Inflammation and Acute Venous Thrombosis

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Abstract

Inflammation is known as a controlled response to an injury that protects against further injury and clears damaged tissue. However, uncontrolled inflammation can lead to a marked breakdown of the extracellular matrix as well as organ destruction. Stewart et al. demonstrated in a vein thrombosis model that leukocytes migrate into vein walls with an intact layer of endothelial cells, subsequently linking inflammation and thrombosis. Since then, multiple studies have reported on the interaction between inflammation and vein thrombosis. This article summarizes published material that assesses the relationship between inflammation and acute vein thrombosis. The article will focus on the role of endothelial cells, leukocytes, platelets, and microparticles in acute vein thrombosis and discuss the role of thrombin, P-selectin, platelet-activating factor, endothelin-1, and nitric oxide from the perspective of inflammation.

Keywords

Inflammation, acute thrombosis, thrombin, selectin, leukocytes

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Venous thromboembolism (VTE) includes both deep vein thrombosis (DVT) and pulmonary embolism (PE). VTE occurs worldwide, and in all age groups and socioeconomic populations in North America and western Europe.[1,2] In a recent study by Heit et al., the estimated total annual cases of VTE occurring in the US exceeded 900,000, of which more than 250,000 were fatal.[3] Despite progress in medicine and biology, no changes in the incidence of VTE were noted during the 25-year cohort study.[3]

Elucidating the intrinsic mechanisms involved in thrombus formation is critical to understanding VTE pathophysiology. Stewart et al. demonstrated leukocyte migration into the vein wall with an intact layer of endothelial cells (ECs) during DVT,[4] linking inflammation and thrombosis. Furthermore, recent studies have reported on the interaction between inflammation and thrombosis.[5–9] This article summarizes published material to assess the relationship between inflammation and acute vein thrombosis. In particular, this article will focus on the role of ECs, leukocytes, platelets, microparticles, thrombin, P-selectin, platelet-activating factor, endothelin-1, and nitric oxide (NO) from the perspective of inflammation.

Inflammation

Inflammation is a complex, highly regulated biological process that can be triggered by several stimuli, such as pathogens, noxious mechanical and chemical agents, and autoimmune responses.[10] In the clinical setting, inflammation is described as redness and swelling with heat and pain. Biologically, these signs and symptoms are explained by an increasing microvascular caliber, enhanced vascular permeability, leukocyte recruitment, and the release of inflammatory mediators.[10]

Inflammation in physiologic states is a controlled response that protects against further injury and clears damaged tissue. In disease states such as sepsis, however, uncontrolled inflammation can lead to a marked breakdown of the extracellular matrix as well as organ destruction.[10] Inflammation can also be acute or chronic—two well-characterized states in which neutrophils and monocytes, respectively, play an important role. Similarly, DVT is a process with acute and chronic phases. In this setting, the main cells observed during acute DVT are the neutrophils. Monocytes are observed during the chronic phase. This parallel between inflammation and DVT is highly suggestive of a relationship.

Inflammation and Vein Thrombosis

Using a vein thrombosis model in a dog, Stewart et al. showed that large numbers of white cells adhered to the vessel walls, passed through the endothelial intercellular junctions, and accumulated in pockets between the endothelium and basement membrane.[4] Since then, several studies have demonstrated the interaction between inflammation and thrombosis, noting the importance of ECs in this link.[5–9] The question of what activates ECs towards a procoagulant effect is fundamental in understanding the pathophysiology of VTE.

Endothelial Cells

ECs play a pivotal role in venous thrombosis in healthy individuals. While arterial thrombosis requires EC disruption with collagen exposure (as

occurs in atherosclerotic plaque rupture), ECs are "intact and no collagen exposure is needed in order to generate thrombi during VTE."[11] One of the main functions of ECs is to modulate the pro- and anticoagulant mechanisms. The anticoagulant properties of ECs include suppressing coagulation (platelet activation and adhesion) and inflammation (leukocyte activation).[9] Despite this, a procoagulant effect is observed during states of EC activation and disturbance, either physical (vascular trauma) or functional (sepsis).[9] Thus, under an adequate procoagulant stimulus, the balance between anti- and procoagulant mechanisms is altered toward procoagulant activity.[1,12,13]

Thrombin

Thrombin (factor II) is a major procoagulant molecule that cleaves fibrinogen into fibrin, forming a fibrin mesh. Specifically, thrombin cleavage α and β chain of fibrinogen releases fibrinopeptides A and B, respectively. This causes fibrin monomer polymerization that stabilizes the initial platelet plug. Thrombin also activates factor XIII, which contributes to thrombus amplification through the activation of platelet factors V and VIII.[14] In addition to this, thrombin itself has an inflammatory effect. Thrombin induces a rapid EC activation, increasing P-selectin expression and prostacyclin and platelet-activating factor secretion.[15,16] Thrombin may also activate transcription factor nuclear factor-kappa B (NF-κB) independently of the well-known pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1).[17,18] This is critical in leukocyte activation and recruitment as it promotes EC secretion of monocyte chemotactic protein-1 and IL-8.[18,19]

P-selectin and Leukocytes

Selectins are cell-adhesion molecules that have critical roles in inflammation and thrombogenesis.[5,9,20] P-selectin is involved in leukocyte rolling and adhesion, which is an early inflammatory mechanism that facilitates leukocyte transmigration.[4,20] Animal studies utilizing rat and mouse thrombosis models have demonstrated the upregulation of P-selectin in the vein wall as early as six hours after thrombus induction.[20] These studies show that P-selectin is a common molecule that links inflammation and thrombosis *in vivo*.

The P-selectin receptor known as P-selectin glycoprotein ligand 1 (PSGL-1) is a glycoprotein expressed on the surface of leukocytes and platelets. It is key in the recruitment of leukocytes and platelets to inflamed tissue. The PSGL-1:P-selectin complex promotes rolling and adhesion of leukocytes and platelets, respectively, which ultimately results in increased vein wall cell infiltration.[21–22]

Chronologically, the initiation of inflammation and/or thrombosis processes occurs almost immediately after EC activation. P-selectin is stored inside the EC, within the Weibel-Palade bodies. Molecules of P-selectin are released upon EC activation, as reported by Wagner et al.[23,24] The exocytosis of Weibel-Palade bodies initiates a rapid translocation of P-selectin to the EC surface, resulting in its adhesiveness for leukocytes and platelets.

Platelets

The role of platelets in arterial thrombosis is well known, but its action in venous thrombosis remains obscure. Platelets are anucleate circulating blood particles derived from bone marrow megakaryocytes.[2] The megakaryocytes extend their cytoplasm into the bone marrow sinusoid and release a small portion of cytoplasm containing alpha and dense granules surrounded by a bi-lipid membrane into the blood circulation.

Under physiological conditions, circulating platelets are at rest (or non-activated), expressing PSGL-1.[24,25] As previously mentioned, thrombin promotes EC activation, resulting in P-selectin expression and platelet-activating factor secretion. EC P-selectin expression allows EC–platelet interaction (platelet PSGL-1:EC P-selectin), and platelets become activated by EC platelet-activating factor.[21,24] Ultimately, platelets excrete the contents of their granules, increasing platelet adhesiveness and potentiating platelet aggregation.[25] P-selectin is contained in platelet alpha granules. These granules are excreted with platelet activation, leading to P-selectin expression. These processes favor leukocyte–platelet cross-talk as a direct consequence of platelet activation.[21,24] It has been shown that platelets release a greater amount of P-selectin compared with ECs.[24] Thus, once platelets are attached to the vein wall, the concentration of available P-selectin increases dramatically, improving the leukocyte recruitment efficiency of the vein wall.[24] In a mouse model, Frenette et al. demonstrated that platelets roll on the vein wall (in a similar manner to leukocytes) after EC activation.[21]

Microparticles

Circulating cell-derived microparticles are small vesicles (<1µm) consisting of plasma membrane surrounding a small amount of cytoplasm that contains cell-specific surface molecules that characterize their cell of origin.[26,27] Procoagulant microparticles derived from ECs, leukocytes, and platelets contribute to the coagulation and amplification of thrombosis.[2,26,28] Microparticles are present in the blood of healthy individuals but increase under specific circumstances, including DVT.[2,29] ECs, leukocytes, and platelets have a very well-structured plasma membrane characterized by a controlled transverse lipid distribution.[30] Specific areas of the plasma membrane have been identified as 'lipid rafts,'[31] a formation of sphingolipids and cholesterol. It has been postulated that lipid rafts enhance signaling functions by providing platforms that physically concentrate receptors and downstream kinases involved in signaling pathways.[32] The membrane of microparticles is rich in lipid rafts.[30]

Moreover, lipid-raft-derived microparticles concentrate tissue factor.[30] Tissue factor and the expression of prothrombinase activity on the membrane and PSGL-1 are involved in the procoagulant activity of microparticles.[9,33] Therefore, the fusion of microparticles with activated platelets promotes thrombus formation in a tissue-factor-dependent manner.

During inflammation, the activation of ECs upregulates the expression of P-selectin on their surface, leading to the formation of EC P-selectin:PSGL-1-leukocyte complexes. These complexes stimulate the production of microparticles from leukocytes, particularly monocytes, along with platelets and ECs. Furthermore, the accumulation of leukocyte markers expressed on the surface of microparticles in the growing thrombi is mediated by the P-selectin:PSGL-1 complex,[9] resulting in a dramatic increase in microparticle concentration at the site of vein wall injury and inflammation.[5]

It has been suggested that microparticles participate in platelet activation. Recent reports have postulated that platelet activation may also result from the interaction of macrophage-1 antigen, found on leukocyte-derived microparticles, via microparticle:glycoprotein Ib (platelet) alpha polypeptide.[34,35]

In addition, a recent comparative proteomics analysis of microparticles showed that galectin-3 binding protein (Gal-3 BP) was significantly upregulated in DVT patients compared with negative controls.[36] Galectins are a family of carbohydrate-binding proteins that have a high affinity for the galactosides present on cell surfaces and extracellular glycoproteins.[37] Galectins are involved in multiple biological functions, including modulation of cell apoptosis, cell activation, and inflammation.[37] It has been suggested that galectins are involved in P-selectin expression.[37] Although the role of galectins and Gal-3 BP in thrombogenesis remains unclear, these observations suggest a new avenue linking inflammation and thrombosis.

Interleukin-6

As previously mentioned, leukocyte diapedesis through an intact layer of endothelial cells has been described in early stages of venous thrombosis (VT), linking acute inflammation and acute thrombosis.[38–42] This event further incites the inflammatory response and alters the composition of local cytokines. Increased plasma levels of interleukin-6 (IL-6), a major inflammatory cytokine, have been demonstrated during VT[43] and post-thrombotic syndrome (PTS)[44]. While these studies suggest that IL-6 may be used as a marker for VT, its role as an active cytokine in the development of PTS has been recently established.[45] Using the mouse inferior vena cava (IVC) ligation model to generate vein thrombosis and the neutralization of IL-6, with an antibody anti-IL6, we studied the role of IL-6 in VT. We found that IL-6 had an acute and a chronic effect in VT. During the acute phase of thrombosis, CCL-2 was significantly decreased, two days after thrombosis in the group where circulating IL-6 was blocked. This was demonstrated at the gene expression level as well as at the protein level.

Moreover, the decrease in CCL-2 caused a reduction in the number of monocytes recruited into the injured area, at an intermediate time-point (six days after VT) indicating a dynamic and linked process. At the chronic time-point (14 days after surgery), a statistically significant decrease in fibrosis was observed in the group treated with anti-IL6 versus control. This was demonstrated using several methods, including Masson's stain quantification of fibrosis. In summary, we have shown a relationship between IL-6, CCL-2, monocyte recruitment, and fibrosis for the first time in the context of VT, exhibiting a potential pathway that started early on in VT and continued to have an impact on chronic VT as well.[45] This is an important step in understanding the link between inflammation and thrombosis in the context of VT, and indeed a potential therapeutic target for PTS.

Platelet-activating Factor

Platelet-activating factor, also known as acetyl-glyceryl-ether-phosphorylcholine (AGEPC), is a molecule secreted by ECs, macrophages, mast cells, and leukocytes.[46] The thrombotic and pro-inflammatory effects of platelet-activating factor have been suggested in several studies. In an animal study using baboons, thrombocytopenia and neutropenia were observed secondary to the intravenous infusion of platelet-activating factor, suggesting both thrombotic and pro-inflammatory effects.[47] In addition, platelet-activating factor was shown to cause aggregation and accumulation of platelets and subsequent changes in local blood flow at the sites of experimental thrombosis *in vivo*.[48]

One of the main roles of platelet-activating factor during inflammation is to activate the leukocytes adhered to the vessel wall, in a paracrine fashion, via adhesion molecules expressed by ECs.[46,49] Activation of leukocytes induced by platelet-activating factor leads to priming for enhanced inflammatory responses, polarization and directional migration, degranulation and oxygen radical generation.

Endothelin-1

Endothelin-1 (ET-1) is a 21 amino acid peptide produced by a variety of cells including endothelial and smooth-muscle cells.[50] ET-1 receptors include ET_A and ET_B.[50–51] ET_A is expressed in inflammatory cells.[52] Specific locations for ET_B receptors have been suggested: in vein ECs for ET_{B1} and vein vascular smooth-muscle cells.[50] Once ET-1 binds to its receptor (Gq-proteins) on the vascular smooth muscle it induces an increase in inositol 1,4,5-triphosphate levels, with subsequent calcium release and muscle contraction.[50] Specifically, it has been shown that EC dysfunction and inflammation contribute to an overproduction of ET-1 in humans.[52] The exact role of ET-1 in VTE, however, remains unclear. Future studies are needed to elucidate the role of ET-1 in venous thrombosis.

Prostacyclin and Nitric Oxide

Finally, prostacyclin and NO are secreted by ECs.[53] These molecules contribute synergistically to vessel homeostasis by reducing the tone and growth of vascular smooth-muscle cells, platelet aggregation, and leukocyte adhesion to the endothelium, thus decreasing the vessel's susceptibility to form thrombi.[2,53] Interestingly, Osanai et al. demonstrated that vessel homeostasis might be maintained through an increase in prostacyclin production in vascular ECs when NO synthesis is impaired.[54] Endothelial NO synthase function has been widely studied in arterial ECs, but there is also evidence suggesting that decreased NO production may play a role in the development of venous disease.[55–57]

Summary

This article has reviewed the current evidence of inflammatory mechanisms in vein thrombosis. The Conrad Jobst Vascular Laboratory at the University of Michigan is currently studying other inflammatory molecules, such us galectins and inflammatory cytokines. As previously mentioned, Gal-3 BP was upregulated in human microparticles obtained from patients undergoing DVT.[37]

Acute VTE is a complex and dynamic process. ECs, platelets, and leukocytes are the main circulatory elements involved in vein thrombosis. Inflammation has been shown to be closely involved in vein thrombus formation. Inflammatory cytokines and thrombin are thought to orchestrate this early phase of DVT. Further understanding of the intrinsic mechanism involved in DVT may help to develop new preventive and/or therapeutic modalities directed to these potential targets. ■

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