Coagulation and the vessel wall in pulmonary embolism

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Abstract: Venous thromboembolism comprises deep-vein thrombosis, thrombus in transit, acute pulmonary embolism, and chronic thromboembolic pulmonary hypertension (CTEPH). Pulmonary thromboemboli commonly resolve, with restoration of normal pulmonary hemodynamics. When they fail to resorb, permanent occlusion of the deep veins and/or CTEPH are the consequences. Apart from endogenous fibrinolysis, venous thrombi resolve by a process of mechanical fragmentation, through organization of the thromboembolus by invasion of endothelial cells, leukocytes, and fibroblasts leading to recanalization. Recent data utilizing various models have contributed to a better understanding of venous thrombosis and the resolution process that is directed at maintaining vascular patency. This review summarizes the plasmatic and cellular components of venous thrombus formation and resolution.

Keywords: thrombosis, thrombus resolution, pulmonary embolism, CTEPH.

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INTRODUCTION

Venous thromboembolism (VTE) is understood as a continuum including deep-vein thrombosis (DVT), thrombus in transit, acute pulmonary embolism (PE), and chronic thromboembolic pulmonary hypertension (CTEPH). With ca. 500–800 cases/million/year, VTE is common and carries high morbidity and mortality, leading to sudden death in about 10% of patients and accounting for ~300,000 yearly clinical episodes and 50,000 deaths in the United States.^{1,2} Still, pulmonary thromboemboli often resolve, with restoration of normal pulmonary hemodynamics.^{3–5} Resolution occurs by mechanical fragmentation,⁶ through organization of the thromboembolus by invasion of endothelial cells (ECs), mononuclear cells, and fibroblasts leading to recanalization, and through endogenous thrombolysis. In 0.1%–9.1%^{7–16} of cases, venous thromboemboli fail to resorb, thereby resulting in CTEPH or in permanent occlusion of the deep veins.

The generation of thrombin from its precursor prothrombin is the central event of blood coagulation, which is essential to hemostasis and the culprit of thrombosis. It represents a highly regulated, dynamic, and rapid process. By contrast, the removal of thrombus results from the concerted action of plasmatic fibrinolysis and a time-consuming, com-

plex vascular remodeling process¹⁷ directed by circulating cells and cells within the vessel wall.¹⁸ Injury of the blood vessel wall, disturbed blood flow, and hypercoagulability are defined as the 3 aspects of Virchow's triad and are thought to be the main drivers of thrombosis. However, compared with the scientific interest in mechanisms of thrombus formation,^{19–21} a new view of thrombosis, as depicted here, will give equal emphasis to the cellular and vascular biology of venous thrombus resolution (Fig. 1).

THE VESSEL WALL AND COAGULATION

The intact endothelium represents a barrier separating platelets from adhesive substrates in the subendothelial connective tissue matrix.²² Disruption of the integrity of the vessel wall by mechanical or functional trauma allows circulating platelets to come in contact with the subendothelial matrix.

Fibrin formation may be triggered by exposure of blood either to a damaged vessel wall (extrinsic) or to blood-borne (intrinsic) factors. Coagulation factor XII (FXII, or Hageman factor) is the main initiator of the intrinsic pathway and is activated by contact with charged surfaces.²³ Unlike deficiencies of other components of the coagula-

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Figure 1. A new view of thrombosis. Activated endothelial cells (ECs) release ultralarge von Willebrand factor (ULVWF) and P-selectin from Weibel-Palade bodies (WPB) and cause platelet and neutrophil adhesion. Platelets recruit tissue-factor (TF) microparticles and generate fibrin. Neutrophils release neutrophil extracellular traps (NETs), thus providing an additional scaffold enhancing thrombus growth. Injured cells release procoagulant extracellular RNA (eRNA). Lymphocytes, macrophages, ECs, and their secretomes, as well as proteases—such as plasmin, DNase, and RNase—and fibrin fragments, play important roles in thrombus resolution. CXCR4: chemokine receptor type 4; EPC: endothelial progenitor cell; HIF-1: hypoxia inducible factor-1; MΦ: macrophages; PAI-1: plasminogen activator inhibitor-1; PAR-1: protease-activated receptors-1; PECAM: platelet EC adhesion molecule; RBCs: red blood cells; SMCs: smooth muscle cells; VE-cadherin: vascular endothelial cadherin; VEGF: vascular endothelial growth factor.

tion cascade, such as factor VII, tissue factor (TF), and factors VIII or IX (which cause the bleeding disorders hemophilia A and B, respectively), FXII deficiency does not lead to bleeding. This supports the hypothesis that fibrin formation *in vivo* is initiated primarily through the extrinsic pathway of coagulation. *FXII*^{−/−} mice have a normal hemostatic capacity. However, thrombus formation in *FXII*^{−/−} mice is defective in venous stasis models, arterial thrombosis and stroke models, and models for PE.^{23–26} Therefore, FXII may become a target for safe anticoagulation with thromboprotective but sustained hemostatic properties. FXII seems to be important for thrombus stability. Clinical data are controversial. A strong and almost linear association of decreasing FXII plasma activity between 90% and 10% with increasing all-cause mortality in a large Viennese patient cohort has been demonstrated. However, a FXII activity rate below 10% does not increase mortality rates, thus resulting in a U-shaped survival curve.²⁷ Larger epidemiological studies in the Netherlands and Switzerland did not find a correlation between FXII deficiency and increased thrombotic risk.^{28,29} Patients

with severe deficiency of factor XI, the substrate for activated FXII, have a reduced incidence of DVT.³⁰

Naturally occurring polyphosphates such as extracellular DNA, RNA, and inorganic polyphosphate are potent activators of the coagulation cascade (Fig. 1) and represent a potential therapeutic target for novel anticoagulation strategies.³¹ Recently, nucleic acid-binding polymers were discovered as molecular scavengers that can counteract the activity of any nucleic acid aptamer, regardless of its sequence, and can inhibit clotting *in vitro* and thrombosis without increasing bleeding in *in vivo* mouse models.³²

MICROPARTICLES (MPs) AND THROMBOSIS

MPs are vesicular structures resulting from the blebbing of activated or apoptotic cells.³³ They express a large repertoire of molecules representative of their parent cells. Like activated and apoptotic cells, MPs are also characterized by the disruption of phospholipid asymmetry, leading to exposure of phosphatidylserine (PS) on the outer leaflet. Consequently, PS can bind to coagulation factors and promote their activation, consistent with a procoagulant potential.

Furthermore, EC-derived microparticles (EMPs) contain TF, the initiator of the extrinsic coagulation pathway.

By addition of increasing amounts of EMPs the clotting time of normal plasma could be reduced, thus mediating thrombin generation.³⁴ EMPs from activated cells triggered TF-dependent thrombin formation in vitro and thrombus formation in vivo in a rat venous stasis model.³⁵ However, EMPs can also expose endothelial protein C receptor and exhibit anticoagulant properties,^{36,37} suggesting that EMPs participate in the tuning of the pro-/anticoagulant equilibrium. EMPs induce proliferation of ECs and inhibit apoptosis of ECs, presenting vascular protective effects.³⁸

Moreover, EMPs released upon activation with tumor necrosis factor- α generate and disseminate plasmin, also by expressing urokinase-type plasminogen activator (u-PA) and its receptor on the surface.³⁹ The MP-induced plasmin generation affects the angiogenic ability of endothelial progenitor cells (EPCs). In a bimodal dose-dependent manner, low amounts of EMPs increased and higher concentrations inhibited tube formation.³⁹

Elevated EMP levels were found in VTE patients,⁴⁰ and platelet-derived microparticle (PMP) levels were increased in PE patients.⁴¹ In another study, it was shown that circulating procoagulant microparticles (CPMPs) and PMPs were significantly elevated in patients with acute PE, compared with those in controls lacking cardiovascular risk factors. However, when CPMP and PMP levels were compared with those of controls who had cardiovascular risk factors, there were no significant differences, emphasizing that the presence of cardiovascular risk factors must be adjusted in conditions with elevated MP levels.^{42,43}

NEUTROPHIL EXTRACELLULAR TRAPS (NETs) AND THROMBOSIS

NETs kill bacteria and contribute to combating infection.⁴⁴ Upon activation, neutrophils undergo a multistep cell death program, so-called NETosis. In brief, enzymes from granules translocate to the nucleus and facilitate chromatin decondensation (e.g., by the action of peptidylarginine deiminase 4 [PADI4]⁴⁵). Internal membranes break down, and cytolysis contributes to NET release. NETs are intact chromatin fibers containing histones and other proteins that form scaffolds (Fig. 1), which can retain large quantities of microbes. The direct contact with antimicrobial proteins leads to fast elimination of infection.^{46,47}

NETs are large structures that can contribute to thrombus formation and may promote thrombus stability similarly to von Willebrand factor (vWF) and fibrin.⁴⁸ When perfused with blood, NETs cause platelet adhesion, acti-

vation, and aggregation. NETs also bind red blood cells (RBCs). RBCs may promote coagulation by exposing PS and altering blood viscosity.⁴⁹ In vitro, NETs stimulate the extrinsic pathway by cleaving TF pathway inhibitor⁵⁰ and stimulate the intrinsic coagulation pathway by binding FXII,⁵¹ thus promoting fibrin formation.

NETs are abundant in experimental deep-vein thrombi in baboons⁵⁰ and mice,^{21,52} where they colocalize with vWF and fibrin.^{50,52} NETs are predominantly found in the red, RBC-rich part of the thrombus (Fig. 1). Treatment of mice with DNase1^{21,52} cleaved the NET scaffold and prevented thrombus formation underscoring the importance of NETs for DVT.⁵⁰

In vitro, NETs provide a tissue-type plasminogen activator (t-PA)-thrombolysis-resistant scaffold for blood clots.⁵⁰ Recalcified blood was incubated with NETs releasing neutrophils. Only when blood was treated with the combination of t-PA, ADAMTS-13, and DNase did complete clot lysis occur. DNase- or t-PA-lysis alone led to partial thrombolysis of the blood clot. Blood clots treated with t-PA lacked fibrin but were held together by a scaffold of extracellular DNA.⁴⁶

CROSS TALK BETWEEN ECs AND PLATELETS

Cross talk between platelets and ECs may occur over a distance (paracrine signaling), via transient interactions, or through receptor-mediated cell-cell adhesion.^{53,54} Platelets release interleukin-1 β ,⁵⁵ transforming growth factor- β (TGF- β), platelet-derived growth factor, and vascular endothelial growth factor (VEGF), each of which may trigger signal transduction pathways in the endothelium. ECs express cell surface receptors or soluble mediators that either inhibit platelet function (e.g., nucleoside triphosphate diphosphohydrolases, prostacyclins, or nitric oxide) or promote platelet activation (e.g., platelet-activating factor).⁵⁴ Studies have demonstrated a critical role for the CD40-CD40L system in mediating reciprocal interactions between platelets and ECs.^{54,56} Platelet activation results in increased expression of CD40 and CD40L.^{56,57} GPIIb-IIIa-dependent adhesion of platelets to the endothelium results in CD40L-induced activation of ECs with secondary induction of TF,⁵⁷ cytokines, adhesion molecules,⁵⁸ matrix metalloproteinases, u-PA, t-PA, and urokinase receptor.⁵⁶ Thus, the platelet indirectly coordinates (via the endothelium) the changes in coagulation, leukocyte trafficking, and extracellular matrix modeling/turnover. At the same time, the interaction between platelets and ECs results in GPIIb-IIIa-mediated outside-in signaling, with secondary induction of CD40L⁵⁹ and P-selectin expression on the platelet surface.⁶⁰ In addition, activated platelets release

soluble trimeric CD40L that may engage platelet CD40 in an autocrine or paracrine manner, resulting in shape change and dense granule and α -granule release.^{54,59} A recent study suggests a role for CD40L in the pathogenesis of pulmonary hypertension.⁶¹ Platelet adhesion causes the secretion of inflammatory factors that stimulate ECs and thereby recruit additional platelets to the growing thrombus.⁶² E-selectins mediate platelet rolling, which is defined as the first loose contact between circulating platelets and vascular endothelium.⁶⁰ In response to inflammatory stimuli, ECs express E-selectin⁶³ and also P-selectin, which is rapidly expressed on the endothelial surface by translocation from membranes of the Weibel-Palade bodies to the plasma membrane.

In vivo studies showed that platelets from mice lacking P- and/or E-selectin roll as efficiently as wild-type platelets. Thus, platelet rolling does not require previous platelet activation, which is in accordance with the concept of endothelial inflammation as a trigger for platelet accumulation.⁶⁴ Recently, homozygosity in the single-nucleotide polymorphism Ser128Arg in the E-selectin gene has been found to be associated with recurrent VTE.⁶⁵ However, this polymorphism was not more frequent in patients with CTEPH than in the general population (I. M. Lang and J. Bernd, unpublished data).

DISTURBED VENOUS BLOOD FLOW AND INFLAMMATION AND THROMBOSIS

Hemodynamic forces and flow patterns modulate EC functions. Sustained laminar flow with high and directed shear stress, as in straight parts of the arterial tree, keeps the endothelium in a quiescent and thromboprotective phenotype.⁶⁶

However, disturbed flow with associated agitated low shear stress at branch points and curvatures generally activates ECs and upregulates atherogenic/thrombogenic EC genes and proteins, leading to localized atherosclerotic lesions. In the venous system, disturbed flow resulting from reflux, outflow obstruction, and/or stasis leads to venous inflammation and thrombosis.⁶⁶ In a state of disturbed flow, ECs change their morphology from laminar-flow-aligned to round shapes, short actin fibers are randomly distributed at cell periphery, and parallel stress fibers are distributed in the central region.⁶⁷ ECs in disturbed flow have higher proliferation and DNA synthesis rates, a higher permeability rate, accompanied by a discontinuous vascular endothelial cadherin and connexin43 distribution, and lower migration capability than ECs under laminar flow. Also, leukocyte adhesion and transendothelial migration are increased with activated ECs, which can be attrib-

uted to increased intercellular adhesion molecule-1, Tie1, and E-selectin expression.^{66,68}

RATE AND SEQUENCE OF THROMBUS ORGANIZATION IN THE DEEP VEINS

We have utilized a mouse model of stagnant-flow vena cava thrombosis to clarify the natural history of thrombus resolution.⁶⁹ In brief, a stenosis is produced in the vein by tying a 4-0 silk suture around the infrarenal inferior vena cava (IVC), distant from nearby branches, to include the 5-0 Prolene suture. The Prolene is then pulled to allow blood to continue to pass up the vein. Branches remain open. Thus, stagnant-flow venous thrombosis ensues. Within 8 hours a thrombus will be formed. In this model, thrombi resolve to approximately one-sixth of their original size by 28 days after IVC ligation. Reduced flow produces a laminar thrombus, allowing the study of cellular kinetics, and this model resembles nonocclusive DVT.⁷⁰ One limitation is the intrinsic overlap between thrombosis and thrombus resolution in this model, which can be addressed by calculating percent change of thrombus area over time to estimate relative resolution. Thrombus formation and resolution in murine models of large-vein thrombosis and in human venous thrombosis involve similar processes, including leukocyte recruitment, both of neutrophils and monocytic cells, activation of the TGF- β pathway, replacement of fibrin and erythrocytes with collagen, vein wall retraction, and neovascularization.⁷¹

Recently, mechanisms of DVT were analyzed with various models in sequence.²¹ Intravital 2-photon and epifluorescence microscopic images have shown blood monocytes and neutrophils crawling along and adhering to the venous endothelium, thus initiating DVT. Myeloid cell-derived TF activates the extrinsic coagulation pathway, resulting in enormous intraluminal fibrin formation characteristic of DVT. Furthermore, thrombus-resident neutrophils are crucial for subsequent DVT propagation by binding FXII and its activation through the release of NETs. Correspondingly, neutropenia, genetic ablation of FXII, or disintegration of NETs inhibits DVT amplification. Platelets interact with innate immune cells via glycoprotein Ib α and initiate leukocyte recruitment and neutrophil-dependent coagulation. The cross talk between monocytes, neutrophils, and platelets is responsible for the initiation and progression of DVT. However, the potential role of these mechanisms in thrombus resolution is unknown.

THE VESSEL WALL AND FIBRINOLYSIS

In a resting state, the endothelial surface is profibrinolytic and helps maintain blood in its fluid state.²² The contri-

bution of ECs to fibrinolysis varies with their metabolic status (i.e., quiescent or activated), their vascular derivation, and the concentration of other hemostatically active molecules in the local plasma milieu.²²

Binding of t-PA to ECs promotes their fibrinolytic activity and stimulates cell proliferation.^{72,73} ECs produce plasminogen activator inhibitor (PAI-1), which is associated primarily with the extracellular matrix, resulting in stabilization of its activity.⁷⁴ PAI-1 is a serine protease inhibitor, and its main function is to inhibit t-PA and u-PA. Quiescent ECs express little or no PAI-1,²² but after exposure to thrombin and inflammatory stimuli, the expression of PAI-1 is highly upregulated, which results in impaired fibrinolytic function.^{75,76}

Binding of thrombin to thrombomodulin (TM) accelerates activation of thrombin-activatable fibrinolysis inhibitor (TAFI). Activated TAFI cleaves basic carboxyterminal residues within fibrin and other proteins, resulting in loss of plasminogen/plasmin and t-PA binding sites and subsequent retarded fibrinolysis.⁷⁷ By regulating the expression of TM, ECs decrease the rate of intravascular fibrinolysis.²²

Fibrin from CTEPH patients showed resistance to plasmin-mediated lysis, compared with fibrin from control subjects.⁷⁸ Fibrinolytic resistance causes persistence of the N-terminus of the β -chain B- β of fibrin. Five fibrinogen variants with corresponding heterozygous gene mutations (dysfibrinogenemias) were observed in 5 of 33 CTEPH patients, whereas 3 unrelated CTEPH patients carried the B- β P235L mutation (Fig. 1).⁷⁹ Differences in the molecular structure of fibrin are believed to contribute to vascular thrombus persistence.⁸⁰

PULMONARY ENDOTHELIUM

Pulmonary artery ECs reside on a thick basement membrane that separates the intima from underlying smooth muscle layers. They interact with as many as 6 adjacent ECs and are aligned in the direction of blood flow. At a distinct vessel diameter of about 25 μ m, pulmonary artery ECs change their phenotype and become microvascular (capillary) ECs. Capillary ECs are separated by only a thin basement membrane from nearby type I pneumocytes, interact with just one neighboring cell, and do not exhibit any flow alignment.⁸¹ For the regulation of vascular tone, the endothelium keeps a well-adjusted balance of endothelial vasodilators and vasoconstrictors.⁸² Although few data exist on the differential expression patterns of normal pulmonary ECs as a distinct vascular compartment, there is evidence of differential regulation and expression of TGF- β , a family of multifunctional cytokines control-

ling cell growth, differentiation, and extracellular matrix deposition in the lung.⁸³ Furthermore, hypoxia-induced hemoxygenase regulation differs in systemic and lung ECs.⁸⁴ Genetic profiling of ECs in pulmonary hypertension has disclosed an increase of 5-lipoxygenase⁸⁵ and endothelin⁸⁶ and a decrease of prostacyclin synthase.⁸⁷

VASCULAR SMOOTH MUSCLE CELLS (SMCs)

SMCs are characterized as highly specialized cells that embody the media of the vessel wall. Physiologically, they maintain vasomotor tone via contraction or relaxation in response to many metabolic and hormonal stimuli, as well as maintaining vessel integrity by proliferation and synthesis of extracellular matrix.^{88,89} Differentiated SMCs (i.e., SMCs with a “contractile phenotype”) are distinct from other cell types because they display a low rate of proliferation and low synthetic activity and express a unique repertoire of contractile proteins, ion channels, and signaling molecules required for contractile function. Unlike terminally differentiated skeletal or cardiac myocytes, SMCs retain remarkable plasticity and can dedifferentiate to SMCs with a “synthetic phenotype” in response to changes in local environmental cues. SMC accumulation in the neointima is a common feature in vascular diseases, such as atherosclerosis, restenosis, and transplant vasculopathy, that contribute to cardiovascular morbidity and mortality.⁹⁰ The current view is that dysfunctional endothelium and/or inflammatory cells produce growth factors, proteolytic agents, and extracellular matrix proteins that can promote media to intima migration of SMCs, accompanied by a contractile-to-synthetic phenotype switch.⁹¹

PULMONARY EC-DEPENDENT FIBRINOLYSIS AFTER PE

Because pulmonary emboli do generally resolve within 6 months,⁹² it has been proposed that the pulmonary circulation harbors remarkable fibrinolytic capacity.⁹³ Proteases of the fibrinolytic system are crucial for the degradation of pulmonary thrombi.⁹⁴ In agreement with this paradigm, t-PA is expressed in ECs and SMCs of human main pulmonary artery, thus becoming a significant source of luminal plasminogen activator activity. In normal human pulmonary artery, there is an elevated t-PA/PAI-1 expression ratio, with free t-PA rapidly accessible for thrombolysis, thus resulting in increased net fibrinolytic activity.⁹⁵ Within hours after PE, a sequential upregulation of fibrinolytic genes occurs in the wall of mainstem pulmonary artery.¹⁷ One speculation for the increased capacity to efficiently lyse pulmonary emboli may be the developmental requirement in humans to compensate for

the increased rate of thromboembolic episodes due to erect posture. The adventitia is an important source for plasminogen activator activity but does not account for differential fibrinolytic gene expression.⁹⁵

VASCULAR REMODELING IN PE

To understand the role of the fibrinolytic system in thrombus organization, human tissues from patients who had died of acute PE were investigated.¹⁷ Several important observations were made. First, different stages of thrombus organization were found in a single individual specimen. Local expression of plasminogen activators and PAI-1 were observed in distinct patterns. The t-PA antigen was primarily detected in regions containing an intact endothelial lining. In accord with published data, u-PA expression was initially restricted to monocytic cells within thromboemboli⁹⁶ and subsequently detected in cells that appeared to be migrating from the vessel wall toward the thrombus, supporting the role of u-PA in cell migration.⁹⁷ High PAI-1 concentrations were detected in ECs directly in contact with fibrin, which is in good agreement with data showing PAI-1 induction by thrombin⁹⁸ and by TGF- β , a polypeptide growth factor released from platelets.^{99,100} PAI-1 expression was observed within neoendothelial cells overgrowing vascular thrombi, as soon as 1 week after thrombosis.¹⁰¹ An embolizing thrombus may cause local EC injury by mechanically impinging on the EC surface. Direct contact of ECs with fibrin has been shown to modulate a number of phenotypic properties, including loss of organization, severing of cell-cell contacts, and cell retraction.¹⁰² In addition, the thromboembolus within the pulmonary vessel acts as a new interface that is subsequently penetrated by cells that are involved in the organization process. These cells are characterized by the concomitant production of proteases and protease inhibitors, initiating the entry of macrophages and facilitating the degradation of fibrin and capillary sprouting.⁹⁷ Furthermore, local hypoxia may upregulate PAI-1 expression¹⁰³ in the remodeling/organization regions within the thrombi and induce expression of connective-tissue growth factor (CTGF) via a hypoxia-responsive element in the CTGF promoter.¹⁰⁴ Recent data have shown that genetic ablation of the *BMPR2* gene in pulmonary endothelium induces in situ thrombosis, suggesting a role for the TGF- β pathway in thrombus organization.¹⁰⁵

CTEPH AND PULMONARY ECs

There exists no evidence that thrombus organization in the pulmonary circulation has different underlying rules than that in the deep veins. CTEPH is characterized by

predominantly major-vessel obstructions,¹⁰⁶ resulting in increased pulmonary vascular resistance. The molecular mechanisms underlying thrombus persistence are unknown.^{107,108} The typical CTEPH thrombus represents a cast of the pulmonary vascular bed, consisting of endothelium, SMCs, fibroblasts, and fresh thrombus. CTEPH is largely understood as of thromboembolic origin. However, patients with CTEPH lack classic plasmatic thromboembolic risk factors.¹⁰⁹ Furthermore, neither systemic¹¹⁰ nor local¹¹¹ imbalances of fibrinolytic proteins in the pulmonary arterial wall have been detected. In addition, it is virtually impossible to induce the disease in animal models by repeated embolizations,¹¹² suggesting alternative, non-thromboembolic hypotheses.¹¹³ The difficulty of inducing CTEPH by repeated release of preformed clots from the IVC of mongrel dogs¹¹² was resolved by a thorough biochemical dissection of factors contributing to increased vascular fibrinolytic activity in these animals.¹¹⁴ It was found that high plasma levels of u-PA activity are present in this species. Furthermore, u-PA is associated with canine platelets and mediates rapid clot lysis. In recent years, it has been recognized that major-vessel remodeling and classic small-vessel pulmonary arteriopathy coexist in CTEPH,^{107,115} suggesting a complex remodeling process involving factors beyond traditional thrombosis.

To dissect the endothelial fibrinolytic system in non-resolving pulmonary thromboemboli, conditions were established to culture ECs from unthrombosed main pulmonary arteries of patients during surgical pulmonary endarterectomies. Cultured patient pulmonary arterial ECs secreted similar levels of t-PA and PAI-1 in the absence and presence of thrombin when compared to donor pulmonary artery ECs.¹¹¹

ANGIOGENESIS IN THROMBUS RESOLUTION

Natural thrombus resolution comprises retraction of the thrombus from the vein wall, inflammatory cell infiltration, and the formation of new vascular channels.¹¹⁶ Platelet EC adhesion molecule (PECAM-1, CD31) and vascular endothelial-cadherin mediate important cell-cell adhesions.^{22,117,118} Enhanced thrombus neovascularization and rapid vein recanalization have been achieved in experimental models after administration of proangiogenic agents, such as VEGF.¹¹⁹ Recently, hypoxia and upregulation of hypoxia-inducible factor 1- α were identified as additional stimulators of venous thrombus recanalization.¹²⁰ These data confirm an integral role for angiogenesis in the organization process of vascular thrombi (Fig. 1).

ENDOTHELIAL PROGENITOR CELLS (EPCs)

EPCs were first described as a minor subpopulation of peripheral blood mononuclear cells with expression of both endothelial (VEGF-receptor) and stem cell (CD34) lineage antigens on the surface and with vasculogenesis-promoting ability.¹²¹ Since then, many studies have focused on the characterization of functional properties of EPCs in either physiological or pathological conditions. Many vascular-tone- and hemostasis-associated functions have been explored and demonstrated in EPCs. For example, the protease-activated receptor-1 (PAR-1) activation in EPCs leads to increased angiogenesis in vitro via the angiopoietin pathway and to increased cell proliferation and migration by promoting SDF-1/CXCR4 expression.^{122,123} Thrombin may modulate the fibrinolytic pathway by activating PAR-1 on EPCs.¹²⁴ EPCs increase the amount of PAI-1, thus inhibiting spontaneous lysis of a fibrin network.¹²⁴ EPCs are recruited into resolving venous thrombi, suggesting a role in thrombus organization and orchestrating thrombus recanalization,¹⁸ possibly via Tie2-expressing monocytes.¹²⁵ Upon in vitro activation with lipopolysaccharide, EPCs isolated from healthy subjects were able to upregulate TF expression and procoagulant activity.¹²⁶

CONCLUSIONS

Recent data utilizing various models have contributed to a better understanding of venous thrombosis and the resolution process that is directed at maintaining vascular patency. Our new understanding is that, compared with the role of plasmatic factors, cellular components in thrombosis and thrombus resolution have been neglected. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in vivo, in parallel with NETs, extracellular RNAs, and MPs (Fig. 1). Mechanisms of thrombus resolution (DNases, the B- β domain of fibrinogen, and angiogenic growth factors) still have to be explored in more detail.

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