derived growth factor [16] and possibly connective tissue growth factor (CTGF) [17].

Role of coagulation proteases and protease-activated receptor-1 in lung fibrosis

Further work has confirmed a major role for thrombin and PAR-1 in lung injury and fibrosis. PAR-1 and thrombin expression is increased in inflammatory and fibroproliferative foci following bleomycin-induced lung injury in rats, and direct thrombin inhibition has been shown to attenuate lung collagen deposition and CTGF messenger ribonucleic acid levels in this model [18]. Similar findings have also been reported with the anticoagulant, activated protein C, in mice [19]. Recent preliminary data obtained using PAR-1-deficient mice further indicate that these mice are protected from bleomycin-induced lung injury. Studies to unravel the key pathways by which PAR-1 contributes to fibrosis are currently ongoing.

Clinical implications

There have, to date, been no studies of anticoagulants in fibrotic lung disease. However, anticoagulants, including tissue factor pathway inhibitor and antithrombin III, have been trialled in humans with sepsis, a major risk factor for the development of ARDS, but the results have been largely disappointing. The results of a recent phase-III, randomised, double-blind, placebo-controlled, multicentre Protein C Worldwide Evaluation of Severe Sepsis (PROWESS) trial of intravenous infusion of activated protein C in severe sepsis (that included patients with ARDS) and subsequent reduction in mortality, resulted in Food and Drug Administration approval for the use of this drug in severe sepsis, despite greater risk of serious bleeding [20]. ARDS subgroup analysis was not presented, so the ability of this agent to protect the lung in ARDS remains to be determined.

Conclusion

To conclude, there is increasing evidence that modulation of the coagulation cascade, and more specifically the profibrotic effects of coagulation proteases, warrant further investigation as potential therapeutic strategies in a number of respiratory conditions associated with excessive deposition of matrix proteins. Targeting the signalling receptors rather than the enzymes involved in their activation may allow selective interference with the cellular responses of coagulation proteases without the bleeding complications associated with the use of traditional anticoagulants. Since protease-activated receptor-1 antagonists are currently being developed and are showing promise as antithrombotic agents in preclinical studies in primates [21], they may be available for use in a variety of disease settings, including fibrotic lung disease, in the not too distant future.

References

1. Howell DC, Laurent GJ, Chambers RC. Role of thrombin and its major cellular receptor, protease-activated receptor-1, in pulmonary fibrosis. *Biochem Soc Trans* 2002; 30: 211–216.
2. Chambers RC, Laurent GJ. Coagulation cascade proteases and tissue fibrosis. *Biochem Soc Trans* 2002; 30: 194–200.
3. Imokawa S, Sato A, Hayakawa H, Kotani M, Urano T, Takada A. Tissue factor expression and fibrin deposition in the lungs of patients with idiopathic pulmonary fibrosis and systemic sclerosis. *Am J Respir Crit Care Med* 1997; 156: 631–636.
4. Idell S, James KK, Levin EG, *et al.* Local abnormalities in coagulation and fibrinolytic pathways predispose to alveolar fibrin deposition in the adult respiratory distress syndrome. *J Clin Invest* 1989; 84: 695–705.
5. Hernandez Rodriguez NA, Cambrey AD, Harrison NK, *et al.* Role of thrombin in pulmonary fibrosis. *Lancet* 1995; 346: 1071–1073.
6. Dik WA, Zimmermann LJ, Naber BA, Janssen DJ, Van Kaam AH, Versnel MA. Thrombin contributes to bronchoalveolar lavage fluid mitogenicity in lung disease of the premature infant. *Pediatr Pulmonol* 2003; 35: 34–41.
7. Gabazza EC, Taguchi O, Tamaki S, *et al.* Thrombin in the airways of asthmatic patients. *Lung* 1999; 177: 253–262.
8. Peyrol S, Cordier JF, Grimaud JA. Intra-alveolar fibrosis of idiopathic bronchiolitis obliterans-organizing pneumonia. Cell-matrix patterns. *Am J Pathol* 1990; 137: 155–170.
9. Eitzman DT, McCoy RD, Zheng X, *et al.* Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J Clin Invest* 1996; 97: 232–237.
10. Hattori N, Degen JL, Sisson TH, *et al.* Bleomycin-induced pulmonary fibrosis in fibrinogen-null mice. *J Clin Invest* 2000; 106: 1341–1350.
11. Wilberding JA, Ploplis VA, McLennan L, *et al.* Development of pulmonary fibrosis in fibrinogen-deficient mice. *Ann NY Acad Sci* 2001; 936: 542–548.
12. Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular

cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* 1991; 64: 1057–1068.
13. Chen LB, Buchanan JM. Mitogenic activity of blood components. I. Thrombin and prothrombin. *Proc Natl Acad Sci USA* 1975; 72: 131–135.
14. Chambers RC, Dabbagh K, McAnulty RJ, Gray AJ, Blanc Brude OP, Laurent GJ. Thrombin stimulates fibroblast procollagen production *via* proteolytic activation of protease-activated receptor 1. *Biochem J* 1998; 333: 121–127.
15. Bogatkevich GS, Tourkina E, Silver RM, Ludwicka-Bradley A. Thrombin differentiates normal lung fibroblasts to a myofibroblast phenotype *via* the proteolytically activated receptor-1 and a protein kinase C-dependent pathway. *J Biol Chem* 2001; 276: 45184–45192.
16. Ohba T, McDonald JK, Silver RM, Strange C, LeRoy EC, Ludwicka A. Scleroderma bronchoalveolar lavage fluid contains thrombin, a mediator of human lung fibroblast proliferation *via* induction of platelet-derived growth factor α-receptor. *Am J Respir Cell Mol Biol* 1994; 10: 405–412.
17. Chambers RC, Leoni P, Blanc-Brude OP, Wembridge DE, Laurent GJ. Thrombin is a potent inducer of connective tissue growth factor production *via* proteolytic activation of protease-activated receptor-1. *J Biol Chem* 2000; 275: 35584–35591.
18. Howell DC, Goldsack NR, Marshall RP, *et al.* Direct thrombin inhibition reduces lung collagen, accumulation, and connective tissue growth factor mRNA levels in bleomycin-induced pulmonary fibrosis. *Am J Pathol* 2001; 159: 1383–1395.
19. Yasui H, Gabazza EC, Tamaki S, *et al.* Intratracheal administration of activated protein C inhibits bleomycin-induced lung fibrosis in the mouse. *Am J Respir Crit Care Med* 2001; 163: 1660–1668.
20. Bernard GR, Vincent JL, Laterre PF, *et al.* Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001; 344: 699–709.
21. Derian CK, Damiano BP, Addo MF, *et al.* Blockade of the thrombin receptor protease-activated receptor-1 with a small-molecule antagonist prevents thrombus formation and vascular occlusion in nonhuman primates. *J Pharmacol Exp Ther* 2003; 304: 855–861.