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Inflammation, Infection and Venous Thromboembolism

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Abstract

The association between inflammation, infection, and venous thrombosis has long been recognized; yet, only in the last decades have we begun to understand the mechanisms through which the immune and coagulation systems interact and reciprocally regulate one another. These interconnected networks mount an effective response to injury and pathogen invasion, but if unregulated can result in pathologic thrombosis and organ damage. Neutrophils, monocytes, and platelets interact with each other and the endothelium in host defense and also play critical roles in the formation of venous thromboembolism. This knowledge has advanced our understanding of both human physiology and pathophysiology, as well as identified mechanisms of anticoagulant resistance and novel therapeutic targets for the prevention and treatment of thrombosis. In this review, we discuss the contributions of inflammation and infection to venous thromboembolism.

Keywords

Inflammation; venous thrombosis; venous thromboembolism; thromboinflammation; coagulation; neutrophil extracellular traps; platelets; endothelium

Subject Terms:

Endothelium/Vascular Type/Nitric Oxide; Peripheral Vascular Disease; Inflammation

Venous thromboembolism (VTE), encompassing both pulmonary embolism (PE) and deep vein thrombosis (DVT), is a leading cause of morbidity and mortality worldwide. VTE is responsible for 300,000 deaths annually in the United States alone and is the third most

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common cause of cardiovascular mortality¹. Following an initial VTE, patients remain at risk of recurrent thrombosis, which is only partially mitigated by treatment with anticoagulants. The fatality rate of PE continues to increase, most notably in adults younger than 65 years of age². Similarly, patients with acute or chronic inflammatory diseases, including sepsis, inflammatory bowel disease, and autoimmune disease, are at higher risk of initial and recurrent VTE^{3, 4}. These concerning trends are despite the introduction of direct oral anticoagulants as well and improved use of VTE prophylaxis in high-risk situations. Taken together, these observations highlight the need for a deeper understanding of the molecular and cellular mechanisms that culminate in VTE in order to identify novel therapeutic approaches.

Virchow's triad of factors predisposing to thrombosis- altered blood flow or stasis, changes in composition of blood (hypercoagulability), and vessel wall damage, have been the foundation for our understanding of the pathophysiology of venous thrombosis. However, inflammatory molecules and immune cells are now recognized as central contributors to thrombosis. In many clinical contexts, the risk factors for VTE, including inflammatory bowel disease, systemic lupus erythematosus, obesity, surgery, cancer, and acute and chronic infection are characterized by dysregulated immune networks that intersect with coagulation³. Immune dysregulation and disruption of the natural antithrombotic blood:vessel interface adds contextual elements to Virchow's triad which may more accurately reflect the modern understanding of VTE^{5, 6}. Current therapeutics for VTE target coagulation factors and are associated with a risk of bleeding, but don't address the key inflammatory processes that lead to thrombosis and leave a significant gap in treatments available to prevent and treat VTE effectively.

The relationship between inflammation and plasma coagulation is an intricate network that balances an organism's response to injury and pathogen invasion with maintaining homeostasis. Perturbation of this balance can tip physiologic responses toward pathological thrombosis and organ damage. The molecular interactions between inflammation and coagulation have been elegantly reviewed by Foley et al⁷. In this Review, we focus on the contributions of inflammation to the pathogenesis of VTE by exploring the nuanced cellular interactions between immune responses and coagulation that result in VTE. We begin by defining central concepts in the relationship between thrombosis and inflammation, followed by a review of the vascular immune cell-based model of venous thrombogenesis.

Hemostasis, immunothrombosis, and deranged thromboinflammation

Hemostasis is a protective process to reduce blood loss after injury through a series of highly regulated steps, initially by sealing the damaged blood vessel and ultimately by supporting vessel repair. Central to this process is the controlled generation of thrombin and fibrin, which is contemporaneously described using a cell-based model of coagulation (Figure 1). Platelets, leukocytes, and endothelial cells are the primary cellular components and they localize coagulation reactions, ensuring activation of coagulation factors occurs at areas of injury. Pathologic thrombosis in veins or arteries occurs when coagulation is triggered either in the absence of vascular injury, or if unchecked coagulation occurs at sites of injury. In the deep veins, the majority of thrombi form on grossly intact endothelium and frequently

within valve pockets of deep veins, where lower blood oxygen levels and blood stasis occur⁸. Thrombi in the pulmonary circulation can occur *in situ*, or as pulmonary emboli when venous thrombi become unstable, detach, embolize to the lungs, and occlude pulmonary circulation.

Similarly, inflammation, an early defense mechanism of the immune system in response to infection, irritation, or injury is necessary for host defense. Characterized by increases in local blood flow, recruitment of leukocytes, and release of chemokines and cytokines, inflammation contains and eliminates pathogens, and facilitates wound healing. Immunothrombosis, the result of reciprocal actions of the immune and coagulation systems, is an emergent response to the entry of infectious pathogens into the vasculature. Following pathogen recognition, local thrombosis and microvascular occlusion aid in control of infection dissemination and pathogen elimination⁹. This thrombotic mechanism of controlling infections is evolutionarily conserved, and ancient organisms that lack an adaptive immune system, such as Horseshoe crabs, rely on clotting factors and a single type of circulating blood cell to ward off pathogen invasion through coagulation of hemolymph¹⁰. Infectious diseases have exerted external pressures that influenced the evolution of the human hemostatic system. For example, the acquisition by Group A Streptococcus of the ability to produce a streptokinase that specifically activates human plasminogen provides a window into the pathogen-host “arms race” in physiologic coagulation factor interactions^{11, 12}. Distinct from physiologic immunothrombosis, exaggerated and uncontrolled activation of the immune and coagulation systems can lead to microvascular and macrovascular thrombosis called “thromboinflammation” and result in tissue damage¹³. The historical separation of the inflammatory and coagulation systems has curtailed a more complete understanding of the mechanisms of hypercoagulability described in Virchow’s triad.

Endothelial activation and local blood:vessel barrier dysfunction

The layer of endothelial cells at the blood:vessel interface is a physical barrier between blood-borne clotting factors and prothrombotic extracellular matrices. The intact endothelium tonically maintains blood fluidity and vascular homeostasis through anticoagulant and anti-inflammatory properties, including the production of prostacyclins, nitric oxide (NO), and the ADP-dissipating ectonucleotidase CD39^{14, 15} (Figure 1). The single cell layer endothelium also exert a fibrinolytic counterbalance to physiologic thrombosis, by producing tissue-type plasminogen activator (tPA)¹⁶ and urokinase plasminogen activator (uPA)¹⁷. The endothelial lining actively resists inflammation by controlling the expression of adhesion and signaling molecules at its luminal surface that facilitate leukocyte recruitment.

Natural anticoagulants on the endothelium that also reduce inflammation include the glycosaminoglycans (principally heparan sulfate (HS) and chondroitin sulfate (CS)), tissue factor pathway inhibitor (TFPI), endothelial cell protein C receptor (EPCR), and thrombomodulin (TM)^{18, 19}.

Following injury or local inflammation, the endothelium loses its anticoagulant and anti-inflammatory properties to eliminate the insult and initiate repair. This phenotypic shift can be triggered by hemodynamic flow pattern changes, cytokine signals, hypoxia, tissue injury, endotoxemia, or pathogen recognition^{5, 18, 20, 21}. Left unchecked, these physiologic responses exert a pathologic effect. Rapid loss of anticoagulant molecules and vascular integrity are accompanied by increases in the expression of leukocyte adhesion molecules and cytokine production. In response to metabolic and cytotoxic insults such as hyperglycemia, endotoxemia and sepsis, matrix metalloproteases dissolve the antithrombotic endothelial glycocalyx coated with HS and CS, resulting in the loss of binding sites for the serine protease inhibitor, antithrombin III (ATIII)^{22,23}. The associated shedding of EPCR and TM further breaks down the endothelial anticoagulant and anti-inflammatory shield by reducing its capacity to activate protein C. The importance of the APC pathway in both inflammation and thrombosis has been reviewed previously^{7, 24}.

The endothelium is also involved in immune cell trafficking and modulating inflammatory responses. Simultaneous with the denudation of the vascular anticoagulant surface, adhesive glycoproteins (P-selectin (P-sel), E-selectin (E-sel), intracellular adhesion molecule-1 (ICAM-1), venous cellular adhesion molecule-1 (VCAM-1)) are rapidly mobilized to the surface of activated endothelial cells and tether leukocytes through ligand-receptor interactions to initiate leukocyte-endothelial crosstalk. P-sel is stored in subplasmalemmal Weibel-Palade bodies (WPB) in endothelial cells, and in the α granules of platelets^{25, 26}. Rapid exocytosis of these intracellular storage depots can be triggered by extracellular signals such as thrombin, histamine from mast cells, bradykinin, tissue necrosis factor- α (TNF- α), bacterial toxins, epinephrine, anaphylatoxins, or disturbed blood flow, and results in translocation of P-selectin to the cell surface²⁷⁻²⁹. These adhesion molecules are important coordinators of thrombo-inflammation. Intravital microscopy studies have identified that early leukocyte-endothelium interactions in veins are facilitated by selectins, ICAM-1, and VCAM-1. P-sel has been proposed as a sensitive and specific clinical biomarker for diagnosis of VTE, and improves prospective VTE risk stratification in patients with cancer who are undergoing chemotherapy^{30, 31}. In animal models, P-sel inhibition is effective in preventing and treating VTE^{32, 33}, and crizanlizumab, a monoclonal antibody that targets P-sel, is used to reduce vaso-occlusive crises in patients with sickle cell disease^{34, 35}. P-sel and E-sel inhibition are now under investigation as treatments for patients with COVID-19 associated vasculopathy^{36, 37}.

Early in endothelial activation, exocytosis of WPB also delivers von Willebrand Factor (VWF) to the endothelial plasma membrane, where it binds to platelets via the glycoprotein Iba (GPIb α) receptor, an interaction that is essential for thrombus formation³⁸. Under low-shear conditions, VWF also mediates leukocyte recruitment via direct binding to β 2 integrins and P-selectin glycoprotein ligand (PSGL)-1, and increases endothelial permeability that may promote leukocyte extravasation³⁹. The important role of VWF in inflammation and thrombosis is highlighted by recent observations of elevated levels of VWF and FVIII in diet-induced obese mice and protection against obesity-induced thrombosis by inhibition of VWF⁴⁰. In large, cohort studies, gene variants associated with plasma VWF levels segregate with incident VTE, and a novel *Vwf* locus was included in a recently validated polygenic risk score for VTE^{41, 42}. Given its rapid mobilization following

WPB release, the level of VWF in circulation is often used as a marker of endothelial activation and vascular inflammation. Indeed, in patients hospitalized with COVID-19, circulating levels of VWF and TM were associated with severity of illness and with mortality⁴³.

Endothelial activation and subsequent alterations in hemostatic balance occur in response to diverse infectious and sterile-inflammatory conditions, including bacterial and viral infections such as SARS-CoV-2, as well as in chronic inflammatory states such as sickle cell disease, diabetes, obesity, lupus, and others^{44, 45}. Circulating pathogen- and damage-associated molecular patterns (PAMPs, DAMPs), cytokines (e.g. TNF- α and IL-1 β), and autoantibodies secreted from leukocytes and platelets bind to receptors on the endothelial surface, triggering intracellular signaling cascades that activate the transcription factors NF κ B and activator protein-1 and produce cell adhesion glycoproteins^{46, 47}. In a positive feedback loop, activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) also increases the expression of numerous procoagulants, chemokines, and cytokines including C-X-C Motif Chemokine Ligand 2 (CXCL2) and CXCL4, TNF- α , IL-1 β , IL-18, and IL-6 amongst other signaling molecules^{48, 49}. Additionally, in *in vitro* studies of endothelial cells, ROS increases the expression of tissue factor (TF) – the receptor and cofactor for factor VII/VIIa necessary to activate the extrinsic pathway of coagulation, overwhelming anticoagulant molecules⁵⁰.

In the context of chronic and acute inflammatory illnesses including COVID-19, a similar pattern of endothelial activation occurs with the shedding of cell surface anticoagulant proteins, release of WBP contents, and expression of cell adhesion molecules, resulting in a multi-organ, systemic thrombo-inflammatory phenotype. These changes facilitate platelet and leukocyte recruitment, dismantle the normal anticoagulant and anti-inflammatory phenotype of the vessel wall and support thrombosis at the blood:vessel interface^{43, 47, 51}. Chronic and acute inflammatory conditions may also contribute to VTE by compounding regional differences in endothelial physiology.

It is worth highlighting that endothelial cell phenotypes can be heterogeneous based on vessel size (macrovascular versus microvascular), and vascular bed (venous, arterial, and lymphatic). The distinct endothelium can be characterized by differential expression of cell surface adhesion molecules and signaling receptors, intracellular signaling molecules and response to stimuli. Endothelial cells from post-capillary venules are particularly responsive to inflammatory cytokines, which induces expression of adhesion molecules and uniquely supports leukocyte interactions^{52–54}. In the venous system, the endothelium in the vein valve sinus is exposed to hypoxia and disturbed blood flow, and maintains a compensatory environment that is carefully tuned to prevent thrombosis⁵⁵. Stasis of blood flow or the introduction of inflammatory stimuli in the valve sinus can overwhelm this delicate antithrombotic balance, denuding natural anticoagulants and promoting an inflammatory milieu that results in thrombosis^{21, 55, 56}.

Leukocyte Contributions to Venous Thrombosis

Glycoprotein adhesion molecules on activated endothelial cells support rapid leukocyte recruitment to sites of injury or inflammation. While not classic components of the

coagulation system, leukocytes are critical for VTE formation and facilitate initiation of coagulation on the endothelium. In a murine flow restricted model of DVT, turbulent blood flow activates the uninterrupted endothelium, enabling leukocyte binding and local inflammation, with subsequent thrombogenesis and leukocyte migration (Figure 2). Similar to human DVT, circulating leukocytes are major cellular components in this model^{38, 57}. Neutrophils and monocytes adhere to intact endothelium within hours of flow reduction⁵⁷, a process dependent on endothelial-derived P-sel binding to PSGL-1 on the leukocyte surface^{58, 57, 59}, and on leukocyte C-X-C Motif Chemokine Receptor 2 (CXCR2) which engages with CXCL1 on endothelial cells⁶⁰. Platelets and myeloid cells release extracellular vesicles that carry coagulants and inflammatory mediators to remote sites^{61–63}. Ultimately, thrombus initiation and propagation under flow restriction are dependent on both classic immune and hemostatic factors, including P-sel, accumulation of neutrophils and monocytes, platelet activation, VWF, and blood-cell derived TF^{57, 60, 64, 65}.

Neutrophils and neutrophil extracellular traps link prototypical host defense mechanisms and venous thrombosis

Neutrophils have historically been defined by their role in innate immunity, in which pathogen recognition through toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors and C-type lectin receptors triggers a swift immune response to contain and eliminate invading pathogens through phagocytosis, degranulation, and formation of neutrophil extracellular traps (NETs)^{66–69}. Neutrophils orchestrate inflammatory and immune responses in venous thrombosis at least in part by releasing NETs, sticky web-like structures comprised of a scaffold of decondensed chromatin decorated with cytosolic and granule proteins. These neutrophil-derived proteins, including histones, cytokines, alarmins, defensins, proteases, and myeloperoxidase, have antimicrobial and other functions⁷⁰. In the absence of NETosis, activated neutrophils release cytokines and other molecules relevant in thrombosis which will be discussed later in this review.

NETs are able to directly activate coagulation, bridging the immune and coagulation systems through immunothrombosis. Although they serve as a vital host defense response to contain infections⁷⁰, when dysregulated, NETs contribute to sterile inflammation, autoimmunity, arterial and venous thrombosis^{57, 71–74}. Extracellular trap production has also been described in monocytes, eosinophils, and basophils, but neutrophils are believed to be the dominant trap producers in venous thrombosis⁷⁵.

Neutrophils and NETs have been implicated in a variety of systemic immune and infectious disorders, many of which are accompanied by high risk of thrombosis such as antiphospholipid syndrome (APS), thrombotic thrombocytopenic purpura (TTP), heparin-induced thrombocytopenia (HIT), preeclampsia, autoimmune diseases, COVID-19, and the recently described SARS-CoV-2 vaccine-induced immune thrombotic thrombocytopenia (VITT)^{71, 76–84}. Evidence of NETs can be detected in human venous, arterial, and microvascular thrombi^{57, 85}. Murine models have confirmed the importance of neutrophils and NETosis in venous thrombogenesis. Depletion of neutrophils, degradation of NETs by administration of DNase, or inability to produce NETs due to deficiency in protein arginine

deiminase (PAD4) are all protective against thrombosis in flow-restricted conditions^{57, 71, 76, 86, 87}.

Diverse stimuli including autoantibodies, cytokines, activated platelets, and a cache of bacteria, fungi, and viruses can initiate signaling cascades that result in NET production in an environment- and stimulus-dependent fashion^{71, 88–91}. Although the intermediate signals that direct neutrophils to undergo NETosis rather than other functional responses remain to be fully elucidated, the steps in neutrophil activation are beginning to be defined^{92, 93}. NETosis is a tightly regulated process that involves activation of surface integrins, receptors, and mechanosensors that trigger intracellular calcium mobilization, ROS generation, shape change, and PAD4-catalyzed citrullination of proteins including histones which maintain chromatin structure⁹⁴. Cytoskeletal changes then disassemble the nuclear envelope, leading to release of decondensed chromatin into the cytosol. Acute cytotoxic stress responses result in shedding of microvesicles and vesiculation of the endoplasmic reticulum. These processes culminate in rupture of the plasma membrane and extrusion of unraveled DNA into the extracellular compartment as NETs^{93, 95}.

Critical activation steps in NETosis can occur in multiple intracellular locations. Neutrophil interactions with antiphospholipid antibodies or *S. aureus* stimulate the production of mitochondrial ROS and subsequent NETosis^{96, 97}. Mitochondrial ROS production in neutrophils from patients with lupus is also triggered by activation of the endoplasmic reticulum stress sensor, inositol-requiring enzyme 1 α (IRE α), which is known to perpetuate inflammation in chronic diseases and may represent an intermediate step between autoantibody signaling and ROS production⁷³. Although citrullination of histones by PAD4 in the nucleus was initially considered indispensable for NETosis, recent studies demonstrating PAD4-independent NETosis suggest this paradigm is species- and stimulus-dependent^{98–100}.

Recent studies have triggered interest in the transmembrane protein, Solute Carrier Family 44 Member 2 (Slc44a2) receptor for its potential role in VTE. Slc44a2 is highly expressed on neutrophils and believed to contribute to VTE, particularly in inflammatory settings. GWAS identified an association between expression of the human neutrophil antigen 3b (HNA-3b) epitope (rs2288904, 461G>A; Arg154Gln) on the *Slc44a* locus and a 30% lower risk of VTE¹⁰¹. Slc44a2 has subsequently been identified as a receptor for VWF¹⁰² and $\alpha_{IIb}\beta_3$ on VWF-primed platelets¹⁰³. Experimentally, Slc44a2 deficient mice develop smaller thrombi and recruit significantly fewer neutrophils to inflamed mesenteric venules^{104, 105}. Mechanistically, the HNA-3a epitope on neutrophils is required for slow rolling and interacting with VWF at venous shear rates, which is further augmented by LPS¹⁰⁵. Taken together, these findings suggest that under venous flow rates and inflammatory conditions, Slc44a2/HNA-3a may be important for neutrophil adhesion and NETosis and warrants further investigation.

Once released, NETs can activate coagulation and limit fibrinolysis by tPA¹⁰⁶. The NET chromatin scaffold serves as a surface for fibrin polymerization, VWF binding, platelet adhesion, and erythrocyte entrapment¹⁰⁶. Fibrin formation may also be amplified by direct fibrin-FXIIa interactions¹⁰⁷. The components of NETs additionally contribute to thrombosis

by presenting coagulation factors (eg. TF, FXII, FXI, thrombin), highly charged molecules (eg. histones H3 and H4, nucleosomes), and myeloperoxidase and serine proteases that degrade natural anticoagulants^{108–111}. Neutrophils, NETs, and NET degradation products can also serve as counterpoints to balance thrombin generation. Neutrophil elastase and cathepsin G, which cleave PSGL-1 from the neutrophil surface, limit neutrophil interactions with the endothelium and platelets¹¹². Extracellular DNA and sterile breakdown products bind to TLR9 on both monocytes and neutrophils, resulting in negative regulation of their prothrombotic functions¹¹³. These observations highlight the nuanced diversity of neutrophil functions in thromboinflammation, which may differ by stimulus, inflammatory milieu, and temporal kinetics.

As NETs are themselves degraded by endogenous DNases, histones and other liberated components may become available to activate endothelial cells and platelets, impair protein C activation, promote thrombin generation, and interfere with thrombin inhibition by antithrombin^{114, 115}. Intriguingly, studies of blood samples from patients with lupus, antiphospholipid syndrome, and COVID-19 have shed light on a new paradigm wherein anti-DNase and “anti-NET” antibodies bind and stabilize NETs by “shielding” them from catalytic degradation by DNases^{109, 116, 117}. Such anti-NET antibodies represent an additional layer of control in thromboinflammation and may also trigger an Fc receptor-mediated immune response, perpetuating the cycle of inflammation¹¹⁸.

Certain neutrophil subpopulations may have a proclivity to form NETs. Aged neutrophils in circulation are more likely to release NETs, rapidly homing to sites of inflammation or infection to induce an immediate effector response¹¹⁹. Additionally, some chronic autoimmune diseases are characterized by a higher proportion of circulating low-density granulocytes (LDGs) that exhibit a proinflammatory secretory phenotype and heightened capacity to form NETs¹²⁰. Finally, in acute inflammatory conditions, emergent granulopoiesis leads to rapid mobilization of immature neutrophils from the bone marrow. The effect of immature neutrophils on inflammation, NETosis, and thrombosis remains to be determined¹²¹.

Neutrophils also contribute to thrombosis independent of NETs by releasing cytokines, DAMPs, and extracellular vesicles (EVs) that result in neutrophil interactions with other cells^{7, 122}. Restricting neutrophil-platelet and neutrophil-endothelial crosstalk in mice by deletion of the P-sel ligand, PSGL-1, reduces venous thrombosis⁵⁹. The myeloid alarmin heterodimer calprotectin (S100A8/9) is an example of a neutrophil effector with diverse functions. Calprotectin modulates calcium signaling, functions as a chemoattractant, and triggers cellular responses by binding to the scavenger and danger signal receptors CD36, TLR4, and receptor for advanced glycation end products (RAGE)¹²³. In preclinical studies, mice lacking the myeloid alarmin protein S100A9 are protected from venous thrombosis¹²⁴. In patients with severe COVID-19, in whom microvascular and macrovascular thrombosis can result in organ failure, circulating levels of calprotectin were elevated and were predictive of respiratory failure^{81, 125, 126}.

Neutrophils have become an interesting target for therapeutic modulation given their multifaceted role in thrombo-inflammation. Although systemic depletion of neutrophils

compromises host defense and is therefore not a viable therapeutic approach, multiple strategies to inhibit the effects of NETs including by sequestering cell-free DNA, nucleosomes, or histones; inhibiting serine proteases; degrading NETs using recombinant DNases; and targeting NET-associated coagulation factors are being investigated as potential treatments for thrombotic and inflammatory diseases^{9, 127}. Precise targeting of PAD4 and arginine deamination has proven to be challenging for clinical translation and a different approach using antibodies to inhibit histone citrulline residues appears more promising as a means to reduce neutrophil activation¹²⁸. The urgent need for therapeutics to inhibit NETosis has led to a resurgence of interest in repurposing existing drugs including colchicine, dipyridamole, and fostamatinib^{129, 130}.

Monocytes, tissue factor, and inflammasome effectors in thrombogenesis

Monocytes, while classically defined by their phagocytic role in innate immunity, contribute to venous thrombogenesis in multiple ways¹³¹. Animal models of DVT differ in their dependence on monocytes for acute venous thrombogenesis. Depletion of all monocytes with clodronate reduces DVT in a FeCl₃ model, while inducible depletion of CD11b+ monocytes affected clot resolution but not acute thrombogenesis^{132, 133}. In flow-dependent models of DVT, monocytes interact with the endothelium and recruit platelets to the site within hours. Although the complete contributions of monocytes continue to be elucidated, the most well-defined roles for monocytes in coagulation are to initiate coagulation through presentation of TF and to potential inflammation through inflammasome activation^{57, 65}.

TF roles in coagulation and immune responses

The transmembrane glycoprotein TF is the primary initiator of coagulation under most physiologic and pathologic conditions, as well as an important mediator in the physiologic response to injury or infection. Concordant with its role as a central regulator of inflammation and thrombosis, preformed TF can rapidly shift functions from cell-signaling to coagulation by conformational activation in a complement-dependent, thiol-disulfide exchange catalyzed by protein disulfide isomerase (PDI) (Figure 3)¹³⁴. TF initiates proinflammatory pathways, primarily through activation of the G-protein coupled protease-activated receptors (PARs), which are variably expressed on platelets, leukocytes, and endothelial cells. Activation of PARs initiates diverse inflammatory signals through release of cytokines (eg. TNF- α , IL-1, IL-6) and growth factors¹³⁵ and induction of adhesion molecules that result in recruitment and activation of leukocytes¹³⁶. The release of cytokines and DAMPs further increases TF expression on monocytes, initiating a self-reinforcing loop with sustained or escalating TF expression and activation of complement.

Activated TF exposed to blood complexes with FVIIa, increasing FVII activity two-million-fold¹³⁷ and resulting in thrombin generation. While three circulating pools of TF exist in the vasculature: cell-associated, EV-associated, and an inactive soluble form that lacks a cytoplasmic tail, TF on monocytes/macrophages and the EVs they generate are indispensable for thrombus accretion in flow-dependent models of DVT^{7, 57, 131}. Under physiologic conditions, coagulation initiation is under tight negative regulation by endogenous anticoagulants including ATIII, the protein C pathway, and TFPI. During

venous thrombogenesis, monocyte TF is activated in response to stimuli from cytokines, chemokines, cell adhesion molecules, pathogens, DAMPs, hypoxia, and tissue damage. Circulating platelet-monocyte aggregates also trigger monocyte TF expression by facilitating transcellular communication through P-sel and $\alpha_{IIb}\beta_3$.¹³⁸ While constitutive TF deletion is embryonically lethal in mice, mice with inducible TF deletion or constitutive expression of low levels of human TF may have a bleeding diathesis but are protected from thrombosis^{57, 65}. Conversely, exaggerated TF activity is responsible for catastrophic thrombosis and lethality following pathogen stimulation⁶⁵. Elevated levels of circulating TF-positive EVs have been detected in patients following surgery or with pancreatic cancer and are associated with thrombosis risk or mortality¹³¹. Viral infections with influenza A, HIV and Ebola virus are also associated with elevated circulating TF¹³¹. Most recently, studies during the pandemic have revealed that increased circulating monocyte TF expression, and TF-positive EVs are associated with mechanical ventilation and death^{138, 139}. Intriguingly, TF mRNA and protein have been detected in neutrophils from patients with COVID-19, and were reduced by complement factor 3 (C3) blockade¹⁴⁰. Inhibition of TF is now being evaluated in a clinical trial (NCT04655586) as a treatment to block thrombo-inflammation in patients with COVID-19. Altogether, these clinical and experimental findings demonstrate the importance of TF for physiologic hemostasis and immune function, but the potential for overwhelming thrombotic and inflammatory damage if there is loss of the natural brakes in thromboinflammation.

Inflammasome activation and venous thrombosis

Proximate to TF activity, monocytes bridge inflammation, infection and venous thrombosis by coordinating inflammasome activation and coagulation. Assembly of the canonical NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome in response to DAMPs and PAMPs, and the resulting prototypical inflammatory cytokine IL-1 β are key steps in the initiation and propagation of venous thrombosis¹⁴¹. Through transcellular communication, activated neutrophils and NETs can license macrophages to produce IL-1 β ¹⁴³ and a bidirectional interdependence between macrophage inflammasome activation and NET formation is plausible¹⁴². TF expression in monocytes can be triggered by pyroptosis, an inflammasome-directed form of lytic cell death in response to pathogens and PAMPs^{65, 143}. The specific canonical or non-canonical inflammasome pathway activated diverges by stimulus, with gram negative rod proteins leading to caspase-1 activation, while LPS triggers a caspase-11 pathway of pyroptosis and TF expression¹⁴⁴. However, both pathways appear to converge on gasdermin D as the final common element required for inflammasome-dependent TF production. Additional, consequences of inflammasome activation and subsequent pyroptosis include release of extracellular histones, caspases, ASC speck proteins, gasdermin D, and other effectors capable of eliciting cis and trans cellular responses integral in host defense and venous thrombosis¹⁴⁴.

Although monocytes have been attributed as the major source of IL-1 β , recent studies point to neutrophils, endothelium, and platelets as additional cellular compartments for inflammasome activation and production of IL-1 β and IL-18¹⁴⁵⁻¹⁴⁷. As neutrophils outnumber monocytes in the leukocyte milieu during acute venous thrombosis, it is conceivable that monocytes and neutrophils cooperate in host defense and thrombosis by

regulating NETosis through an inflammasome-operated “molecular switch”, which may be internally or externally derived. Endogenous thromboinflammatory checkpoints align the multiplicity of mechanisms that lead to thrombosis. Reduced expression of the ectonucleotidases CD39 or CD73, or activation of hypoxia-inducible factor alpha (HIF-1 α) can release the brakes on inflammasome activation, NETosis, and vascular quiescence, resulting in thrombosis^{64, 141, 148, 149}. Indeed, immune checkpoint inhibitors widely used to treat patients with cancer have recently been associated with an increased risk of VTE¹⁵⁰. These are important areas that warrant further investigation as the therapeutic use of checkpoint inhibitors and inflammasome modulators are advanced in patient care.

Platelet roles in inflammation and infection

Platelets are abundant, circulating anuclear cells derived from megakaryocytes that have conventionally been recognized for their prominent roles in hemostasis and arterial thrombosis. They aggregate at sites of endothelial injury to support hemostasis by recruiting other platelets, forming an adhesive platelet plug as a physical barrier, facilitating the generation of thrombin, and serving as a surface for coagulation to prevent blood loss¹⁵¹. Defects in platelet function such as storage pool disorders or thrombocytopenia confer patients with a higher risk of bleeding complications, whereas hyperactive platelets are associated with arterial thrombotic conditions such as myocardial infarction and stroke^{152, 153}. However, a more complex role for platelets in maintaining homeostasis has emerged in the past two decades, with platelets being identified as important mediators in a variety of biologic functions including inflammation, immunity, and venous thrombosis^{153–155}.

Platelets contribute to the pathogenesis and sequelae of inflammatory diseases that are associated with a higher risk of VTE, including psoriasis, cancer metastases, transplant rejection, and infectious diseases such as sepsis, malaria, dengue and SARS-CoV-2 infection^{153, 156–158}. During thromboinflammation, platelets not only provide a surface for and regulate coagulation, but they also interact with the immune and complement systems and release cytokines relevant in thrombogenesis. The essential role for platelets in venous thrombosis is demonstrated in experimental models of DVT, in which depletion of platelets, or isolated deficiency of either GpIb or VWF eliminates thrombosis in blood flowing through venous circuits^{38, 57}. The clinical significance of platelets in VTE is further emphasized by the reduction in recurrent VTE in patients treated with an antiplatelet agent^{153, 159, 160}.

Following endothelial disruption during normal hemostasis, platelets adhere to specialized receptors on the underlying matrix and initiate signals to achieve hemostasis and alert the immune system (reviewed by van de Meijden and Koupenova)^{152, 153}. The sequences in platelet activation that follow include calcium influx and cytoskeletal rearrangements that lead to a shape change, and enrichment of surface receptors for adhesion and clotting proteins. During venous thrombosis, platelets directly adhere to collagen via $\alpha_2\beta_1$ integrin (GPIa/IIa) and glycoprotein VI, and to the endothelium through VWF-GpIb and P-selectin-PSGL-1 interactions. Indirect platelet-endothelial associations occur when platelet-leukocyte aggregates form through receptor-ligand interactions eg. platelet surface C3 binding to

leukocyte CD11b/CD18^{161, 162}. Platelet receptor-specific stimuli leads to release or expression of a cache of cytokines, coagulation, and signaling molecules including a range of bioactive eicosanoids¹⁵². The primary platelet storage depots are α -granules, dense granules, and lysosomes. The biorepository content of α -granules includes coagulation factors, chemokines, adhesion proteins and regulators of angiogenesis, while the cargo of dense granules includes ADP, ATP, calcium, serotonin, and other mediators. Following recruitment to the endothelium, platelets promote inflammation and subsequent venous thrombosis through several signaling pathways including mammalian target of rapamycin complex 1 (mTORC1), ROS, and activation of the inflammasome complex resulting in release of the cytokines IL-1 β and IL-18^{146, 147, 163, 164}. Platelet aggregation and release of granular contents also localize platelets to leukocytes adherent to the endothelium and licenses leukocyte signaling that can amplify venous thrombosis.

As the interactions between the hemostatic and immune functions of platelets are unraveled, they provide growing insight into the mechanisms by which platelets participate in venous thrombogenesis during acute and chronic inflammatory conditions. In autoimmune diseases such as rheumatoid arthritis and psoriasis, in which dysregulated thrombosis can occur, platelets are hyperactivated and induce inflammation at the vessel wall and in circulating leukocytes^{151, 156}. In patients with glioblastoma, platelet cell-surface lectin-like receptor type-C2 (CLEC-2) interactions with podoplanin can trigger platelet aggregation and venous thrombosis¹⁶⁵. In the context of inflammation, platelets are recruited and activated by circulating PAMPs and DAMPs through TLRs and purinergic receptors at the plasma membrane^{21, 166}. In turn, platelets release their own stores of DAMPs including high mobility group box 1 (HMGB1) and S100A9 that amplify the normal hemostatic and immune response^{124, 167}. HMGB1 recruits monocytes and additional platelets to sites of venous thrombus accretion and stimulates NETosis which can potentiate thrombus propagation¹⁶⁸. Platelets also stimulate NETosis through the CD40L-CD40 receptor axis^{89, 169}, and through the release of inorganic polyphosphates (polyP) from platelet dense granules¹¹¹. Potent activation of NETosis and the inflammasome complex by platelets can aid in control of pathogens, but can also create a hypercoagulable nidus^{90, 170}.

PolyP are linear polymers of inorganic phosphates, derived from endogenous pools such as platelets and exogenous pools such as bacteria. Although polyP were only recently discovered in mammalian systems, a developing body of research has shown that polyP has diverse biological functions, including procoagulant and proinflammatory signaling¹⁷¹. Platelet-derived polyP are released from dense granules as short-chain polymers, 60–100 phosphates in length similar to those released by mast cells and basophils. Platelet-sized polyP can activate the contact pathway (via factor XII and factor XI with thrombotic and inflammatory sequelae), activate factor V, enhance the activity of thrombin-activatable fibrinolysis inhibitor (TAFI) which delays clot lysis, modulate migration and function of immune cells including neutrophils, monocytes, and macrophages, and regulate endothelial cell function^{172–175}. Deficiency of polyP such as occurs in the dense granule storage disorder, Hermansky-Pudlack syndrome are associated with defective FXII-dependent clotting and thrombosis¹⁷³. PolyPs also activate canonical inflammatory pathways through NF- κ B and β -catenin signaling, increasing endothelial permeability, expression of cell adhesion molecules and apoptosis¹⁷⁶. Notably, both mammalian- and pathogen-derived

polyP have size dependent-functions ranging from acting as a potent procoagulant to an effective immune cell modulator¹⁷¹. As a counterpoint to its roles in coagulation and inflammation, platelet-sized polyP can also inhibit complement activation¹⁷⁷. The molecular mechanisms that regulate polyP synthesis, degradation, and its divergent effects on biological systems remain to be fully investigated.

During bacterial and viral infections, platelet-leukocyte interactions may precede platelet aggregation. This suggests that a parallel pathway to that found in autoimmune disease exists in pathogen responses to facilitate rapid cellular communication and amplify the immune response and trigger thrombosis. When encountering pathogens, platelets rapidly mobilize host defense responses and aggregate on the surface of bacteria. These interactions trigger the release of alpha granules which contain antimicrobial proteins such as β -defensin 1, platelet basic protein, and connective tissue-activating peptide 3, as well as the inflammatory mediators that are released during sterile inflammation¹⁷⁸. Severe, viral infections, such as with the influenza H1N1 or the SARS-CoV-2 virus, are accompanied by markedly increased risk for microvascular and macrovascular thrombosis in both, venous and arterial circuits⁸¹. The platelet transcriptome in COVID-19 is directed toward a hyperimmune response marked by antigen presentation and mitochondrial dysfunction¹⁵⁸. During these infections, the increase in circulating platelet-monocyte and platelet-neutrophil aggregates correlates with increases in platelet activation, tissue factor activity, NET formation, and severity of illness¹⁵⁸. Similarly, virus particle engulfment by platelets can trigger secretion of complement component C3 and GM-CSF, which trigger NETosis in healthy neutrophils¹⁶². Pathogen-antibody immune complexes, or dysregulated autoantibodies can trigger thrombin generation through platelet Fc γ RIIA signaling¹⁷⁹. Platelets also release platelet factor 4 (PF4), which binds to glycosaminoglycans on the pathogen surface¹⁸⁰. Following adhesion to glycoproteins, PF4 undergoes a conformational change and exposes a new epitope for antibody binding. Pathogen-bound or PF4-bound antibodies are presented to platelets in a self-amplifying loop, to phagocytes for clearance, and to B-cells for maturation and clonal expansion. Platelet-activating antibodies to PF4 have been detected in patients with bacterial periodontal disease, sepsis, and acute COVID-19^{157, 181, 182}. Engagement of this host defense response paradigm is also believed to occur in the recently described VITT following exposure to certain SARS-CoV-2 vaccines^{183, 184}. Exposure of PF4 to highly negative-charged constituents of the vaccine may trigger conformational changes in PF4 and antibody binding similar to the cycle of platelet activation and coagulation observed following bacterial infection or in HIT⁸³. Although rare, understanding the immune mechanisms that lead to VITT will be important to inform autoantibody-driven thrombosis in other diseases.

Bidirectional interactions between platelets and complement additionally contribute to thromboinflammation. Both C3a and C5a bind to activated platelets, triggering the release of alpha granules¹⁸⁵. Additionally, C3 is required for platelet activation and mice deficient in C3 have prolonged bleeding after vascular injury, have decreased fibrin and platelet deposition, and are protected against DVT¹⁸⁶. Notably, C5-deficient mice do not have abnormalities in platelet activation, platelet deposition nor hemostasis, however, they had reduced fibrin formation and DVT. Platelets reciprocally regulate complement activation. C3, C1 inhibitor, and factor H are stored in α -granules¹⁸⁶. C1 inhibitor, an acute phase

protein, inactivates C1r and C1s in the classical pathway, as well as associates with P-selectin and interferes with leukocyte rolling^{187, 188}. Additionally, polyP enhances the interaction of C1 inhibitor with C1s, slowing C2 and C4 activation¹⁸⁹. Factor H binds to the active and inactive forms of GpIIb-IIIa on platelets and regulates activation of the alternative complement pathway¹⁹⁰. Finally, platelets also express complement regulatory proteins which limit excessive complement activation¹⁹¹. These findings highlight possible differences in therapeutic effects and risks associated with different complement factor inhibitors in the treatment of thromboinflammatory diseases^{192, 193}. Roles for complement signaling have also been established in arterial disease. In atherosclerotic plaques, de-differentiated smooth muscle cells (SMC) overexpress C3, activate the anaphylatoxin arm of the complement cascade, and induce vascular inflammation¹⁹⁴. Notably, despite the presence of opsonin C3b on their surface, the SMCs avoid immune detection by expressing high levels of the “don’t-eat-me” signal, CD47 during M1 macrophage skewing. Blockade of CD47 in patients with cancer using a monoclonal antibody appears to reduce carotid artery inflammation¹⁹⁵. Clinically, elevated C3 levels are associated with both an increased risk of venous and arterial thrombosis, and future studies will determine whether complement pathways integrate venous and arterial disease^{194, 196}

By connecting multiple, conserved host defense response mechanisms, platelets responding to “danger” or pathogen signals aid in host defense, but can also fuel a disastrous cycle that culminates in thrombus initiation and propagation.

Extracellular vesicles

EVs, sub-micron sized phospholipid vesicles shed from a variety of cell types, have become a topic of significant interest for their ability to deliver thrombo-inflammatory signals to locations that are distant from the initial site of inflammation, infection, or thrombosis. Circulating EVs carry archetypic molecules from their parent cell including membrane proteins such as integrins, phosphatidylserine, adhesion proteins (eg. P-sel, PSGL-1), receptors (e.g. TF, TM, EPCR), and intracellular molecules such as cytokines, DAMPs, RNA and microRNAs. Consistent with their parent cells, EVs have complex biological effects on the vasculature. Platelets and megakaryocytes are the primary source of circulating EVs in healthy individuals, although most other vascular cells are also capable of producing EVs⁷. Platelet-EVs remain the predominant EVs in diseased states, where they may serve procoagulant functions by activating the tenase and prothrombinase complexes and by interacting with leukocytes via P-sel to trigger TF expression or NETosis (reviewed in Foley et al)⁷. EVs released by activated monocytes can carry a cargo of TF, phosphatidylserine and other signaling molecules that initiate inflammation and coagulation in remote locations¹⁹⁷. Similar to platelet-EVs, EVs bearing PSGL-1 from monocytes can ligate P-selectin on platelets and the endothelium⁶¹. During the interaction of EVs with other cells, origin cell cargo such as TF may fuse with the plasma membrane of recipient cells and become available for activity¹³¹. A recent study suggests that perivascular cells may be an additional source of circulating TF-positive EVs following oxidative stress and may contribute to intravascular coagulation and lung inflammation¹⁹⁸. Cancer cell-derived EVs expressing high levels of TF and PSGL-1 injected into mice associate with platelets and the endothelium, enhancing DVT formation. Similar transcellular communication through EVs

can shuttle neutrophil-derived arachidonic acid into platelets for thromboxane production and endothelial activation^{7, 122}. Consistent with this role for EVs, phosphatidylserine on the surface of EVs from platelets, monocytes, and neutrophils may activate endothelial cells and platelets to amplify thrombosis. Intriguingly, platelet-EVs may incorporate into bone marrow where they may have the opportunity to functionally reprogram this hematopoietic niche⁶². The diverse functional capabilities of EVs are a subject of intense investigation to better understand their relevance to disease and leverage these for diagnostic and therapeutic purposes.

Roles for adaptive immunity in VTE formation and resolution

The contributions of the innate immune system to the pathophysiology of VTE have been firmly established; however, the role of the adaptive immune system, specifically B and T cells, in VTE formation and resolution remains incompletely understood. The most well-characterized contribution of the adaptive immune system to VTE is in patients with autoantibody-driven thrombosis as discussed above. The endogenous and/or exogenous signals that trigger autoantibody production and the specific mechanisms by which they exert prothrombotic effects are unclear. While both B and T cells have been detected in murine DVT during the thrombus accretion and resolution, their function hasn't been studied until recently. SCID mice, which lack functional B and T cells, develop DVT that are of similar size, cellular composition, and clot resolution as their wild-type counterparts, suggesting the presence of B and T cells is not required for VTE formation or resolution in a flow-restriction model¹⁹⁹. In two mouse studies, depletion of T cells did not affect initial clot formation, but influenced clot resolution^{200, 201}. However, one study found that the depletion of T cells impaired clot resolution while the other study demonstrated T cell depletion accelerated clot resolution^{200, 201}. The divergent results between these studies could depend on the mouse strain, timing of intervention, and global T-cell versus effector-memory T cell depletion. Platelets can also present antigens to T-cells via major histocompatibility class-1 (MHC-1) expression, and platelet-T-cell interactions are associated with altered cytokine production in patients with COVID-19¹⁵⁸. Additional research will be necessary to more clearly define the interactions between the adaptive and innate immune systems in VTE.

Clonal hematopoiesis, inflammation, and VTE

Over the lifespan of an individual, hematopoietic stem cells can accumulate somatic mutations that provide the cells with a competitive fitness advantage. This process, called clonal hematopoiesis of indeterminate potential (CHIP), leads to a disproportionate increase in the circulating number of the advantaged stem cell clones with age (reviewed in detail by Jaiswal and Ebert)²⁰². CHIP is associated with an increase in the risk of developing hematopoietic malignancies and mortality secondary to cardiovascular events. The majority of individuals with CHIP have variants in either *Tet2*, *Dnmt3a*, *Jak2*, or *Asx11*, which are believed to contribute to a proinflammatory vascular environment. Studies in animal models suggest an increase in IL-1 β and other cytokines may contribute to the inflammatory milieu. Notably, the most frequent *Jak2*^{V617F} variant in myeloproliferative malignancies is associated with a 12-fold increase in VTE risk, possibly due to excess NET formation²⁰³. It

is unclear whether one or more mutations in *Tet2*, *Dnmt3a*, and/or *Asx11* are associated with venous thrombotic events^{204, 205}. Although the implications of CHIP for VTE and other vascular disorders are not ready for translation to the clinic, the relationship between CHIP and vascular thromboinflammation warrants further investigation.

Coagulation and fibrinolytic pathways overlap with inflammation

While traditionally, TF was thought to be exclusively responsible for *in vivo* hemostasis, the contact pathway is now appreciated to have important roles in both thrombin generation and inflammation^{173, 206}. Under inflammatory conditions, the contact system is initiated via activation of FXII, with loss of some negative regulatory mechanism. Physiologic activators of FXII during inflammation including RNA, DNA, activated platelets^{7, 108, 175}, NETs⁵⁷, HS²⁰⁷, and LPS, polyP or peptidoglycans on the surface of bacteria²⁰⁸. FXIIa can then trigger two distinct pathways: the extrinsic pathway via activation of FXI that culminates in thrombin generation; and the kallikrein-kinin pathway via generation of PK, which cleaves HK and culminates in liberation of bradykinin (BK) with activation of diverse potent proinflammatory pathways (reviewed by Foley and Renné)^{7, 209}.

Exploration of the contact system has exposed the overly simplistic classification of factors as either pro- or anti-thrombotic, or pro- or anti-inflammatory. Contained within these binaries is the expectation that absence of a procoagulant factor will result in hypocoagulable state with bleeding and excess procoagulant factor will result in hypercoagulability. FXII exemplifies the complexities of factors with roles in both the coagulation and inflammatory systems. Inhibitors of FXII and FXIIa are protective against thromboembolic disease^{210, 211}, while conversely, patients with gain-of-function mutations in the *F12* gene²¹² do not have an increased risk of clotting, but have hereditary angioedema (HAE), a serious inflammatory disease due to excess generation of BK²¹². These observations generated excitement regarding the potential to target FXI and FXII in thromboinflammatory conditions. However, they also demonstrate the possibility for unexpected side effects of inhibiting factors that work at the interface of inflammation and coagulation.

Regardless of the mechanism of thrombus formation, breakdown of thrombi through fibrinolysis is essential to physiologic hemostasis, however, it can also contribute to inflammation. Plasmin, the central fibrinolytic protease, has been traditionally defined by its role in fibrin polymer degradation. It also has vital functions in degradation of the extracellular matrix, tissue remodeling, cell migration, and tuning of inflammation through cleavage of fibrin, cytokine induction and complement activation²¹³. The primary negative regulator of the fibrinolytic system is plasminogen activator inhibitor-1 (PAI-1), which inhibits both tPA and uPA. High levels of PAI-1 are a risk factor for thrombosis and are observed in inflammatory diseases without a concomitant rise in tPA or uPA⁷. Conversely, individuals with lower levels of PAI due to a rare loss-of-function mutation have longer life spans and reduced incidence of thromboinflammatory diseases²¹⁴.

Dysregulation of fibrinolysis can have thrombotic implications due to a hypofibrinolytic state, but also due to uncontrolled inflammation. The specific role of fibrinogen and

plasminogen in inflammation have been reviewed in greater detail^{213, 215, 216} and we will briefly focus on plasminogen. The localization of the plasminogen activators, tPA and uPA, to different compartments support plasmin's diverse functions. Additionally, plasminogen receptors on cells localize cell-based plasminogen activation through generation of local high concentrations of plasminogen²¹⁷. For example, the plasminogen receptor, annexin A2, has increased cell surface expression in response to certain inflammatory stimuli.²¹⁸ On endothelial cells, annexin A2 forms a tetramer complex with S100A10, serving as a co-receptor/chaperone for tPA and plasminogen and accelerating plasmin generation more than 60-fold. Conversely, plasmin activates macrophages through the annexin A2-S100A10 tetramer complex, which results in JAK1/TYK2 signaling, STAT3 activation, NFκB activation, and the release of proinflammatory cytokines. Thus, interactions between plasminogen and annexin A2 support both fibrinolysis and a proinflammatory response²¹⁹. Decreased annexin A2 protein expression has been associated with VTE in patients with a positive family history but no identifiable thrombophilia²²⁰ and in patients with VTE compared to healthy controls²²⁰. Anti-A2 antibodies have been found in patients with cerebral venous thrombosis²²¹, thrombotic APS²²², and an early report suggests in COVID-19 as well²²³. Overall, these findings posit that annexin A2-mediated cell surface fibrinolysis may be an important mediator in hemostasis and thrombosis.

Plasmin and the complement system also activate and regulate each other. During inflammatory states, plasminogen can also be activated by components of the complement system.²²⁴ Conversely, plasminogen can directly bind C3, C3b, and C5, cleave some complement factors into inactive fragments, and act as a complement inhibitor via its lysine residues²²⁵. This inhibitor function has been coopted by pathogens to avoid effective host killing. For example, *Moraxella catarrhalis* expresses plasmin on its surface that degrades C3b and C5b²²⁶. The importance of the fibrinolytic system to the immune response has been demonstrated experimentally in both mice deficient in fibrinogen or plasminogen^{11, 227, 228}. Notably, the phenotype associated with plasminogen deficiency is abrogated with concomitant fibrinogen deficiency, demonstrating the dependence of plasminogen on fibrinogen (likely through fibrinolysis) for many of its inflammatory functions^{227, 229}. While components of the fibrinolytic system have clear roles in both fibrinolysis and inflammation, it remains uncertain if dysregulation of its inflammatory roles contributes to thrombosis.

Targeting Thromboinflammatory Pathways

The complex relationship between immune function and coagulation has evolved to stop bleeding, prevent infection, and promote wound healing. Leukocyte and platelets interact with activated endothelium and each other, initiating a diverse set of inflammatory and thrombotic pathways that are stimulus- and context-dependent. Without timely negative regulation, unregulated reciprocal activation of the immune and coagulation systems can result in catastrophic thrombotic and inflammatory damage to organs and tissues. The current paradigm for prevention and treatment of VTE with anticoagulants alone is not universally effective. Even aggressive pharmacologic fibrinolytic therapy and mechanical thrombectomy of VTE insufficiently address the underlying inflammatory milieu, tissue damage, and risk of recurrent thrombotic diatheses. A major limitation to the use of more potent antithrombotic agents is the concomitant risk of bleeding. As a more nuanced

understanding of molecular mechanisms at the interface of coagulation and inflammatory systems is acquired, novel and perhaps more insightful applications of prior inflammatory targets can be tested for the prevention and treatment of VTE. Indeed, treatment of patients with elevated inflammatory biomarkers with a statin reduced the risk of VTE²³⁰. The ideal therapy to prevent or treat VTE will suppress excess inflammation and coagulation without disrupting hemostasis or triggering immune deficiency. As highlighted in this review, interventions targeting a number of inflammatory steps in murine models of DVT, including the inhibition of cell adhesion receptors, NETosis, platelet activation, and cytokine signaling, decrease thrombosis. As research discoveries shed new light on the nexus of inflammation and coagulation, there is renewed enthusiasm to identify and develop therapies targeting thromboinflammation that can be translated to the clinic for improved patient care.

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Non-standard Abbreviations and Acronyms

ADP	adenosine diphosphate
APS	antiphospholipid syndrome
ATIII	antithrombin
BK	bradykinin
C3	complement factor 3
CHIP	clonal hematopoiesis of indeterminate potential
CLEC-2	cell-surface lectin-like receptor type-C2
COVID-19	Coronavirus disease 2019
CS	chondroitin sulfate
CXCL	C-X-C Motif Chemokine Ligand
CXCR	C-X-C Motif Chemokine Receptor
DAMPs	damage-associated molecular patterns
DVT	deep vein thrombosis
E-sel	E-selectin

EPCR	endothelial cell protein C receptor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPIIbα	glycoprotein Iba
HAE	hereditary angioedema
HIF-1α	hypoxia-inducible factor alpha
HIT	heparin-induced thrombocytopenia
HMGB1	high mobility group box 1
HNA	human neutrophil antigen
HS	heparan sulfate
ICAM-1	intracellular adhesion molecule-1
IFN	interferon
IREα	inositol-requiring enzyme 1 α
LDGs	low-density granulocytes
mTORC1	mammalian target of rapamycin complex 1
NETs	neutrophil extracellular traps
NF-κB	nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NLRP3	NOD-like receptor family pyrin domain containing
NO	nitric oxide
NOD	nucleotide oligomerization domain
P-sel	P-selectin
PAD4	protein arginine deiminase
PAI-1	plasminogen activator inhibitor-1
PAMPs	pathogen-associated molecular patterns
PARs	protease-activated receptors
PDI	protein disulfide isomerase
PE	pulmonary embolism
PF4	platelet factor 4
polyP	inorganic polyphosphates

PSGL-1	P-selectin glycoprotein ligand-1
RAGE	receptor for advanced glycation end products
ROS	reactive oxygen species
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
Slc44a1	Solute Carrier Family 44 Member 2
SMC	smooth muscle cells
TAFI	thrombin-activatable fibrinolysis inhibitor
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TLRs	toll-like receptors
TM	thrombomodulin
TNF-α	tissue necrosis factor-alpha
tPA	tissue-type plasminogen activator
TTP	thrombotic thrombocytopenic purpura
uPA	urokinase plasminogen activator
VCAM-1	venous cellular adhesion molecule-1
VITT	vaccine-induced immune thrombotic thrombocytopenia
VTE	venous thromboembolism
VWF	Von Willebrand Factor
WPB	Weibel-Palade bodies

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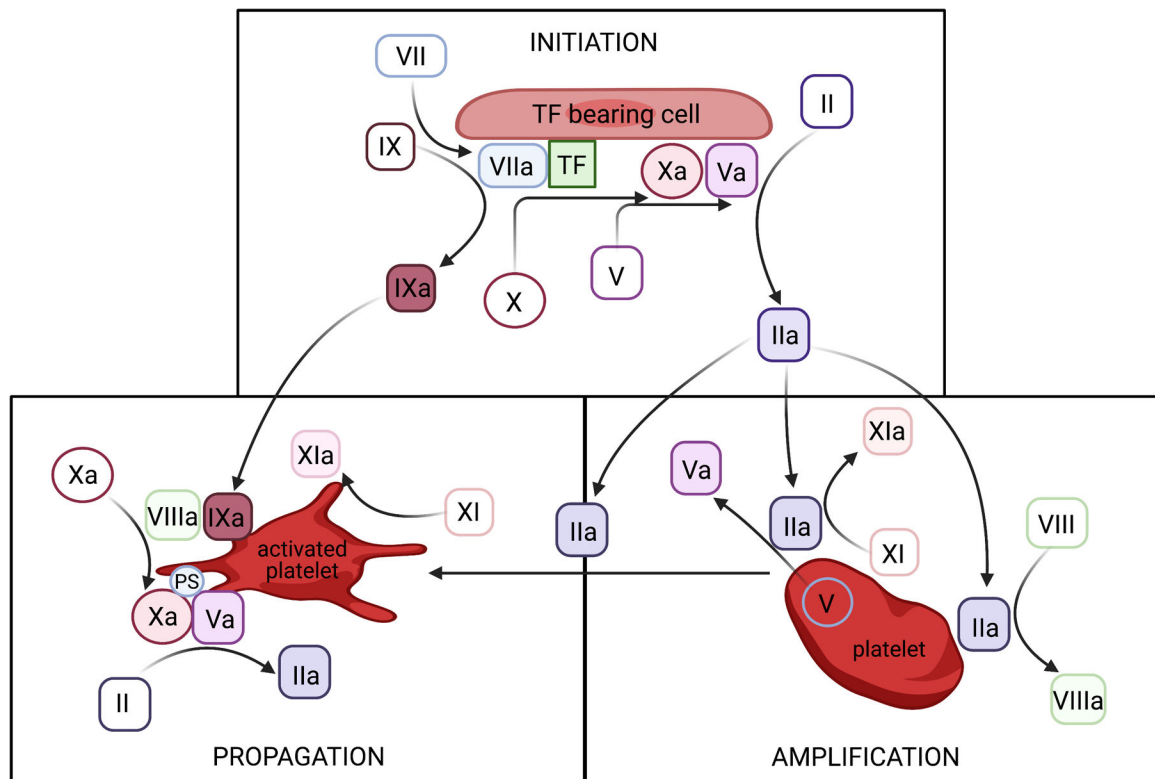


Figure 1: Endothelial Activation.

The quiescent endothelium shown on the left wall of the vein is an antithrombotic surface. Endothelial cells prevent or limit coagulation through expression of heparan sulfate, tissue factor pathway inhibitor (TFPI), CD39, and CD73, as well as release of tissue plasminogen activator (t-PA), prostacyclin and nitric oxide. Additionally, expression of endothelial protein C receptor (EPCR) and thrombomodulin (TM) exert anti-inflammatory effects and negatively regulate thrombin generation. Shown on the right wall of the vein, endothelial cells, in response to injury or functional perturbations, endothelial cells undergo shift to prothrombotic surface. There are increased expression leukocyte adhesion molecules (P- and E-selectin) and tissue factor (TF), loss of vascular integrity and expression of antithrombotic molecules, and production of cytokines. (Illustration credit: Erina He).

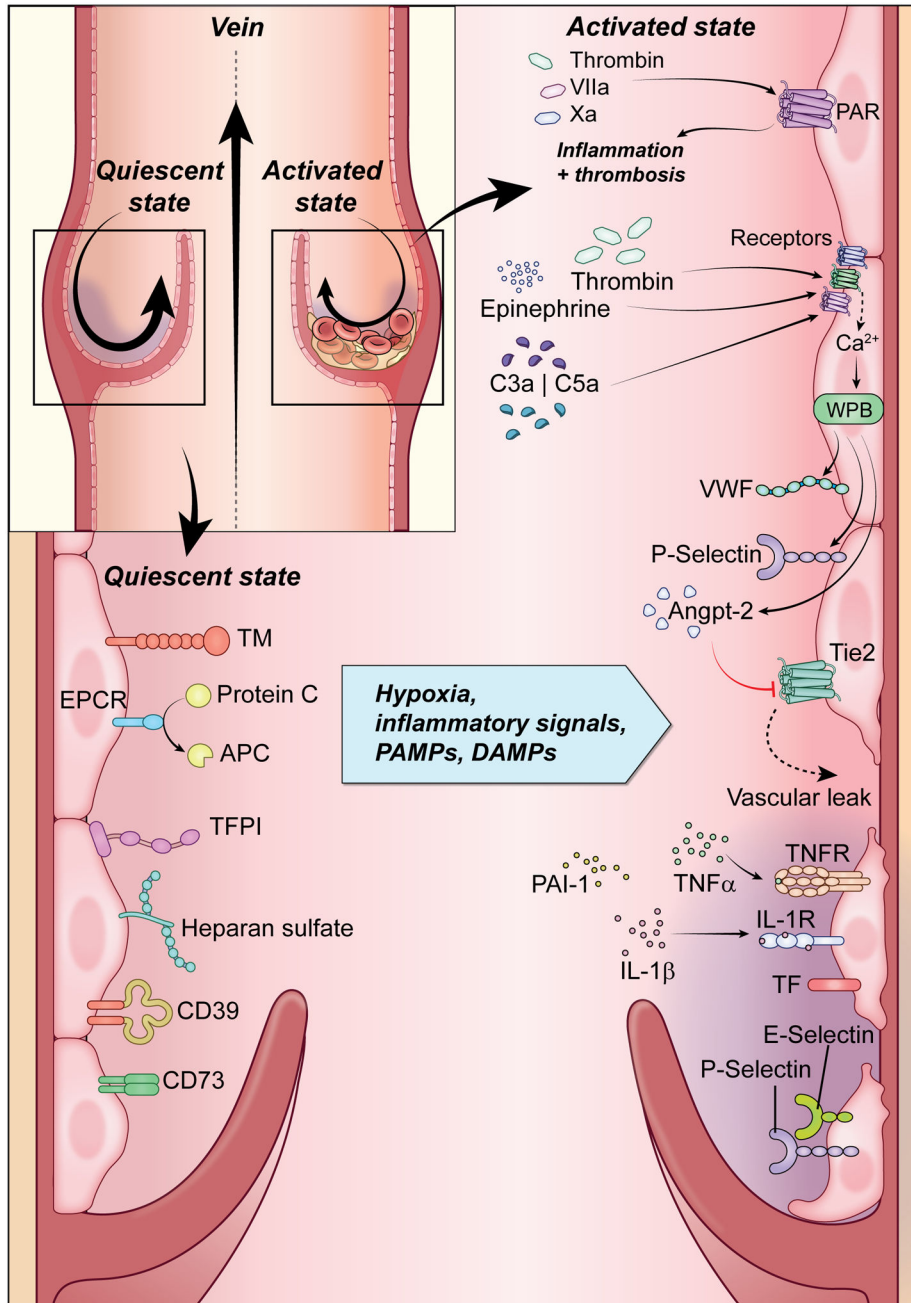


Figure 2: Leukocyte and Platelet Recruitment and Activation. Increased adhesion molecules on endothelium including C-X-C Motif Chemokine Ligand 1(CXCL1), P-selectin and von Willebrand Facotr (VWF) support leukocyte and platelet rolling and binding. Inflammatory cytokines, pathogen- and damage associated molecular patterns (PAMPs and DAMPs), reactive oxygen species (ROS), and growth factors activate induce tissue factor (TF) expression on monocytes with resulting initiation of coagulation. Diverse stimuli including autoantibodies, cytokines, activated platelets with release of platelet factor 4 (PF4) and high mobility group protein B1 (HMGB1), and pathogens trigger neutrophils to undergo NETosis. On the scaffold of decondensed chromatin decorated with

cytosolic and granule protein, there is VWF and platelet binding, erythrocyte entrapment, and activation of coagulation through presentation of TF, thrombin and factor XI (FXI), and auto-activation of FXII with resulting fibrin polymerization and thrombosis. There is simultaneous negatively regulate fibrinolysis through degradation of tissue factor pathway inhibitor (TFPI) and antithrombin (ATIII). (Illustration credit: Erina He).

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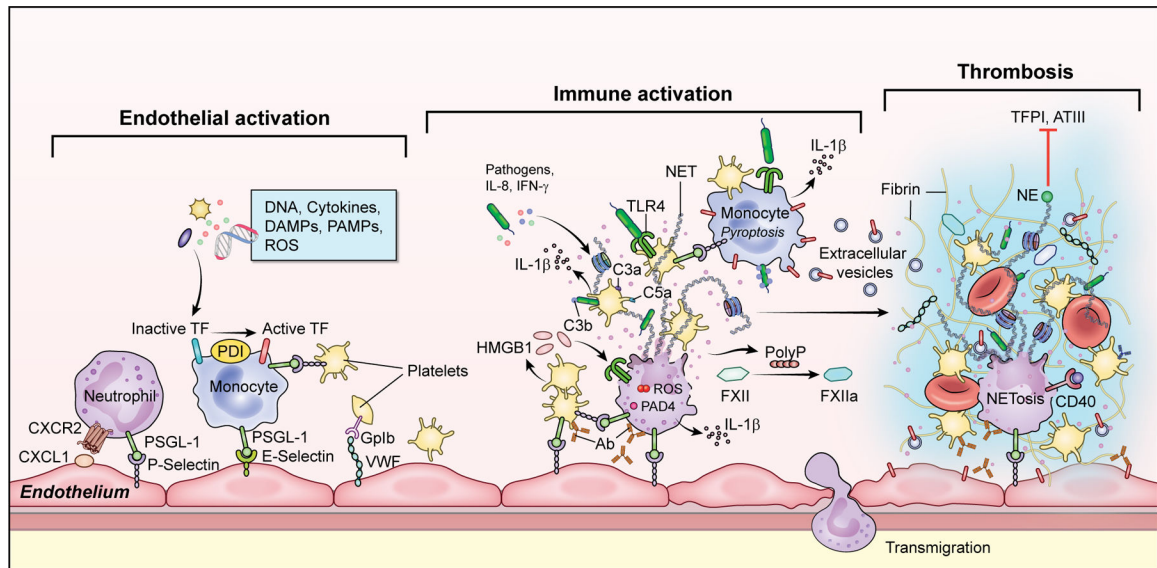


Figure 3: Cell-based model of coagulation.

Initiation phase: On the surface of a tissue factor (TF)-bearing cell, small amounts of thrombin (II) and factor IXa (IXa) are generated that then migrate to the surface of platelets. Amplification phase: Factors V, VIII, XI and platelets are activated. Propagation: On surface of activated platelet, activated factors assemble and generate thrombin burst. PS – phosphatidylserine. (Illustration made using Biorender)