

Review Article**Pulmonary Embolism and vascular injury: What is the role of thrombin?***Filip A. Konecny****Abstract**

Lungs possess dynamic and complex coagulation-thrombolytic and inflammatory sets of innate reactive responses, which generate changes that cause a dramatic alteration in its parenchyma. Clinically, since its steps are reciprocally inter-connected, the pulmonary embolic injury is very complex to describe as one exclusive progression. For example, each step in a coagulation-fibrinolytic-inflammation-integrated pathway produces more side-steps, which later plays an important role in a specific lung damage description. In this clinical review, the enzyme thrombin, an ontogenetically very old and key blood coagulation serine protease is centrally placed as a mediator, activator and up-regulator of post-pulmonary embolic damage to reveal its enormous flexibility in post-pulmonary embolism damage and its reparative processes. Additionally, some beneficial aspects of its control throughout its inhibition are discussed.

ABBREVIATIONS: ADP adenosine 5 diphosphate; APC activated protein C; APTT activated partial thromboplastin time; ASA Acetylsalicylic acid; AT III antithrombin; C-1INH C-1Inhibitor; EC endothelial cells; eNOS endothelial nitric oxide synthase; EPCR endothelial protein C receptor; FDP's fibrin degradation products; fibrin stabilization factor FSF; FM Fibrin monomer; GAG Glycosaminoglycans; GP glycoprotein; HC II Heparin cofactor II; HIT heparin induced thrombocytopenia; HMWK High Molecular Weight Kininogen; LDUH low dose unfractionated heparin; LMWH low molecular weight heparin (s); LV left ventricle; MAP mean arterial pressure; MLCK myosin light chain kinase; NO nitric oxide; PA pulmonary artery; PARs Proteinase (or protease)-activated receptors; PC protein C; PAI-1 plasminogen activator inhibitor; PE pulmonary embolism; PF 4 platelet factor 4; PLA-2 phospholipase A 2; PN-1 Protease Nexin; PT prothrombin time; RBC red blood cell; RV right ventricle; SMC'S' s vascular smooth muscle cells; t-Pa tissue plasminogen activator; TAFI thrombin activatable fibrinolysis inhibitor; TF tissue factor (factor III); TFPI Tissue factor pathway inhibitor; TM Thrombomodulin; TNF tumor necrosis factor; TXA 2 thromboxane A2; VEGF vascular endothelial growth factor; vWf von Willebrand factor.

KEY WORDS: Antithrombin, endothelial cells, pulmonary embolism, vascular injury.

JRMS 2007; 12(4): 203-216**Dynamics facets of Lung injury**

Lung injuries are dynamically multifaceted with slow and only partial pulmonary reparative development, probably due to the ontogenic stages of lung maturation. For example, it has been well documented that ventilatory support of neonates causes long-term bronchial problems ¹. Chan et al. ² depicted that volume ventilation in newborns could promote neonatal thrombosis via lung tissue factor (TF-factor III) release and volutrauma-activated intravascular coagulation ². Subsequently,

chronic hypoxia was shown in humans and animals to cause chronic pulmonary hypertension with thickening of the pulmonary arteriolar walls, particularly the smooth muscle and the adventitia ³. Adventitia was portrayed as a contributor to the narrowing of the vascular lumen due to its thickness augmentation ⁴. The complexities of wall thickening and the intracellular pathways leading to post-inflammatory hyperplasia and dysplasia are just now beginning to be understood. Surprisingly, the replication of adventitial cells comes

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first and often exceeds that of the smooth muscle² and the degree by which extracellular matrix controls cellular replication within the vascular wall⁴. As SMC's suffer cell death through the apoptotic pathway, phosphatidylserine that is exposed on the cell membrane facilitates the assembly of a prothrombinase complex that accelerates the generation of thrombin. Apoptotic SMC's thrombin production incites a positive response that increases the speed of the production of new SMC's after vascular injury, as they respond to many stimuli, including mechanical forces, hypoxia, thrombin and mitogenic cytokines. Additionally, activated thrombin action, through its membrane G protein-coupled receptors GPCR-PAR receptor(s), can induce EC barrier dysfunction and enhance actin-myosin interaction, central stress fiber formation, EC contractions, gap formations and barrier disruption to rapidly increase lung vascular permeability by activation of Rho-kinase (RhoK) and the Ca²⁺/calmodulin-dependent myosin light chain (MLC) kinase (MLCK)⁵.

Directly in relation to lung injury, these factors can be divided into airways-related factors (high shearing forces of the airway epithelium, shedding and depletion of the surfactant, osmotic pressure damage of the pneumocytes, periodic over-inflation stress, low blood supply of damaged parenchyma cells) and factors of damaged vessel wall, including thrombosis and fibrinolysis with activation of TF/ factor VIIa, Xa, thrombin, fibrin, TM, APC, tissue factor pathway inhibitor (TFPI), Antithrombin ATIII, Heparin cofactor (HC II), C-1Inhibitor (C-1INH) or PAI-3, Protease Nexin (PN-1), platelet activation, secretion, activation of Prekallikrein, High Molecular Weight Kininogen (HMWK), tissue plasminogen activator (t-Pa) and others. Both lung injury factors are equally important because they control the quality of blood-gas transport, blood gas exchange and ultimately, lung homeostasis with control of its immune response. It is evident that a variety of cells and cytokines participate in the response to lung injury. To recognize all its complexity, it will be required to integrate

this premise into a multi-disciplined research cooperative endeavor. Moreover, the inhibitory mechanisms that can be applied early to stop additional lung vascular damage might be through inactivation of thrombin. Consequentially, this will decrease membrane G protein-coupled receptor(s) GPCR-PAR activity, causing lung vascular permeability. Reducing platelet activation or performing early, limited TF activation may further stop additional lung vascular damage.

Lung uniqueness in response to injury

Physiologically in lungs, the higher trans-bronchial pressure must be present in the airways to achieve a given lung volume needed during spontaneous breathing, and thus, the tidal volume is dispensed unequally. The volume of the lungs has a central influence on its pressure-flow relationship. When trans-alveolar pressure and volume of the open alveoli increases, during the exercise for example, tidal volumes could reach very high and unexpected values. Resistance falls during this volume stretch, while SMC's contracts in airways and thus makes it difficult to narrow back the airways' lumen. Furthermore, amplified forces could easily inflict tissue damage when they fail to maintain lumen patency and are repeated at the high-rate tidal cycles. Moreover, in diametrically smaller airways, which are usually surrounded by a non-inflating, collapsed lung tissue, high inequality of central airway pressures persist with relatively little or no opposing adjacent alveolar pressure. Therefore, when airways suddenly break open, while subdued to high shearing forces, disruption of the airway epithelium is often observed. Injury inflicted upon the alveolar tissue might also be caused by a sudden rise of pressure of non-inflated, resting alveoli, but also by repeating incoming high tidal volumes, or by high repeating ventilation phases without ability to exhale. Ontogenetically, because of fragility, the lungs are enclosed deep in the chest cavity and protected by the structure of the rib cage. Delicate arrangement of air-blood exchange structures makes them unique of the other internal organs. Structur-

ally, lungs are composed of an interwoven lattice of microvascular pulmonary capillary endothelium, subendothelial matrix and airway epithelial lining. Consequently, three separate compartments are involved in gas exchange: the alveolar space, interstitium and blood.

Phenotypic properties of the lung capillary EC and the subendothelial matrix vary ⁶. Remarkable EC heterogeneity exists throughout the pulmonary vasculature. In the case of the pulmonary artery, the unique tissue and blood surrounding EC microenvironment are often exposed to mixed venous blood gases, and are hence covered by diametrically thicker basement membrane. Conversely, capillary EC are exposed to arterial blood gases, so only a thin-protein matrix is firmly associated with Pneumocytes type I. Heterogeneity in EC function is evident along all segments of the lung vascular tree, including the arterial and small pre-capillary segments. This heterogeneity is obvious in functional studies and in the variable location-specific protein manifestation. Another example of site-specific phenotypic-heterogeneity of EC is the pulmonary arterial endothelium, which expresses greater amounts of endothelial nitric oxide synthase (eNOS), and produces more NO than capillary endothelium. This most likely reflects the importance of NO in maintenance of low pulmonary vascular tone ⁷. After birth, structure of the original fetal pulmonary arteries changes into adult vasculature. Transformed arterioles have less-smooth muscle and thinner walls. Distally along the new arterioles during maturation, smooth muscle thins and becomes sporadic. It then disappears, giving rise to the low pressure, low resistance vascular bed ⁸.

In the absence of endothelial damage, intact EC surfaces assure antithrombotic, anticoagulant, electrical and fibrinolytic excellence until injured. When injured, the surface becomes highly procoagulant and prothrombotic. Thrombin-challenged human EC monolayers demonstrate increased formation of actin stress fiber and loss of barrier integrity, reflected by decreased electrical resistance ⁹ through what

is probably a partial activation of GPCR-PAR receptor (s) ⁵. The tight control that is regularly performed by the lung EC produces both prostacyclin and NO that has potent vasodilative action. ADP must be metabolized before this activation. Additionally, granules with antagonist action, i.e. prostacyclin PGI₂, and ADP-ase, show an inhibitory activity that can directly affect platelet function and expanding inflammation. Alveolar capillary EC share antigens with a peripheral blood monocyte/macrophage subset capable of presenting soluble antigens and triggering autologous mixed lymphocyte reactions. Alveolar capillary EC are HLA-DR⁺, OKM1⁻, and OKM5⁺ ⁶. In addition, EC often express IL-1. However, the antigens are absent, or only weakly visible on the vascular EC of medium and small vessels. In contrast, f. VIII/vWf antigen (FVIII Ag), which is produced in vascular EC, is heavily present in the EC of medium and small vessels, but only weakly represented in the alveolar capillary EC ⁶. Ontogenetically, EC without SMA-positive cells develop into a capillary network surrounding the budding components of distal airways before communicating with proximal vessels. EC of the capillary network are mainly positive for vWf during the early gestational stages, but modify their phenotypes to those of mature lungs (vWF negative and TM positive) during the terminal sac phase ¹⁰. Studies of lung capillary EC show hence difference in expression of certain membrane receptors, which supports the uniqueness of lung vasculature and its response to injury ^{6,11,12} and subsequent response to inflammation.

The lung EC are currently accepted for their capability to switch from anti-inflammatory to highly proinflammatory through Janus cell surface receptors ¹³. Even though lung EC do not produce immune mediators and are not considered immune modulator cells, it is the local response, including cytokine production that may play a role in lung innate immunity. The far less central lung EC response to injury and inflammation, compared to professional response from specialized first-line defense

cells, is to operate as a first line of resistance before more specialized cells come into play. In isolation however, the innate immune response defending lungs at any given time is likely to be shared between its receptive cells (EC) and is expected to be orchestrated as a joint effort of both cell types (EC and professional immune cells) with the lung EC as a first-line immune shield.

Embolic pulmonary damage and the post-embolic effect of thrombin

The structural composition of pulmonary emboli differs, and in most cases can be connected to factors such as thrombus age, its cross-linked stage and site of thrombus development and expansion. There is particular consideration of heart mechanical mincing action by papillary muscles and valves, occurring while the thrombus passes through the heart cavities and is finally expelled into the pulmonary artery (PA). The presence of thrombin-active sites that open on the emboli and successfully pass through the heart cavities would be most responsible for the seriousness of lung injury. It is known that aged, platelet-rich, and well-organized thrombi formed under flow conditions are more resistant to thrombolysis than fresh, thrombin-fibrin and red blood cell-rich clots formed under conditions of stasis¹⁴, which may, in fact, limit the capacity to generate a clinically appropriate pulmonary embolism animal model. Initially, clot structure might be different if the embolic source is a thrombus engrafted in a popliteal, femoral, after disruption of an atherosclerotic lesion, or formed while passing through multiple valves' orifices in venous vasculature. Fibrin deposits in lung circulation provide temporary scaffold into which mesenchymal and other cells migrate from still partially-circulating blood through the area, leading to an extensive lung vascular and parenchymal remodeling and destroying the fine structure of lungs accompanied by presence of an extensive collagen deposition produced by migrated cells.

Thrombin is one of the key enzymes in post-embolic lung damage, as it is vigorously involved in inflammation and acute lung injury

(ALI). Microthrombi and fibrin in intra-alveolar and interstitial compartments create an expansion force by enabling the entrapment of circulating cells with plasma that contains fresh fibrin. Hence, this mechanically damages fine pulmonary microvasculature. A variety of mechanisms, such as an exposure of plasma components to TF expressed by EC, macrophages, alveolar epithelial cells, or fibroblasts and SMC's in the injured area, leads to an intra-alveolar activation of coagulation and thrombin production. TF is an extracellular lipoprotein bound to almost all cells in organisms that synthesize it and present it on their cell-surfaces for later binding of VIIa. TF activation amplifies the initiation of clotting by enhancing exposure of coagulant phospholipids and generation of thrombin, further inhibiting fibrinolysis by elevating plasminogen activator inhibitor 1 (PAI-1) and by decreasing the natural anticoagulant pathways, which largely downregulates the protein C (PC). The complex of extra-vascular TF/f. VIIa initiates thrombin production through activation of Prothrombinase complex (ff. Va, Xa, phosphatidyl inositol, serine, prothrombin, Ca²⁺). Subsequently, thrombin occurs on platelets and on other extra vascular cells traveling through the bloodstream. Thrombin at this stage is able to activate f. XI to further increase its production, while enhancing platelet activation and haemostatic plug formation to minimize bleeding. At the same time as it is transported on platelets, thrombin might be responsible for disseminative coagulation processes occurring in the vital organs, including the lungs.

Therefore, the process after lung injury is multifaceted. By further activating thrombin and activating platelets to form loose platelet plugs, injured lungs become TF and f. VII coagulation-active. Platelets bind to both, the exposed and the newly formed collagen while releasing adenosine 5 diphosphate (ADP), and Thromboxane (TXA₂), which activate more platelets, serotonin phospholipids and lipoproteins. When platelets bind to collagen by (GP Ib/IX) with the help of bridging α granules

they release vWf, and stabilize factor VIII. Collagen they adhere to releases calcium ions and leads to the activation of enzymatic phospholipase A2, which splits membrane phospholipids and activates arachidonic acid, leading to more leukotrienes, prostaglandins and TXA₂ production. TXA₂, a very unstable inflammatory factor in the lung capillary system¹⁵, creates lung vasoconstriction and bronchoconstriction along with other prostaglandin PGD₂ α released by enzymatic phospholipase A₂ degradation. TXA₂ furthermore increases platelet aggregation and enhances the contraction of the bronchial smooth muscles with the bronchoconstriction, which leads to limitation of gas and metabolite exchange. Given the amount of TXA₂ released, its physiological antagonist prostacyclin insufficiently inhibits TXA₂, which is metabolized through cyclooxygenase enzymatic degradation of Arachidonic Acid. This causes imbalances of blood-gas exchange and leads to stronger bronchoconstriction and severe pulmonary hypertension and more lung mechanical injury. This might lead to a cardiovascular collapse because of an enormous increase of left ventricle workload. If only a small amount of TF/f.VIIa is activated, only partial vasoconstriction in the injured area is observed, making it difficult for blood to perfuse through the obstructed lung vascular segment. Reperfusion damage occurs after the blood pressure is able to open the obstruction (figure 1).

Characteristically, thrombin-serine protease generated from a circulating prothrombin after tissue injury, converts fibrinogen to fibrin. During this process, thrombin binds GPCR-protease-activated receptors PARs (PAR-1, PAR-3, and PAR-4) and promotes numerous other cellular effects; i.e. release of inflammatory mediators, chemotaxis, proliferation and GF release, smooth muscle contractions, EC barrier dysfunction, central stress fiber formation, EC contractions, gap formations, barrier disruption, increase lung vascular permeability, endothelial release of NO and vWf, platelet aggregation, procollagen production, prostanoid synthesis and release and fibro-

proliferative processes. PAR-1 is expressed in human arteries, almost exclusively in the endothelial layer¹⁶, where macrophages, vascular smooth muscle cells, and mesenchymal-appearing intimal cells are abundantly present. In the tunica media of pulmonary arteries, thrombin enhances SMC's proliferation and causes endothelium-dependent relaxation through activation of PAR-1 or a PAR-1-like receptor. Reception by PARs is universally expressed in a variety of cell and tissue types, including lung fibroblasts, airway smooth muscle and epithelium, platelets, osteoblasts, connective tissue, vascular smooth muscle and endothelium, keratinocytes, stomach, intestine, kidney, neurons, astrocytes, and skeletal muscles. Reception by PARs through thrombin activation controls the action of post-pulmonary embolism, including coagulation, thrombosis, thrombolysis and mitogenesis response. Thrombin-mediated activation of PAR-1 has been shown to stimulate the tyrosine phosphorylation of the GF receptors, activating MAPK cascade, stimulating trans-activation of GF receptors, promoting cell survival, and enhancing mitogenesis. When thrombin proteolytic cleavage generates a new tethered ligand (SFLLRN) that interacts with the extracellular loop-2 of the receptor, it further activates downstream signal transduction cascades¹⁷. It induces EC barrier dysfunction through multiple signaling pathways and cytoskeletal targets, including Rho-kinase (RhoK)¹⁸ and the Ca²⁺/calmodulin-dependent myosin light chain kinase (MLCK)^{5,19}. MLCK was recently shown as an important factor of vascular permeability in lungs through the novel mechanism of PAK-PIX-GIT1 activation of Erk in a model of an injured mouse lung²⁰. Increased RhoK and MLCK activation enhances actin-myosin interaction, stress fiber formation, EC contraction and barrier disruption with increased vascular permeability. Thrombin activates the small GTPase Rho-kinases, leading to MLC phosphorylation, RhoK-mediated phosphorylation and inactivation of the myosin-associated phosphatase. This process most closely characterizes the primary mechanism

of prolonged EC contraction and increased vascular permeability through PAR-1, and TGF- β in an $\alpha v\beta 6$ integrin-specific manner ²¹. The effect is PAR-1-specific and is mediated by RhoA and Rho kinases. Therefore, PAR-1-thrombin mediated enhancement of $\alpha v\beta 6$ -dependent TGF- β activation could be one mechanism by which lung circulating thrombin activation contributes to the development of lung injury ²¹. Thus, the molecular mechanisms of thrombin-induced EC stress fiber formation, contraction, and barrier disruption plays an undisputable role in lung vascular permeability.

Nevertheless, the effect of thrombin on pulmonary epithelial cell barriers is still unknown. Despite an extensive lung alveolar damage, type II alveolar cells are able to survive and restore the epithelium of the airways ²². The epithelial cell and EC intercellular junctions are tight and adherent junctions, which are closely related to the actin cytoskeleton barrier. Thus, the barrier-protective effect of thrombin on epithelial cells described by Kawkitinarong in 2004 further suggests a critical role of cell-specific cytoskeletal remodeling and tight-junction regulation in EC and alveolar epithelial barrier regulation ⁹. In that respect, the role of thrombin must be fully elucidated. Unlike other GPCR, which require ligand binding for activation, PARs might be activated upon cleavage by thrombin and by other serine proteases such as, factor Xa, complex of ff. VIIA-Xa, Cathepsin-G, plasma MMP-1 or trypsin. At a low concentration of the thrombin/ thrombomodulin (TM) complexes, thrombin might stimulate activation of the thrombin activatable fibrinolysis inhibitor (TAFI). In that reaction, TAFI helps to stabilize the thrombus by inhibiting partially degraded thrombus-associated thrombin to be further divided, and thus ensures thrombus stability. At higher concentrations, thrombin activates protein C (APC). APC subsequently reduces factors V and VIII activity, and diminishes thrombin generation (Fig. II) ²³. However, it is not fully understood at this time, whether other serine proteases are capable of lung PAR receptor

stimulation with the generation of the same thrombin-like response. Comparable to other GPCR, downstream PARs signaling cascades include many kinases and intracellular messengers, such as Gq/11-mediated increase in cytoplasmic free Ca²⁺ ([Ca²⁺]-cyt) by inositol 1,4,5-trisphosphate and DAG and activation of PKC and calmodulin kinases (CaMKII and CaMKIV); G12/13-mediated activation Rho/Rho kinase and c-Jun NH2-terminal kinase; and G12 β -mediated Ras/MAPK and PKB ¹⁷. Therefore, the direct effect of thrombin on vascular tunica intima goes hand in hand with forming loose platelet plugs and new thrombin activation, EC rounding, and endothelial production of cytokines, growth factor (GF), and matrix proteins through GPCR-PAR-1 signaling, causing Erk phosphorylation. This enhances the expression of early growth response-1 and c-Fos, two immediate early genes that have been implicated in inflammation and tissue remodeling in response to injury ²⁴.

Important inhibitors of thrombin in lungs

Demonstration of pulmonary embolism when induced by the injection of thrombin shows an increasing amount of platelets in lung circulation. Massive thrombi localization can be seen lodged in both large and small lung arteries ²⁵. Thrombin converts fibrinogen to fibrin and incorporates platelets and fibrinogen into the thrombus. When bound to TM, thrombin no longer activates fibrinogen. In lungs, TM represents the integral membrane glycoprotein (GP), which serves as a cofactor for protein C activation. TM binds to the same site on thrombin that would normally bind fibrinogen, f. V, or platelets, and the complex activates the PC pathway ²⁶. Protein C is finally activated by TM-thrombin complex at a different site on the thrombin molecule. As thrombin is bound to TM, it is rapidly cleared from lung microcirculation. System of PC (figure II) is highly involved in the anticoagulant, fibrinolytic and anti-inflammatory mechanisms that are present in the lungs. These mechanisms naturally control inhibition of factors Va and VIIIa, and activation of thrombin. This anticoagulation principle is based on thrombin,

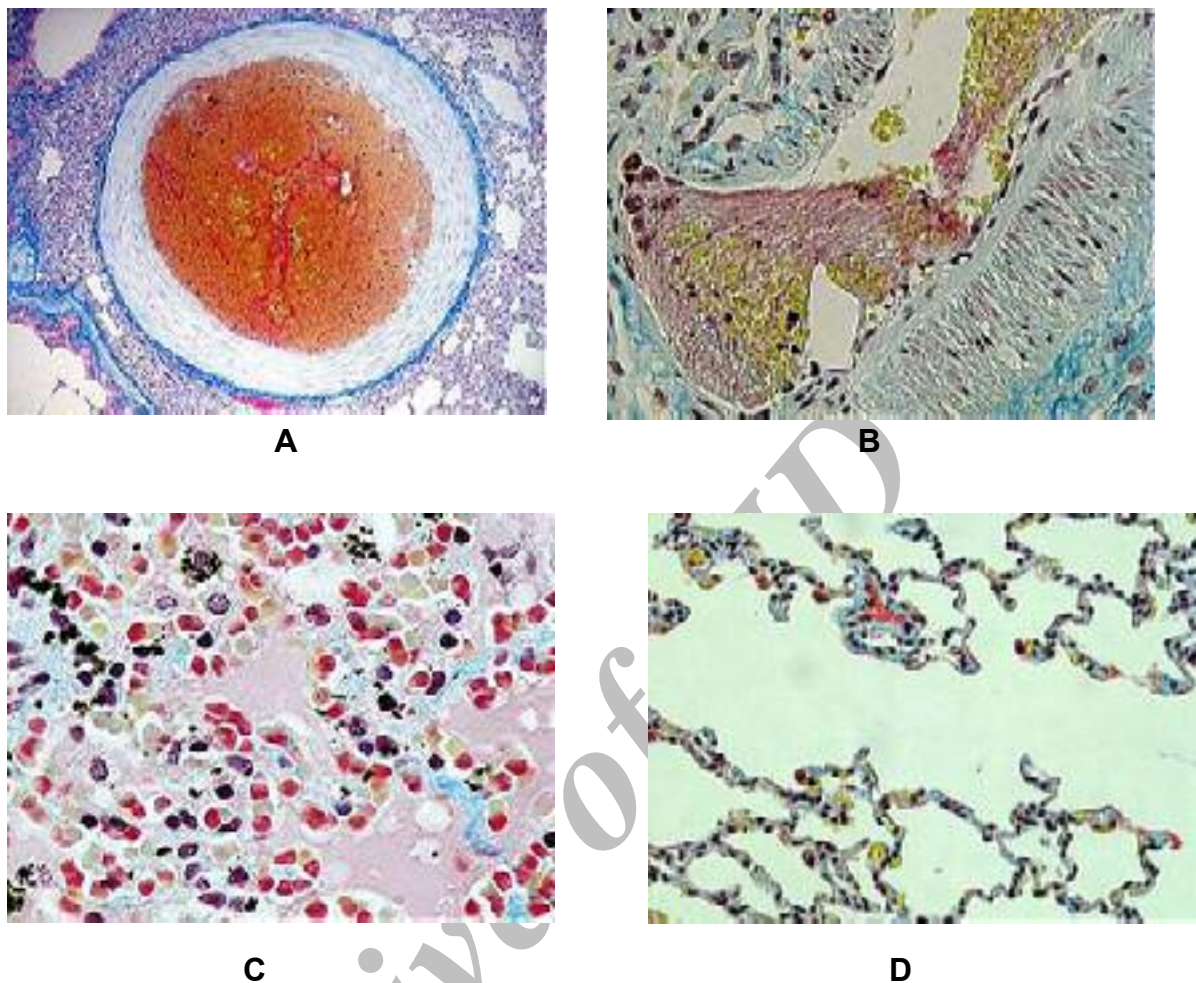


Figure 1. Characteristic picture of post-embolic pulmonary vasculature and parenchyma M.S.B. stain (martius/scarlet/blue) for connective tissue and fibrin. Nuclei brown/black; Collagen blue; red blood cells yellow; muscle and fibrin red. Thrombus-organized fibrin in lung vasculature (A) (10X) obstructs vessel lumen and creates damages of endothelium (B) (20X). The enormous force that mechanically grinds down fragile lung vascular endothelium, with the presence of clumped platelets, causes flooding of the alveolar areas directly in vicinity of thrombus (C) (40X). Some fibrin will still appear in bordering alveolar areas, probably due to new thrombin-fibrin formation (D) (40X). Further picture (C) represents alveolar flooded severe ischemic area, where an increase of oxygen-free radicals and an increase of intracellular calcium, leading to a significant alteration of the cellular metabolism know as reperfusion. Therefore, the oxygen paradox can be observed the in ischemic alveolar area. Oxygen is mandatory for cell survival, but it may also be responsible for a direct lung injury.

which is not coagulation active if TM binds it to EC surface. Instead, the complex of TM-thrombin and Ca^{2+} activates PC to APC. APC inhibits factor Va and VIIIa with the assistance of protein S. Moreover, APC and PAI-1 bind to the tight complex, which stimulates fibrinolytic t-Pa activity²⁷. APC further inhibits thrombin activation of the active thrombin fibrinolytic inhibitor (TAFI), to which the thrombin is a

potent activator²⁸. Thus, TAFI inactivation could be controlled by thrombin inhibition.

The importance of TM in pulmonary thrombosis depicts experiments with a genetically TM-/- mouse²⁹, where the absence of TM causes multiple organ thrombosis. As described by many, the amount of TM in lung EC varies^{10-12,30}. The microcirculation contains regions of TM abundance, such as in alveolar

capillaries, connective tissue and juxta-alveolar zones¹². The TM concentration in microcirculation is approximately 500nmol, compared to large vessels, where its concentration rapidly decreases to about 0.1-0.2 nmol³¹. Recently, the recombinant TM was developed to suppress an intravascular coagulation and pulmonary thrombosis in an animal model³². However, further studies are necessary to understand the role of TM in post-pulmonary embolism. At a low concentration of the thrombin/TM complexes, thrombin stimulates activation TAFI. The enzymatic role of TAFI is to remove lysine residues of partially degraded fibrin, and to protect thrombin against newly formed lysis within the newly formed thrombus. The most recent studies of TAFI propose that its additional role is to deactivate the complement-derived anaphylatoxin, C5a³³. In lung systemic vasculature, the rapid TAFIa activation and subsequent rapid inactivation of C5a would be physiologically favorable because it would protect lung microcirculation from the complement-mediated injury. The complex of TM - thrombin activates TAFI to TAFIa by proteolysis at Arg, which results in the release of activation peptide from the catalytic domain and exposure of the complete activation site. The other coagulation and fibrinolytic enzymes (APC, t-Pa, u-Pa, kallikrein and factors VIIa, IXa, and Xa) are incapable of activating TAFI. Activated TAFI further inhibits thrombolysis by cleaving off partially degraded fibrin from the site of its lysis. At higher concentrations however, thrombin activates protein C (APC). With the assistance of protein S, APC subsequently decreases the activity of factors Va and VIIIa, therefore diminishing thrombin generation²³.

The whole APC pathway will be initiated when thrombin binds to TM on the surface of the EC. An endothelial protein C receptor (EPCR) uses the thrombin-TM complex to augment protein C activation³⁴. Inflammatory mediators such as TNF α can downregulate EPCR, TM and IL-6, which can depress levels of protein S. Inflammatory mediators disable the EPCR, which is shed from the endothelium

and binds to an activated neutrophils. Inhibition of the PC pathway increases cytokine amplification, EC injury and leukocyte extravasations, processes that could be decreased by infusion of APC³⁵. In vitro, APC inhibits TNF α expansion from monocytes blocks leukocyte adhesion to selectins. EPCR can undergo translocation from the plasma membrane to the nucleus during this translocation process, where it redirects gene expression³⁵. It can carry APC to the nucleus, possibly to modulate the inflammatory mediator responses in the endothelium. The anti-inflammatory qualities of APC are based on its ability to inhibit induced pulmonary vascular injury by restraining accumulation and transmigration of leucocytes and pulmonary alveolar macrophages (PAM)^{32,36}. The inflammatory-based changes develop as well, due to alteration of lung EC systems such as TM/APC³⁷. Like APC, endothelial PC receptor (EPCR) shows anti-inflammatory activities. The EPCR-APC has a function in antigen presentation. EPCR-APC shows some similarities with MHC class 1/CD1 protein lipid antigen presentation family^{38,39}. Another important inhibitor of thrombin is the tissue factor pathway inhibitor (TFPI) because of its early inactivation on an injured EC matrix. TFPI is also known as antithromboplastin, anticonvertin or the inhibitor of the extrinsic coagulation pathway. It plays a substantial role in the regulation of extrinsic coagulation started by TF, representing the first feedback inhibitory reaction (figure 2). TFPI possesses three separate serine protease inhibitory regions. One binds f. Xa, the second binds f. VIIa-TF complex, and the function of the third is unknown. TFPI is a direct inhibitor of f. Xa and its binding reaction is augmented by heparin. Its inhibitory reaction aims first towards f. Xa and then f. VIIa for unknown reasons. This reaction is probably due to the TF-complex, which first activates f. X and f. Xa and later catalyzes the conversion of f. VII to VIIa. However, the activation of f. X is probably its priority. EC in lungs synthesizes TFPI, and the majority of TFPI stays localized in the EC caveolae, managed by the glycosylphosphatidy-

linositol-anchorage mechanism⁴⁰. The remaining TFPI is secreted into the blood. It is reported that only severe damage of the lung EC causes significantly increased TFPI levels in plasma⁴¹.

Another thrombin inhibitor, yet very important in lung coagulation, is Antithrombin III (AT III) (figure 2). AT III is a single-chain heparin-binding glycoprotein that acts within plasma as the major inhibitor of thrombin not attached to the surface. It interferes with several plasma proteases, including kallikrein and ff. IXa, Xa, XIa, and XIIa, thus playing a central role in regulating lung coagulation⁴². AT III belongs in the group of acute-phase proteins with half-life in plasma between 45-60 hours, which shortens to about three hours during pathological conditions⁴³. During an acute phase of lung injury, AT III reduces accumulation of fibrinogen and hence controls procoagulant activity of bronchoalveolar lavage fluid⁴³. AT III is considered a major plasma inhibitor of thrombin and the complex of TF/f. VIIa. Heparin and other heparinoids from the vascular surfaces bind to AT III reversibly, creating a conformational change and transform AT III into the complete inhibitor. This complex additionally inhibits other factors, i.e. IX, X and XII. AT III activation and binding follows the release of heparin, as heparin is secreted locally upon injury of the damaged EC. Heparin release has several limitations such as its short half-life, the increase of bleeding, the inability to inhibit clot-bound thrombin, and the harmful immuno-effect of heparin-induced thrombocytopenia (HIT). The biophysical limitation of bound ATIII/heparin complexes rest in its inability to access and inactivate either f. Xa in the prothrombinase complex, or thrombin already bound to fibrin or subendothelial surfaces. Factor Xa bound to a platelet is also protected against inhibition by the AT III/Heparin complex. Therefore, when thrombin bound to its surfaces is unsuccessfully inactivated, heparin has to be neutralized. However, the process of its neutralization is currently not completely understood. It is speculated that it could be due to a neutralization of

areas on AT III and thrombin. Interestingly, platelets may limit their anticoagulant effect by releasing PF4, a protein that neutralizes heparin. Because of the anticoagulant effect of heparin, which is based on its ability to increase the inhibitory activity of AT III, a deficiency of AT III requires replacement therapy. ATIII deficiency is mostly congenital in origin, but can also be acquired. When AT III binds to heparin-like GAG on EC, it induces production of prostacyclin and mediates an anti-inflammatory effect⁴⁴. The role of AT III in the inhibition of thrombin generation in the lungs needs to be further studied before a full explanation can be reached.

Two other inhibitors of thrombin are known to be essential in vascular environment. One of them is heparin cofactor II (HC II), a serine protease inhibitor with a molecular weight of 65.6 kDa, which is synthesized by the liver and circulates in plasma at a concentration of 1.0 $\mu\text{mol/L}$ ⁴⁵. HC II is a more potent thrombin inhibitor than AT III in the presence of heparin and dermatan sulfate. HCII has been detected in the intima and media of normal human arteries, where dermatan sulfate is deposited. The inactivation rate of thrombin by HCII increases by about 1000-fold after binding to dermatan sulfate⁴⁶. Regrettably, HC II does not possess anti Xa action and has only a local effect. There is a likelihood that HC II action increases as ATIII activity decreases. HCII might neutralize the actions of the thrombin generated at injured vascular walls if smooth muscle cells and fibroblasts by the matrix of vascular intima and media secrete dermatan sulfates. Protease nexin 1 (PN-1) is a 43- to 45-kDa protease inhibitor that belongs to the serpin superfamily. PN-1 is secreted by a wide range of cultured cells, including platelets, but is barely detectable in plasma and forms inhibitory complexes with thrombin, u-Pa and trypsin. Furthermore, it is a potent inhibitor of the blood coagulation Factor XIa⁴⁷. FXIa-PN1 complexes are shown to be internalized and degraded by human fibroblasts⁴⁷. The potential inhibitory activity towards thrombin is multiplied by the presence of heparin or

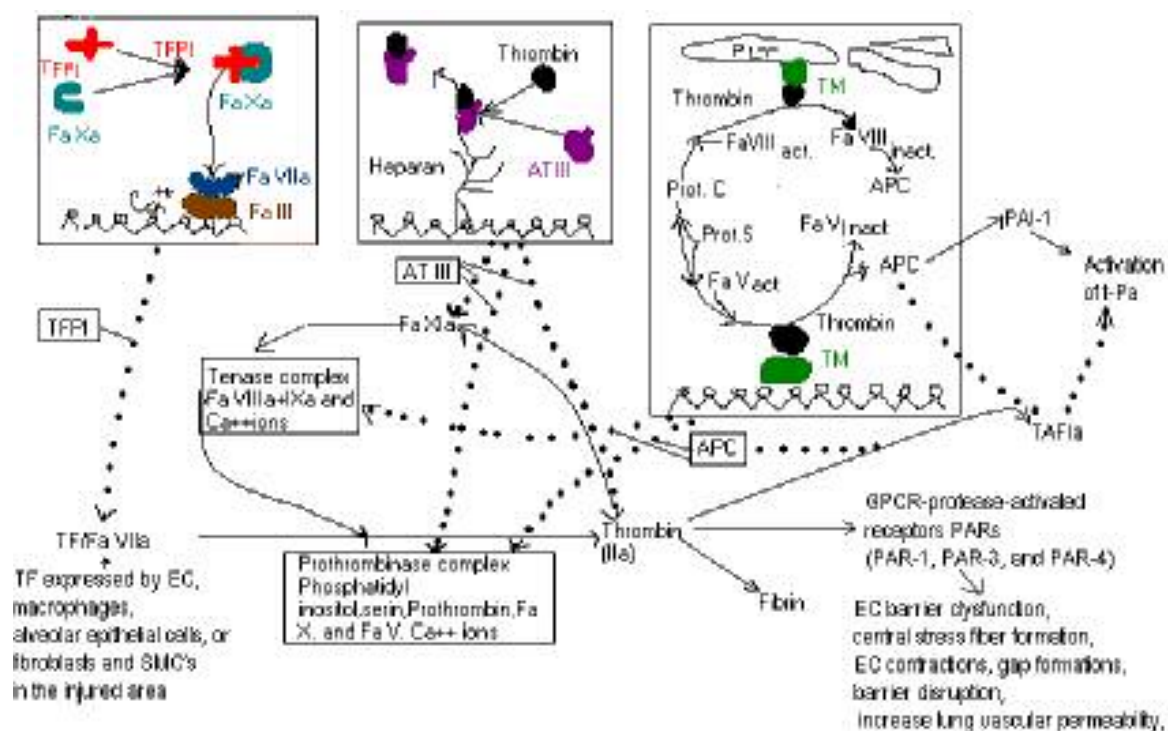


Figure 2. Three important anticoagulant mechanisms in lung vasculature. **Color versions of figures are available for download at the journal homepage**

Table 1. Platelet granules- modified from ⁵⁶.

α Granules are most abundant type	Platelets- specific protein (PF 4 and β-thromboglobulin) Adhesive glycoproteins (vWf, fibrinogen, fibronectin, thrombospondin and vitronectin) Coagulation inhibitor (Protein S) Mitogenic f. (platelet derived grow factor (PDGF), transforming growth factor (TGF), endothelial cell grow factor (ECGF), epidermal growth factor (EGF) Permeability f., Chemotactic f. Coagulation f. (f. V., f. XI, F.VIII) Fibrinolytic inhibitors (PAI-1, α2 AP, TAFI) Other proteins (albumin and Immunoglobulins) P-Selectin Ions of potassium TAFI (thrombin activatable fibrinolysis inhibitor)
Dense granules a storage of adenine nucleotides	Pyrophosphate, Serotonin, Calcium ions, ADP,ATP, Adrenalin, Noradrenalin, Phospholipids, Serotonin, α-2AP
Lysosomal granules	Acid hydrolases (elastase, collagenase, cathepsin and endoglycosidase) Factor XIII

dermatan sulfate. Heparin engages formation of the PN-1 covalent complexes with thrombin. PN-1 interaction with heparan sulfates accelerates thrombin inhibition. In addition, it has

been reported that PN-1 also interacts with collagen IV, which decreases the rate of inhibition of urokinase and plasmin without affecting the rate of thrombin inhibition by PN-1 ⁴⁸. As a

result, PN-1 is considered only a local cellular thrombin inhibitor. Very little is known about its role in the vascular environment.

Taken together, as the lung coagulation process progresses through its stages, plasma leakage with coagulation proteins and platelets escape and fill the alveolar space. This causes new thrombin, fibrin and platelet aggregation inside of alveoli due to a variety of factors, such as mechanical distension of alveoli, damage of the alveolo-capillary membrane, collapse of neighboring alveoli, platelet activation and its granules secretion (table 1). The following activation of TAFI or platelet agonists such as serotonin, collagen, thrombin, platelet activating factor (PAF), adrenalin and metabolites of arachidonic acid, leads to more leukotrienes, prostaglandins, TXA₂ production and initiation of the vasoconstriction and inflammation. ADP released from platelet granules modifies their membranes and stimulates fibrinogen to bind through their GP IIb/IIIa receptor. Produced directly from coagulation, thrombin leads to more platelet aggregation and conversion of fibrinogen to fibrin via FSF (protransglutaminase, fibrin stabilization f. XIII). Fibrinogen from coagulation and (or) secreted and released from platelets supports the aggregation through GP IIb/IIIa binding. GPIIb/IIIa also mediates platelet attachment to vWf and subsequent aggregation. ADP released by thrombin from platelets increases binding to fibrinogen and thrombin, which acts as a platelet aggregator. While thrombin on platelets is in its active stage, induction of PARs could be observed leading to fibroblast activation and EC stress fiber formation, contraction and barrier disruption. In that reaction, PAR-1 activation causes a brief cathepsin-regulated Ca²⁺ increase in lung fibroblasts. PAR-1 can then increase PGE₂ synthesis and release through cyclooxygenase 2 production. PGE₂ synthesis, therefore, might serve as a mediator in preventing lung fibroblasts activation and by creating bronchodilatation; lungs can thus be partially rescued from the effect of TXA₂. The effects of thrombin in the lungs expand beyond the scope of its functions as co-

agulation, thrombolysis and platelet aggregation. In-vitro, low concentration of thrombin activates more platelets, while higher concentrations induce mitogenetic cell transformation and EC changes that help to release plasminogen activators. Thrombin is one of the most complex enzymes in post-pulmonary embolism due to its effect on granulocyte chemotaxis, cell proliferation, pro-collagen and cytokine production, prostanoid synthesis and release smooth muscle contraction and vascular tone regulation. Through its receptors, thrombin stimulates endothelium-dependent vasodilatation, mediated by activation of nitric oxide production⁷. Its presence on vascular smooth muscle cells (SMC's) or EC stimulates SMC's extracellular matrix collagen accumulation^{49,50}. It possesses an inflammatory effect through secretion of cytokines Il-6, Il-8 and monocyte chemotactic protein-1 by activation of PAR-1, and 2^{51,52} and is one of the most potent stimuli for non-hypoxic vascular endothelial growth factor (VEGF). It upregulates VEGF receptors on endothelial cells⁵³. Further, it upregulates heat shock proteins like Hsp70, Hsp90, and Hsp27⁵⁴.

Dotted line represents native anticoagulants and their inhibitory effects. From left, the inhibitory effect of TFPI that binds to Factor Xa. TFPI and f. Xa react on cell membrane with f. VIIa, which further increase concentration of TFPI and its inhibitory effect towards (f. VII+ f. III) complex. On right, ATIII on the surface of a heparan molecule. AT III changes structure after it binds to heparan,, which facilitates inhibition of thrombin. The third picture describes anticoagulant properties of ACP. Thrombin-TM complex facilitating conversion of protein C to activated APC with help from protein S. APC then inactivates f. V and f. VIII. APC is then degraded by PAI-3 (PCI) or α 1-AT. Protein C and protein S (PS) are vitamin - K dependent GP. Hepatocytes and EC produce protein C. Its concentration in plasma increases with age. The major inhibitor of protein C is α 1-AT, α 2-MG, α 2-AP, elastases and cathepsin G and protein C inhibitor-PCI (PAI-3). PCI was found increased in bronchoalveolar lavage and

it probably plays a role in the inhibition of fibrinolysis⁴⁸. PCI can inhibit activated protein C, and thrombin bind in TM-thrombin complex, urokinase, f Xa, f XIa and TAFIa. Protein S is produced by hepatocytes, EC and megakaryocytes. It could be found in alfa granules of platelets (table I). Its concentration is age-dependent. About 40 percent circulate unbound in plasma, and the rest, about 60

percent of PS, is inhibited and bound to C4b-binding protein (complement factor-protein of acute phase of inflammation)⁵⁵. Protein S has its own inhibitory ability unrelated to APC activity. It is known that its inhibition of prothrombinase complex is directly through factor Xa. Binding to factor V and Va inhibits activation of factor Xa, and its activity towards to factor VIII.

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