Although viewed separately, coagulation and inflammation are highly integrated, delicately balanced biological systems with extensive cross talk that optimizes the organism’s response to injury and invasion by pathogens. Dysregulation of any one component in these systems can affect the entire balance, resulting in a wide range of illnesses that may feature varying degrees of excess inflammation and thrombosis. The molecular and cellular connections between coagulation and inflammation are steadily being delineated, and these advances are uncovering novel insights into the mechanisms underlying disease and revealing new, more specific, and safer therapeutic strategies. In this article, we provide a brief review of selected key pathways in which links between coagulation and inflammation have been reported. We begin with the central player and common trigger, tissue factor (TF), that provides a bridge between these pathways. Downstream events are then discussed, including inflammatory signaling mediated via thrombin and protease-activated receptors (PARs), participation by innate immune pathways, including toll-like receptors (TLRs) and complement, the controversies surrounding microparticles (MPs), the recruitment of negative regulatory systems, such as the protein C (PC)–thrombomodulin (TM) mechanism, the role of neutrophil extracellular traps (NETs), and the activation and regulation of the fibrinolytic system. We end with a discussion of contact activation, a system that has garnered renewed interest, with findings that hold promise for the development of safer antithrombotics. Taken together, this review will provide a glimpse into the many opportunities...
Tissue Factor

TF is positioned at the nexus of coagulation and inflammation, providing a trigger for initiation of the host response to injury or invasion by pathogens. The fundamental importance of TF to normal physiology is underscored by the embryonic lethality observed in TF knockout mice. Cross talk between the coagulation system and pathways that modulate the inflammatory response can almost all be traced to this pivotal receptor.

TF is a type I integral membrane glycoprotein and a member of the cytokine receptor superfamily. Exposure of TF to blood is a critical determinant for most of its functions. Its strategic location in cells throughout the subendothelium of the vasculature, with particularly high levels in brain, skin, lungs, gut, and placenta, as well as in monocytes, makes it readily recruitable. In response to chemical or physical damage, inflammatory cytokines (e.g., tumor necrosis factor-α [TNFα], IL-1β), infectious agents, oxygen-free radicals, or other injurious stimuli, TF expression is rapidly upregulated and de-encrypted by perivascular cells (adventitial fibroblasts, smooth muscle cells, and pericytes) and by circulating monocytes, so that it becomes exposed to the circulation (Figure 1). This allows it to complex with small amounts of circulating factor VIIa, enhancing the latter’s catalytic activity and triggering coagulation by activating coagulation factors IX and X. With sufficient TF exposure and sustained injury, the amount of thrombin generated can be amplified to exceed a threshold beyond several negative regulatory influences to promote platelet activation, fibrin clot formation, and a myriad of proinflammatory events, the latter primarily mediated via activation of PAR cell signaling pathways (see below). This includes, for example, recruitment and activation of monocytes, neutrophils, and platelets, induction of leukocyte adhesion molecules by endothelial cells, release of proinflammatory cytokines, and activation of complement. The TF–factor VIIa complex also stimulates PAR signaling, which induces release of inflammatory cytokines and chemokines in vitro and in vivo. These, in turn, further upregulate TF by monocytes and exposed subendothelial cells, fueled by other damage-associated molecular patterns (DAMPs) that are generated during infection, inflammation, or stress. DAMPs engage TLRs on immune cells, including endothelial cells and platelets, inducing the release of proinflammatory cytokines and chemokines, upregulation of leukocyte adhesion molecules, and expression of TF. If the host is unable to resolve/dispose of the initiating insults that triggered TF exposure, a positive feedback loop ensues, whereby TF expression is sustained and amplified through the action of cytokines, chemokines, and complement factors, augmented by interactions with activated leukocytes, endothelial cells, and platelets.

Models of human disease using genetically modified mice have largely validated the importance of the described pathways, providing major insights into the diverse roles of TF in coagulation and inflammation. Mice with reduced levels of TF that are exposed to lethal doses of endotoxin are protected against thrombosis, inflammation, organ failure, and death. The cytoplasmic domain of TF seems to play a key role in this pathway, in that mice lacking this structure respond to endotoxin challenge with prolonged survival and impaired recruitment and activation of leukocytes. Inhibition of formation of the TF–factor VIIa complex attenuates myocardial ischemia–reperfusion injury in mice, in concert with reduced NF-κB signaling, TLR4 activation, and expression of proinflammatory mediators. TF-PAR2 signaling is also implicated in the chronic inflammatory disorder associated with obesity, inducing gluconeogenesis, lipogenesis, and release of inflammatory cytokines. In mice, this pathway contributes to diet-induced obesity by decreasing metabolism and energy expenditure and augmenting adipose tissue inflammation and insulin resistance.

In spite of the preceding observations, caution must be exercised before drawing the conclusion that all TF-mediated pathways are damaging. To that point, although blocking TF/thrombin/PAR1 signaling is protective in mouse models of myocardial ischemia–reperfusion injury, it is detrimental in Coxsackievirus B3-induced viral myocarditis. Such complexity, likely due in part to differential responses to a range of stimuli, compounded by differing concentrations during the course of the stress, may help to explain the challenges that have been met in designing TF-targeted therapies to prevent or treat sepsis. Indeed, although exogenous administration of inhibitors of TF and TF–factor VIIa have been successfully used in preclinical and clinical trials to dampen inflammation-mediated coagulation, efficacy has only been demonstrated that exist for further exploration and the identification of novel diagnostics and strategically designed treatments.
in animal models (reviewed in van den Boogaard et al20). TF pathway inhibitor is the major endogenous inhibitor of the TF pathway, binding to and neutralizing TF–factor VIIa in a factor Xa–dependent manner.21 It is constitutively expressed by microvascular endothelial cells and is also found in monocytes, smooth muscle cells, platelets, fibroblasts, cardiomyocytes, and plasma.22 Although administration of TF pathway inhibitor has shown efficacy in several animal models of sepsis,23 no benefit was shown in randomized clinical trials in humans.24 Most studies of the role of TF in coagulation refer to its residing in the vessel wall. However, TF also exists in the circulation, that is, it is blood-borne.25 Current evidence suggests that there are 3 different pools (reviewed in Cimmino et al26), that is, cell-associated, in MPs, and in a soluble form because of alternative splicing. The major cellular source of circulating TF is the monocyte. It remains debatable as to whether TF is expressed or, alternatively, acquired by neutrophils, and controversy also persists with regard to its expression in platelets. MPs are heterogeneous submicron particles enclosed by a phospholipid bilayer that can be released from essentially any activated cell. TF may be incorporated or captured by these MPs. There is much debate surrounding which cells release the most TF-enriched MPs, with monocytes being the leading candidate.27–29 The role of TF-bearing MPs in coagulation and inflammation is a topic of major interest, and a more comprehensive discussion is provided in a separate section later. Finally, soluble TF that lacks the transmembrane domain exhibits procoagulant activity and may incorporate into thrombi.30 Overall, there remains much to be learned about blood-borne TF, the extent of its participation in coagulation, immunity and inflammation, and whether it might be a viable therapeutic target or a prognostic tool, as has recently been suggested for severe sepsis.31

It is well established that the key trigger for activation of coagulation is TF, and that initiating events that lead to its exposure to blood drives an escalating circle of events in which inflammation and coagulation positively feed back on each other. If not stopped by natural inhibitory systems or therapeutic interventions, tissue damage featuring vascular thrombosis and inflammation ensues.

**Thrombin and PARs**

The serine protease thrombin, a key downstream product of TF-initiated coagulation, plays a central role in the efficient control of hemorrhage and also to the development of debilitating venous and arterial thrombosis, while simultaneously exhibiting profound proinflammatory properties.8 Thrombin exerts its hemostatic and thrombotic effects through the coagulation–inflammation axis, where it cleaves fibrinogen to generate an insoluble fibrin clot; amplifies its own production through pro-cofactor activation; and activates various cell types via proteolytic cleavage of PARs (reviewed in Coughlin32). PARs are expressed in many cell types, including platelets, endothelial, immune, and epithelial cells, astrocytes, and neurons. PARs are unique among cell surface receptors in that they are activated by proteolytic cleavage, which unMASKS a
The PAR family of receptors contains 4 members (PAR1–4) and signal via the G protein–coupled signaling pathways initiated by $G_{12/13}$, $G_i$, and $G_q$ families. Thrombin efficiently cleaves PAR-1, -3, and -4, resulting in cellular activation, whereas PAR-2 is primarily activated by factor Xa. The broad expression pattern of PARs and their ability to couple to $G_{12/13}$, $G_i$, and $G_q$ explains their involvement in such a diverse range of physiological and pathophysiological processes, including hemostasis, thrombosis, inflammation, and cancer progression, to name a few. It is important that activation of PARs be tightly regulated because thrombin (and other coagulation and noncoagulation enzymes) may cleave and activate many copies of PAR before it itself is inhibited. Furthermore, the ligand is anchored to the same receptor that it activates, meaning that there is no diffusional regulation, which could potentially lead to an exaggerated response. To address these issues, PARs are rapidly desensitized and endocytosed after activation. They are trafficked to the lysosome for degradation instead of being recycled to the surface. In nucleated cells such as endothelial cells and fibroblasts where sustained PAR activation and signaling is required, PAR is translocated to the cell surface from an intracellular store.

Although PARs are expressed throughout the body, those expressed on platelets, leukocytes, and endothelial cells are the most pertinent to hemostasis, thrombosis, and inflammation. Human platelets contain PAR-1 and PAR-4 (mouse platelets contain PAR-3 and PAR-4), and both are important for platelet activation because blockade of a single PAR-1 or PAR-4 is not sufficient to prevent platelet activation after stimulation with high concentrations of thrombin. By contrast, blockade of both PAR-1 and PAR-4 inhibits platelet activation even at high concentrations of thrombin. On thrombin-mediated PAR activation, platelets release the contents of their granules, undergo morphological change and aggregation, mobilize intracellular calcium, and translocate the adhesion molecule P-selectin along with the prothrombinase-binding phosphatidylerine to the platelet surface. Release of proteins, such as factor V, fibrinogen, and factor XIII, enhance thrombin generation and clot stability, whereas ADP and thromboxane-A2 can activate neighboring platelets via P2Y_{12} and thromboxane receptors, respectively. After platelet activation is induced by one or more agonists, $\text{cPLA}_2$ becomes localized on the platelet surface, allowing for the binding of fibrinogen and von Willebrand factor, which supports platelet aggregation and signaling. All of these processes are prothrombotic and, therefore, may contribute to hemostasis and thrombosis.

On the inflammatory side, thrombin can also activate PAR-1 on endothelial cells and fibroblasts to trigger production of monocyte chemoattractant protein-1, TNF-\(\alpha\), and IL-1\(\beta\), and IL-6, PAR-1-dependent signaling also causes endothelial cells to become activated, resulting in P- and E-selectin expression and expression of monocyte chemoattractant protein-1, IL-8, plasminogen activator inhibitor-1 (PAI-1), and $\text{IIb/IIIa}$. Together, these endothelial effects mediate platelet and leukocyte recruitment and adhesion and rolling on the endothelium, which are processes known to occur during the early stages of venous thrombosis. Adherent leukocytes and platelets are susceptible to PAR-mediated activation, resulting in IL-6 and IL-8 production by fibroblasts and monocytes and increased platelet effects described earlier. Altogether, these processes form a potent positive feedback loop that amplifies inflammation and procoagulant processes at the vascular surface.

The central role of thrombin and platelets in thrombosis make them prime targets for anticoagulant therapies, and many such therapies are widely used. In recent years, novel drugs targeting thrombin (dabigatran) or PAR-1 (vorapaxar) have been approved based on large clinical trials showing a reduction in thrombosis. These drugs, like all other anticoagulants, increase the risk of bleeding to various degrees, leaving us searching for the silver-bullet therapy that protects against thrombosis without impairing hemostasis. Identifying a target that decreases thrombosis without affecting hemostasis is a challenging task both conceptually and practically. Because thrombosis has a strong inflammatory component, this elusive drug target may lie in the inflammatory pathways triggered by these essential hemostatic players, thrombin and PAR-1.

### Protein C–Thrombomodulin–Endothelial Cell Protein C Receptor

PC is a circulating vitamin K–dependent zymogen precursor for activated protein C (APC), a serine protease that is essential to prevent unrestricted and unchecked activation of coagulation and inflammation. Its generation relies on cleavage by thrombin, catalyzed by endothelial anchored cofactors, TM (≈1000-fold), and the endothelial protein C receptor (EPCR; ≈20-fold). Deficiency of PC and variants of EPCR augment the risk of deep vein thrombosis and thromboembolism and augment the response to inflammatory stimuli.

There are multiple mechanisms by which the PC-TM-EPCR system modulates inflammation, innate immunity, and tissue repair (Figure 2). As APC is generated by the thrombin–TM–EPCR complex, the enzyme competes with PC for binding to EPCR. When dissociated from EPCR, APC binds to protein S (PS) on the phospholipid surface of activated platelets and endothelial cells, where APC cleaves and inactivates coagulation cofactors VIII/VIIa and V/Va, interfering with formation of the tenase and prothrombinase complexes, respectively, and suppressing further generation of thrombin and factor Xa. Inactivation of factor VIIIa is augmented by PS and factor V, whereas cleavage of factor Va requires only PS. When thrombin is bound to TM, it can no longer activate platelets or endothelial cells, cleave fibrinogen or factor XIII, activate factor V, or induce signaling via the PARs. When unbound, thrombin exhibits a broad range of proinflammatory properties, some mediated via NF-\(\kappa\Beta\) pathways or the PARs. Overall, TM is a biological switch, determining whether thrombin’s output will be procoagulant/proinflammatory or anticoagulant/anti-inflammatory. In a similar manner, EPCR is a determinant of the output of APC; when bound to EPCR, APC is unable to inactivate factors Va/VIIa; however, in this form, APC transmits anti-inflammatory, cytotoxic, and regenerative signals. Free of EPCR, APC also promotes fibrinolysis by neutralizing PAI-1. The resultant increase in plasmin may regulate fibrin clot size, clear cross-linked fibrin, and recruit inflammatory cells to the site of injury to promote clot resolution and healing. Overall, TM and EPCR co-operate
as partners in determining the balance between coagulation and inflammation, providing complementary mechanisms that can respond to a range of pathophysiologic stresses in different vascular beds.

In several animal models, APC, PC, PS, or EPCR dampens release of inflammatory cytokines, leukocyte trafficking, capillary leak syndrome, and coagulation activation. APC suppresses nuclear translocation of NF-κB and activation of AP-1. Interfering with this pathway abrogates expression of cell surface leukocyte adhesion molecules and leukocyte–endothelial cell interactions. APC also dampens release of pro-inflammatory cytokines, including TNFα, IL-1β, IL-6, IL-8, and macrophage inflammatory protein-1α by monocytes/macrophages, reduces expression of TF, and inhibits neutrophil activation and chemotaxis. These pathways are largely dependent on the integrity of EPCR. Mice that overexpress EPCR are resistant to endotoxin-induced sepsis, whereas those with low levels are more sensitive. EPCR acts as a cofactor for PAR-mediated cleavage of PAR-1. Depending on the context, the other PARs (-2, -3) also participate, mediating anti-inflammatory and cytoprotective effects. Signaling may also require other receptors (eg, Mac1, apolipoprotein receptor 2, EGF receptor, Tie 2; reviewed in Griffin et al). APC-induced PAR activation also induces expression of inhibitors of apoptosis (IAP-1, A20) and TAFIa, respectively. APC bound to endothelial protein C receptor (EPCR) induces protease-activated receptor (PAR)-mediated anti-inflammatory/cytoprotective signaling, upregulating inhibitors of apoptosis (IAP-1, A20) and sphingosine-1 phosphate (S1P) signaling and dampening cytoskeletal release from endothelial cells. APC suppresses coagulation by cleaving factors V/Va and VII/VIIa. TAFIa dampens fibrinolysis and inflammation. The lectin-like domain (LLD) also has direct anti-inflammatory properties (Illustration Credit: Ben Smith), BK indicates bradykinin; and HMGB1, high mobility group box-1.

In response to injury or infectious/inflammatory stimuli and the release of damage-associated signals (eg, histones), the ternary coagulation complex comprising TF, factor VIIa, and factor Xa binds to PAR-2 via interactions with EPCR. This induces PAR-2-TLR4-mediated cross talk that yield a pro-inflammatory interferon gene expression response program. Exogenous APC may displace factor VIIa or factor Xa from EPCR, thereby uncoupling the pro-inflammatory PAR-2 signaling. Notably, this requires both PS and a proform of factor V that is cleaved at R506. Interestingly, the R506Q mutation of FV (FV Leiden) seems to confer a survival advantage in mice and humans in response to various infectious organisms. The structure of TM confers its domains with distinct properties (reviewed in Morser). The EGF-like domain, required for thrombin-mediated generation of APC and activated thrombin activatable fibrinolysis inhibitor, sequesters thrombin from its myriad pro-inflammatory effects. Activated thrombin activatable fibrinolysis inhibitor inhibits fibrinolysis and inactivates pro-inflammatory mediators, bradykinin, osteopontin, and complement anaphylatoxins, C3a, and C5a. Mice lacking the N-terminal lectin-like domain have heightened sensitivity to endotoxin-induced cytokine release and are more sensitive to ischemia–reperfusion injury, inflammatory arthritis, and exposure to lipopolysaccharide/Shiga toxin. The lectin-like domain sequesters pro-inflammatory high mobility group box-1, preventing it from binding to the Receptor for Advanced Glycation End Products, TLR-2, and TLR-4. It also binds to the carbohydrate Lewisα on lipopolysaccharide of organisms, inducing their phagocytic clearance and preventing signaling via CD14. Finally, TM dampens complement activation, enhancing factor I/factor H–mediated inactivation of C3b. In humans, mutations in TM (not restricted to the lectin domain) confer an increased risk of developing complement-mediated atypical hemolytic uremic syndrome.

Overall, TM and EPCR are key players in limiting the recruitment and amplification of procoagulant and proinflammatory pathways, an effect that is abrogated in the setting of inflammation/infection or injury (reviewed in Conway).
TM and EPCR are also cleaved by neutrophil proteases to yield soluble fragments that may be useful as biomarkers and have therapeutic utility. Insights gained are yielding promising therapies for thrombotic and inflammatory disorders, including sepsis, disseminated intravascular coagulation (DIC), arthritis, colitis, pneumonitis, ischemic stroke, and diabetic nephropathy (reviewed in Martin et al67).

**Innate Immunity**

Once a pathogen penetrates the body’s physical barriers (ie, skin or mucosal membranes), a second wave of innate immunity is launched against the pathogen. Although the innate immune system is nonspecific, this system, which includes TLR signaling pathways and complement, is activated in response to broad pathogenic classes known as DAMP and pathogen-associated molecular patterns (eg, lipopolysaccharide, mannose and pathogenic DNA/RNA, etc). The nonspecific nature of the innate immune system unfortunately means that it regularly engages in friendly fire, causing inflammation and damage to host cells and organs. TLRs and the complement system have recently gained notoriety in the pathogenesis of thrombosis because these systems are inextricably linked to the pathways/proteins that activate or enhance coagulation (Figures 3 and 4).

**Toll-Like Receptors**

TLRs were discovered in the mid-1990s68,69 and represent a subclass of the pattern recognition receptor family that facilitates the innate immune response against a broad spectrum of molecules. To date, a total of 13 TLR family members have been described (TLR1-11 in humans and TLR1-13 in mice). TLRs are present on endothelial cells, platelets, antigen-presenting cells, such as macrophages and dendritic cells among other cell types, and are a key component of the innate immune system owing to their ability to potently trigger inflammatory pathways70 (Figure 3). The TLRs have also been implicated in playing a regulatory role in phagocytosis.71

Most TLRs form homodimers72,73 or heterodimers in the case of TLR2 (with TLR1 or TLR6), which enables them to interact with their ligands. All TLRs, except TLR3, signal through a MyD88-dependent pathway.74 On ligand binding to the TLR dimer, a conformational change occurs that causes the adaptor protein MyD88 to be recruited. MyD88 then recruits interleukin-1 receptor–associated kinases that initiate events that ultimately lead to NF-κB liberation and translocation into the nucleus (reviewed in Kawai and Akira14), where it drives the production of inflammatory cytokines, such as TNFα, IL-6 and IL-12 and IL-1β. TLR3 signals exclusively via the TIR-domain-containing adapter-inducing interferon-β (TRIF)–dependent pathway, and TLR4 uses this pathway in addition to its MyD88-dependent signaling.75 On dimerization of TLR3 (or TLR4), TRIF is recruited as an adaptor protein to the cytosolic tails of the dimer. TRIF activates both TANK-binding kinase 1 and receptor interacting serine/threonine protein kinase 1, which split the signaling pathway—TRIF/TANK-binding kinase 1—ultimately driving interferon (type I) production, whereas TRIF/receptor interacting serine/threonine protein kinase 1 induces NF-κB liberation and translocation into the nucleus in a manner similar to that of the MyD88-dependent pathway.

The TLR family members described to date show great diversity with respect to the ligands that they bind. The TLRs can be roughly divided into 3 groups based on their ligand specificity76: (1) those that interact with lipids and lipopeptides (TLR1, -2, -4, and -6); (2) those that interact with proteins (TLR5 and mouse TLR11); and (3) those that interact with DNA and RNA (TLR3, -7, -8, and -9). Although all TLR family members recognize and bind molecules that are foreign to the body, they also recognize endogenous molecules that are typically compartmentalized with cells. Most of these molecules, which include DNA, RNA, and heat shock protein, do not typically encounter TLRs unless nonphysiological cell death occurs.

**Figure 3.** Toll-like receptors (TLRs). TLR dimers bind their ligands (eg, fibrinogen, lipopolysaccharide [LPS], heat shock proteins [HSPs], DNA/RNA), resulting in the recruitment of MyD88 or TRIF (depending on the TLR subtype). These accessory proteins activate pathways that ultimately result in the production of inflammatory cytokines, including, for example, interferon (IFN), tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-12, and IL-1β, which in turn can promote coagulation. RIPK indicates receptor interacting serine/threonine protein kinase 1; and TRIF, TIR-domain-containing adapter-inducing interferon-β.
The mechanisms by which the TLRs, as a family, contribute to thrombosis are not fully understood. Research on TLR2 and TLR4 in models of thrombosis, however, has yielded many interesting findings relevant to coagulation and thrombosis. TLR4 has been shown to mediate TF expression in antiphospholipid antibody (anti–β2GP1)–mediated thrombosis, in diabetes mellitus, in hypercholesterolemia in mice and monkeys, in lipopolysaccharide-induced endotoxemia and microvascular thrombosis, and in arterial thrombosis in mice. TLR2 routinely plays a supporting role in TF expression in models where TLR4 is also implicated. In addition to inducing TF expression, TLR2 and TLR4 also mediate platelet activation and increase coagulation in response to extracellular histones. Fibrinogen may act as a ligand for TLR-mediated signaling. TLR9 has also been implicated as an initiator of TF expression in vitro by human coronary artery endothelial cells and in mice, illustrating that TLR-mediated inflammation augments coagulation through more than just TLR2 and TLR4. It is anticipated that additional TLRs will augment both TF expression along with procoagulant molecules in relevant models and diseases; however, further work is required to establish the molecular mechanisms that will ultimately link other TLRs to thrombosis.

Complement

The complement system forms an essential component of the innate immune response. The complement system is activated via the classical (CP), lectin (LP), or alternative (AP) pathways (Figure 4). The CP and LP are homologous, and their initiation is triggered on binding of C1q (CP) or mannose-binding lectin or collectins (LP) to DAMPs or pathogen-associated molecular patterns, respectively (reviewed in Gal et al). As C1q or mannose-binding lectin binds to the DAMP or pathogen-associated molecular pattern, their associated enzymes—Clr and Cls and mannos-binding lectin–associated serine proteases—become autoactivated and drive downstream complement activation. Cls and mannos-binding lectin–associated serine proteases–2 catalyze the cleavage of C4 and C2 into their respective a and b split products. C4b contains a thioester bond that becomes exposed on its liberation from C4. The thioester is hydrolyzed, often resulting in opsonization of C4b to surfaces (ie, cells or pathogens) in the vicinity of complement system initiation. C4b binds C2a to form a C3 convertase, C4b2a, which catalyzes cleavage of C3 into a and b split products. C3b, like C4b, may become opsonized to neighboring cells or pathogens, whereas C3a is released as an anaphylatoxic peptide. The AP also contributes to C3a and C3b generation via its own C3 convertase, C3bBb (reviewed in Pangburn and Muller-Eberhard). The AP does not require engagement of a DAMP or pathogen-associated molecular patterns to become activated, and therefore, its activity simply ticks-over, generating low levels of C3 split products. This low-level of constitutive activation is considered to be a form of immune surveillance. C3 tick-over occurs when complement factor D forms a ternary complex with factor B and C3b (generated by the CP, LP, or AP) or C3(H2O), an uncleaved C3 molecule with a water-hydrolyzed thioester. C3(H2O) shares the same, albeit lower, cofactor activity as C3a and C3b when acting as a convertase cofactor. Factor D cleaves factor B into its split products a and b, resulting in the generation of a serine protease Bb and soluble fragment Ba with unknown function. The C3bBb complex is an AP convertase that also catalyzes cleavage of C3 into a and b split products. As the local concentration of opsonized C3b reaches a critical threshold, the substrate specificity of the C3 convertases (C4b2a for the CP/LP and C3bBb for the AP) shifts from C3 to C5. C5 convertases, denoted as C4b2aC3b and C3bBbC3b for the CP/LP and AP, respectively, cleave C5 into a and b split products. C5a is released as an anaphylatoxic peptide, and C5b initiates the terminal pathway (TP) of complement. C5b rapidly binds to C6 to form the meta-stable C5b,6 complex. In rapid succession, the C5b,6 complex rapidly and tightly binds to C7 (C5b-7), C8 (C5b-8), and multiple copies of C9 (C5b-9) to form the membrane attack complex (or C5b-9), which causes the osmotic lysis of cells.

The complement system as a whole is a major contributor to inflammation and thrombosis. The anaphylatoxins C3a and C5a (and, to a lesser extent, C4a) bind to their G protein–coupled receptors, C3aR and C5aR, respectively, and mediate many inflammatory and prothrombotic processes. Both C3a and C5a are capable of inducing endothelial cells to express IL-8, IL-1, and RANTES. C5a also triggers exposure of cell adhesion molecules such as P-selectin that may act as an important inflammatory mediator facilitating the adhesion of neutrophils to the endothelium. C3a and C5a are also pivotal to the recruitment and activation of innate immune cells, such as monocytes, neutrophils, and macrophages and inducing changes in endothelial permeability. These anaphylatoxins can either directly activate innate immune cells or contribute to their activation indirectly through the stimulation of other cells, such as the endothelium, to produce inflammatory mediators, including IL-6, IL-8, monocyte chemoattractant...
protein-1,94 and others. TP complexes (C5b-7, C5b-8, and C5b-9) also activate cells, causing the production of inflammatory mediators (reviewed in Morgan95). Complement directly induces a prothrombotic phenotype through C5a-mediated TF expression on neutrophils90 and endothelial cells and von Willebrand factor secretion from endothelial cells,91 C5b-7 induced TF expression on monocytes,11 and C5b-8/C5b-9-mediated platelet activation.92 There are numerous points of intersection between complement and coagulation that may cause a prothrombotic phenotype in diseases, such as paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome.93 Although it is known that excessive C5 cleavage causes thrombosis in these patients, the precise mechanism is poorly understood.

The mechanisms by which the innate immune system modifies thrombosis risk are poorly understood, although great strides have been made in recent years to close the knowledge gap. Both TLRs and complement have been shown to contribute to platelet activation and TF expression in vitro and in animal models. Further research is required to confirm these important findings and their link to thrombosis in the clinic.

**Microparticles**

As previously introduced, MPs are phospholipid membrane vesicles that exhibit a wide range of effects on coagulation and inflammation, thus providing an important bridge that is increasingly recognized as of potential clinical importance.

MPs range in size from 0.05 to 1 μm diameter and are normally shed from different cells, but release is increased when activated by various pathological inflammatory and thrombotic stimuli. They are formed by disrupting the phospholipid bilayer of the plasma membrane, budding off as a bleb, while retaining a membrane skeleton. MPs are defined by their size and the antigens that are specific to the parent cell. Thus, they may be derived from any cell in the vasculature, including platelets, leukocytes, erythrocytes, endothelial cells, smooth muscle cells, but also by adipocytes, cardiomyocytes, and cancer cells. They circulate in the plasma, but are found in all body fluids, including saliva, urine, bile, tears, synovial fluid, and cerebral spinal fluid. MPs are distinguished from exosomes,95 which are released from multivesicular bodies, are generally smaller (20–100 nm), cup-shaped, more dense, and less heterogeneous. There are some overlapping functions.95 MPs can also be confused with apoptotic bodies,96 which are remnants of dead cells, larger than MPs, of higher density and lack the biological properties of MPs.

MP release is energy-dependent and regulated and induced by stresses, including hypoxia, exposure to reactive oxygen species (ROS), ischemia, and inflammatory mediators. Moreover, circulating levels of different MPs undergo circadian changes.97 The molecular basis of MP formation is not completely understood, but involves caspases and calpain, with modification of cellular architecture and cytoskeleton, which requires movement of lipid transporters (flippase, flippase, scramblase).98 The life span of MPs in the circulation is ~30 minutes.99 Clearance of MPs may occur via direct phagocytosis, after opsonization, by endocytosis or by phospholipase-mediated degradation.

The cargo that MPs carry is dependent to a large extent on the parent cell and also on the stimulus.100 However, soluble antigens from different cells may associate with MPs from other cells, thereby making it difficult to identify the origin of the MP. MP constituents can be membrane receptors (eg, integrins, TM, EPCR, PARs, TF), adhesion molecules (eg, P-selectin, P-selectin glycoprotein ligand-1, cytoskeleton proteins, cytokines, proteases, growth factors, major histocompatibility complex molecules), RNA, and microRNAs,101 and the relative functional availability of these would likely determine the effects of the MPs in different biological systems.

The majority of MPs (70%–90%) are believed to be derived from platelets or megakaryocytes102 and are referred to as platelet-derived microparticle (PMPs).103 Because platelets have diverse properties in innate immunity, inflammation, and hemostasis/thrombosis,104 it is not surprising that PMPs would similarly have complex effects on these systems, with altered expression being associated with disease.105 Overall, MPs have profound effects on the vasculature and coagulation. PMPs may exhibit procoagulant properties, partly because of the lipids of the membrane skeleton most often (but not always106) being enriched in phosphatidylserine, providing a surface for assembly of the tenase and prothrombinase complexes.107 There is ongoing debate as to whether PMPs or endothelial-derived MPs express TF.7,28 However, platelets seem to have the capacity to capture and incorporate TF or TF-rich vesicles to promote coagulation. Thus, TF-enriched MPs (mostly monocyte-derived) that also display P-selectin glycoprotein ligand-1 can interact with P-selectin expressed by activated platelets. This leads to fusion of the MPs to the platelet surface, acquisition of TF, and the MP membrane, with subsequent augmentation of the TF-VIIa proteolytic activity.29 Moreover, a positive feedback loop ensues, whereby MPs from activated platelets can induce monocytes to release procoagulant TF-expressing monocyte-derived MPs,108,109 overall providing a partial explanation for the procoagulant properties of MPs. That these MPs may be active in vivo is supported by the finding in mice that they can deliver TF to the site of injury during the initial phase of thrombus development.110 PMPs and MPs from erythrocytes can also induce thrombin generation in a factor XII–dependent manner,111 a finding that is intriguing in light of recent interest in developing antithrombotic therapies that interfere with activation of the intrinsic pathway, that is, factors Xla112 and XIla,113

Endothelial-derived MPs may affect vascular function and contribute to endothelial dysfunction in inflammatory diseases via several means, including, for example, by suppressing production of nitric oxide and prostacyclin (PGI2), by dampening nitric oxide bioavailability, and augmenting vascular permeability.114,115 Neutrophil-derived MPs can induce myeloperoxidase-mediated damage of endothelial cells.116 MPs from platelets and other cells may promote monocyte and neutrophil adhesion to endothelial cells117 and, in vivo in rodent models of myocardial ischemia, are angiogenic, probably mediated by the release of angiogenic factors (vascular endothelial growth factor, basic growth factor, sphingosine-1 phosphate)118 and TF-initiated signaling pathways.119,120 PMPs also contain respiratory competent mitochondria and thus may serve as a substrate for release of inflammatory mediators.121
MonoMPs and neutrophil-derived MPs that expose phosphatidylserine on their surface can activate endothelial cells and also interact with and activate resting platelets, helping to propagate the thrombus.122

In spite of some limitations in the models and the challenges in characterization of the MPs in several reports, there are strong clinical data to support the notion that MPs promote coagulation in cardiovascular disease. Mice that were injected with TF-bearing MPs responded with increased thrombin generation, elevated thrombin–antithrombin levels, and a drop in platelet count. Cancer cell–derived MPs bearing TF and P-selectin glycoprotein ligand-1 that were infused into mice accumulated at the site of a carotid artery injury and shortened the time to occlusion.123 In a model of deep vein thrombosis, injected cancer cell–derived MPs expressing high levels of TF induced a higher incidence of thrombosis than MPs with low TF. The infused MPs localized to NETs and also expressed P-selectin glycoprotein ligand-1, allowing them to bind to TF and P-selectin presented by endothelial cells and platelets, thereby triggering coagulation.124 Consistent with these findings, elevated circulating MP levels parallel D-dimers and P-selectin in deep vein thrombosis125 and are a risk factor for coronary artery disease and atherothrombosis in humans.

Increased MP levels have also been correlated with disease activity in diabetes mellitus, cancer, infections, inflammation, autoimmune disorders, and hypertension (reviewed in Suades et al129). In inflammatory arthritides, MPs are increased in the blood and synovial fluid, where they stimulate synovial cell release of proinflammatory IL-6 and IL-8. Although they may exhibit anti-inflammatory properties, in inflammatory and infectious diseases, MPs trigger complement activation, facilitate leukocyte adhesion and trafficking, induce cytokine release, and modulate endothelial cell nitric oxide and PGI2. They may deliver arachidonic acid to endothelial cells and infectious diseases, MPs trigger complement activation, and modulate endothelial cell nitric oxide and PGI2 release of proinflammatory cytokines, bacteria, viruses, endotoxin, and cholesterol crystals. In response to infections, NETs provide a means of entrapping and eliminating invading microbes with the scaffold of chromatin fibers that are associated with microbicidal proteins, proteases, and ROS.130

During clot formation, NETs colocalize with fibrin and von Willebrand factor,135 where they provide a scaffold for thrombi that are resistant to fibrinolysis.136,137 They bind to platelets and endothelial cells, inducing their activation, which in turn escalates further NETosis. Histones are a major constituent of NETs, enhancing activation of coagulation.138 They promote platelet and endothelial cell activation139 and induce the release of procoagulant polyphosphate from platelets via TLR-2- or TLR-4-dependent signaling pathways.81 They bind to TM and PC and interfere with the generation of APC.140 APC normally digests histones,141 but when NET-bound, the histones are resistant to degradation. Histones also interfere with antithrombin-mediated neutralization of thrombin and promote autoactivation of prothrombin to thrombin.142 DNA released with NETs provides a surface for autoactivation of factor XIIa, with subsequent amplification of proinflammatory and procoagulant contact system pathways. The cell-free DNA also interferes with tissue-type plasminogen activator (tPA)–mediated generation of plasmin on the clot surface143 and, during sepsis, modulates clot structure and impairs fibrinolysis.144 Neutrophil-derived proteases and ROS exhibit multiple proinflammatory and procoagulant properties, suppressing expression and function of TM145 and degrading natural inhibitors of the coagulation cascade, including TF pathway inhibitor.146

Neutrophil Extracellular Traps
As the name implies, NETs are secreted from activated neutrophils, but also eosinophils, basophils, and possibly monocytes. They are evolutionarily conserved web-like structures129 comprising DNA, histones, proteolytic enzymes, and other associated proteins,130 and they are implicated in the pathogenesis of both inflammatory and thrombotic disorders. Thrombi are rich in fibrin, red blood cells, platelets, and leukocytes. Early in thrombogenesis, platelets and neutrophils are recruited to the site of vascular injury, triggered in part by the local release of von Willebrand factor, P-selectin, chemokines, and chemoattractants. There, in response to one or more triggers, neutrophils undergo a stepwise process, known as NETosis.131 Enzymes such as neutrophil elastase and myeloperoxidase132 shift from cytoplasmic granules into the nucleus, where they degrade histones and promote the unwinding of chromatin. Preprocessing of ROS in the granules may first be required.133 Granules and nuclear membranes are broken down, and cytolytic of the cell proceeds, with release of the NETs. These web-like structures trap circulating cells, and their associated constituents (histones, DNA, proteases, proteins) exert multiple proinflammatory and procoagulant effects, participating in the pathogenesis of both venous and arterial thrombosis.134

There are several environmental triggers for NETosis, probably with shared intracellular molecular signaling mechanisms. Interaction of neutrophils with platelets seems to be a necessary initiating event, although endothelial cells may suffice. NETosis is provoked by, for example, hypoxia, ROS, cytokines, bacteria, viruses, endotoxin, and cholesterol crystals. In response to infections, NETs provide a means of entrapping and eliminating invading microbes with the scaffold of chromatin fibers that are associated with microbicidal proteins, proteases, and ROS.130

The critical role of NETs in the pathogenesis of thrombosis has been validated in several animal models, with recent strong evidence of a role in humans. Administration of DNase that degrades NETs can protect mice from thrombosis.43,147 Moreover, mice that are deficient in peptidylarginine
deaminase 4 and thus unable to form NETs are resistant to thrombosis. After induction of deep vein thrombosis in baboons, NETs were detected in the thrombus and the plasma. Recent evidence of a definitive role of NETs in venous and arterial thrombosis is now emerging. Circulating free DNA and NETs were detected in patients with primary antiphospholipid antibody syndrome, within the clot of thrombosed stents, and in thrombotic material isolated from coronary arteries during acute myocardial infarction, the latter which were decorated with TF. Finally, at surgery or autopsy, NETs have been localized to human venous thrombi. In all cases, the NETs have been associated with evidence of inflammation, most prominently with infiltration of neutrophils.

The neutrophil constitutes a first-line of defense against infections and is an early responder to stimuli that lead to tissue injury, including inflammation, infection, trauma, cancer, and thrombosis. It follows (and is now recognized) that NETs participate in an array of disorders— Infectious, malignant, thrombotic, inflammatory—and provide a common mechanism by which these pathologies and relevant processes that facilitate wound healing and angiogenesis (reversibly and coordinately degrading fibrin and initiating immune modulation of extracellular matrix proteins). Independent of its activation on cells is reliant on plasminogen receptors. Most cell types, including endothelial cells, leukocytes, and platelets, contain relatively high levels of plasminogen receptors on their surface. This class of receptor, which counts at least a dozen members in its family, binds to both plasminogen and plasminogen activators, localizing them in relatively high concentrations on the cell surface (reviewed in Plow et al). This results in increased efficiency of plasminogen activation. As an example, Annexin A2 is usually located in the cytoplasm, but with certain stimuli its expression level increases, causing it to form a tetrameric complex with S100A10 (2-A2, 2-S100A10). After phosphorylation of A2 by tyrosine kinases, the complex is translocated to the cell surface where it interacts with plasminogen and increases the catalytic efficiency of activation by almost 100-fold. Annexin A2-S100A10 tetramer exposure and the plasmin generation that follows induces signaling pathways that drive inflammatory responses, such as NF-kB translocation to the nucleus and expression of TNFα, IL-1, and IL-6.

Plasminogen System/Fibrinolysis

The fibrinolytic system plays a critical role in the resolution of blood clots, but paradoxically, this system can also promote inflammatory processes that have been implicated in thrombosis.

The plasminogen system drives fibrinolysis by gradually and coordinately degrading fibrin and initiating immune processes that facilitate wound healing and angiogenesis (reviewed in Collen and Pepper). The fibrinolytic system is activated immediately upon fibrin formation. Thrombin generated during hemostasis or thrombosis activates the endothelium to express and secrete intracellular pools of the plasminogen activators tPA and urokinase-type-plasminogen activator (uPA). tPA and uPA both cleave plasminogen to generate plasmin, the principal enzyme of fibrinolysis. Plasmin can in turn cleave both tPA and uPA into 2-chain versions that activate plasminogen more efficiently than their single chain counterparts. Fibrin facilitates the interaction between tPA and plasminogen and, therefore, acts as a cofactor for tPA-mediated plasminogen activation. Although uPA binds fibrin with low affinity, 2-chain uPA activates plasminogen with a 10-fold greater efficiency in the presence of fibrin than in its absence. Despite this modest increase in plasminogen activation, uPA is generally considered to activate plasminogen by a fibrin-independent mechanism. In addition to tPA and uPA, enzymes of the contact pathway have also been shown to activate plasminogen. As plasmin is generated, it cleaves fibrin to generate C-terminal lysine residues that enhance the interaction between tPA, plasminogen, and fibrin, thus contributing to a further enhancement of plasmin generation on the fibrin clot. The fibrinolytic inhibitor thrombin activatable fibrinolysis inhibitor (or carboxypeptidase B2) removes these lysine residues, which attenuates plasminogen activation.

Despite plasmin generation and fibrinolysis being almost synonymous, plasmin generation does not occur exclusively on the surface of fibrin (reviewed in Kolev and Machovich). Plasmin may be generated on a wide variety of cell types, leading to enhanced fibrinolysis, extracellular matrix remodeling, activation of matrix metalloproteinases, cellular activation, and inflammation. The mechanism of plasminogen activation on cells is reliant on plasminogen receptors. Most cell types, including endothelial cells, leukocytes, and platelets, contain relatively high levels of plasminogen receptors on their surface. This class of receptor, which counts at least a dozen members in its family, binds to both plasminogen and plasminogen activators, localizing them in relatively high concentrations on the cell surface (reviewed in Plow et al). This results in increased efficiency of plasminogen activation. As an example, Annexin A2 is usually located in the cytoplasm, but with certain stimuli its expression level increases, causing it to form a tetrameric complex with S100A10 (2-A2, 2-S100A10). After phosphorylation of A2 by tyrosine kinases, the complex is translocated to the cell surface where it interacts with plasminogen and increases the catalytic efficiency of activation by almost 100-fold. Annexin A2-S100A10 tetramer exposure and the plasmin generation that follows induces signaling pathways that drive inflammatory responses, such as NF-kB translocation to the nucleus and expression of TNFα, IL-1, and IL-6.

Other plasminogen receptors, such as enolase-1, Plg-R, H2B, and others, increase plasminogen activation by similar mechanisms, leading to a robust proinflammatory response. The urokinase-type plasminogen activator receptor (uPAR) also increases plasminogen activation by concentrating uPA on the surface of cells. The plasmin generated plays a critical role in fibrinolysis and in the direct and indirect degradation of extracellular matrix proteins. Independent of its roles in fibrin and ECM degradation, uPA-uPAR interactions induce a conformational change that enables uPAR to interact with various adaptor molecules, including integrins, that activate cell-signaling pathways, leading to cellular migration and differentiation and inflammatory cytokine production. Interestingly, uPAR is often localized to the leading edge of inflammatory cells, which promotes plasmin generation and extracellular matrix degradation along the cell’s axis of migration. Integrins are also localized in the same region, which enhances the nature and downstream effects of their interactions.

Both cellular and fibrin-based plasmin generation can activate the complement system, which mediates inflammation via numerous mechanisms. Plasmin can directly activate C3 and C5, generating the inflammatory anaphylatoxins C3a and C5a and C5b, the initiator of membrane attack complex formation. The effects of complement activation are described in more detail in a separate section. Plasminogen can also cleave complement iC3b, which destroys its ability to down-regulate IL-12 expression in macrophages.

Given the broad range of effects that plasmin and plasminogen activators mediate, it is essential that their activity be tightly regulated. The serine protease inhibitors alpha2-antiplasmin and PAI-1 are essential to ensure that uPA and tPA activity remains tightly controlled and that excessive plasmin generation...
does not occur. Several inflammatory molecules, including lipopolysaccharide, IL-1, TNF-α, TGF-β, basic growth factor, very low-density lipoprotein, lipoprotein(a), angiotensin II, thrombin, and phorbol esters, have been shown to increase PAI-1 mRNA and protein levels without affecting plasminogen activator levels. An increase in PAI-1 levels without a counteracting rise in tPA or uPA is therefore prevalent in inflammatory diseases (reviewed in Juhan-Vague et al173), diabetes mellitus, and obesity174 and is considered an independent risk factor for thrombosis.175 The connection between inflammation-induced PAI-1 increases, and thrombosis is supported by the genetic polymorphism, PAI-1 4G/5G, that is associated with both increased plasma PAI-1 and thrombosis.175

The question of whether plasmin(ogen) system-induced inflammation contributes to thrombosis remains unanswered. Addressing this issue will require elegant experiments that dissect and delineate the opposing roles of the plasmin(ogen) system in fibrinolysis and inflammation/thrombosis.

**Contact Activation and Kallikrein–Kinin**

The paradigm that the TF–factor VIIa–mediated coagulation pathway is solely reponsible for hemostasis in vivo has been revisited in recent years because accumulating evidence implicates the contact activation pathway in triggering both thrombin generation and inflammation. With this information, novel insights are providing unique opportunities for the development of safer approaches to treat thrombo-inflammatory disorders.176

The contact activation system and the kallikrein–kinin system, often referred to collectively as the plasma contact system, is required for rapid generation of enzymatic activity on the surface of pathological surfaces, damaged cells, and invading pathogens and can trigger coagulation and inflammation. The key components are factor XII, plasma prekallikrein (PPK), and high molecular weight kininogen (HK).176

Factor XII circulates in the blood as a zymogen. The contact pathway is initiated when factor XII comes in contact with a negatively charged surface and undergoes a conformational change, causing it to autoactivate, yielding small amounts of factor XIIa177 (Figure 5). The efficiency of autoactivation is enhanced in the presence of HK and PPK, the majority of which circulates as a complex. Several candidate physiological activators of factor XII have been identified. These include the surface of damaged blood vessels and cells, invading pathogens, RNA,178 DNA, NETs,43 aggregated misfolded proteins,179 negatively charged polysaccharides, such as chondroitin sulfates on cell surfaces, heparin sulfate released from mast cells,180 and the highly anionic polyphosphate that is released from dense granules of activated platelets and expressed on the surface of bacteria.177,181

As factor XIIa is generated, it recruits HK to the charged surface and cleaves it to its active form HKa, which enhances its surface-binding affinity. HK/HKa also binds to uPAR, gC-1qR, and cytokeratin, inducing release of cytokines and chemokines.168 With PPK bound to HKa, factor XIIa also cleaves PPK to generate the serine protease, kallikrein,182 which in turn feeds back to activate more factor XIIa, amplifying generation of factor XIIa and kallikrein. Free PPK also may bind to endothelial cells, where vessel wall–associated prolylcarboxypeptidase can directly generate kallikrein.176

With sufficient factor XIIa, factor XI is activated, triggering thrombin generation and fibrin formation via the intrinsic pathway, with dampening of the fibrinolytic pathway via activation of thrombin activatable fibrinolysis inhibitor.158 Kallikrein-mediated activation of factor XII therefore provides a catalyst for optimal downstream activation of the intrinsic coagulation system.183 Kallikrein also proteolyses HK to liberate bradykinin, a potent vasodilator, vascular permeability-inducing, and proinflammatory peptide.184 Bradykinin binds to G protein–coupled receptors bradykinin receptor 2 and bradykinin receptor 1 that are expressed on endothelial cells and activated leukocytes and stimulates production of nitric oxide and PGI2 and release of tPA from endothelial cells,185 all of which dampen coagulation, platelet activation, and fibrin deposition. In vitro, bradykinin also suppresses endotoxin-induced TF expression by vascular endothelial cells, and

![Figure 5. Factor XII activation at the nexus of coagulation and inflammation.](http://circres.ahajournals.org/)

On contact with highly anionic surfaces (not shown), factor XII (FXII) autoactivates to generate small amounts of FXIIa. FXIIa recruits high molecular weight kininogen (HK/HKa) that is complexed with prekallikrein (PPK). FXIIa activates PPK to kallikrein (PK), which in turn generate more FXIIa and activates HK to release bradykinin (BK). BK binds to its receptors (B2R) on endothelial cells and neutrophils, triggering proinflammatory responses. FXIIa also activates FXI in the coagulation pathway, inducing further thrombin (IIa) generation. When free, IIa induces coagulation and inflammation. When bound to thrombomodulin (not shown), IIa promotes generation of activated protein C (APC) and TAFIa. Not shown are activating surfaces and C1-inhibitor, the major negative regulator of FXIIa, FXIIa, and PK. TAFI indicates thrombin activatable fibrinolysis inhibitor.
exogenous administration of bradykinin prevents thrombosis in vivo in a mouse model.\textsuperscript{186} Although most bradykinin is derived from kallikrein-mediated cleavage of HK, other serine proteases, including plasmin, thrombin, and factor XIIa, can contribute. Mice lacking factor XII do not have a hemostatic defect and have bradykinin levels that are diminished by \textasciitilde 50\%, underlining the important position of factor XII at the nexus of contact activation–induced coagulation and inflammation,\textsuperscript{187} and thus a potential site for therapeutic intervention (see below).

The major negative regulator of factors XIa, XIIa, and kallikrein is the serine protease inhibitor C1-esterase inhibitor.\textsuperscript{188} Hereditary angioedema, a potentially life-threatening condition caused by a functional deficiency of C1-esterase inhibitor or a gain of function mutation in factor XII, leads to episodic swelling of the mucosa of the oropharynx, larynx, or intestines. During episodes, bradykinin levels are elevated and thus increase vascular permeability. In spite of elevated factor XIIa levels and increased D-dimers during acute episodes,\textsuperscript{189} hereditary angioedema is not associated with a heightened risk of thrombosis. This is likely related to preferential activation of the vasculoprotective kallikrein–kininogen pathway, evidenced by the increased bradykinin levels and resultant increased generation of PGI\textsubscript{2}, nitric oxide, and plasmin, with reduced TF expression. In line with this hypothesis, there is at best conflicting data on whether elevated factor XII levels increase the risk of arterial thrombosis in humans.\textsuperscript{190,191} In contrast, epidemiological studies indicate that elevated levels of factor XIa are a risk factor for venous thrombosis,\textsuperscript{192} stroke,\textsuperscript{193,194} and possibly myocardial infarction.\textsuperscript{195,196}

Patients with deficiencies of factor XII, HK, or PPK do not have excess bleeding,\textsuperscript{197,198} whereas those with low factor XI have a bleeding disorder.\textsuperscript{199} Mice lacking Factor XII, HK, or PPK also do not have a hemostatic defect. Indeed—and highlighting the potential of the contact factors as therapeutic targets—these contact system-deficient mice exhibit resistance to venous and arterial thrombosis, to ischemic stroke, to polyphosphate-induced thromboembolism,\textsuperscript{181} and to the adverse effects of endotoxin.\textsuperscript{200} As expected, these mice also have reduced bradykinin levels, which results in downregulation of bradykinin receptor 2, a major negative regulator of PGI\textsubscript{2}. With reduced bradykinin receptor 2, the increased PGI\textsubscript{2}, in turn, upregulates transcription factors Sirt1 and KLF4, leading to suppression of TF and enhanced expression of TM,\textsuperscript{201} thereby preventing thrombosis. Similarly, mice lacking bradykinin receptor 2 are protected against thrombosis.\textsuperscript{202} In this case, however, the mice have elevated levels of bradykinin, which induces angiotsensin-converting enzyme activity. That, in turn, augments angiotinsin II and angiotensin-(1–7) which bind to their cognate receptors, AT2R and Mas, resulting in an increase in PGI\textsubscript{2}. With subsequent upregulation of TM and downregulation of TF, the vasculature is again protected against thrombosis.

The preceding findings highlight the complex relationship between coagulation and inflammation as regulated by the contact activation and kallikrein–kinin systems. Delineating the mechanisms underlying their initiation and propagation, coupled with genetic studies in mice and humans, is uncovering safer approaches to treat/prevent thrombotic disorders. In that respect, antibodies that inhibit factor XII have been successfully tested for efficacy in thrombosis models in mice and nonhuman primates, and notably, these have not been complicated by excess bleeding.\textsuperscript{203–205} As the biochemical links are uncovered, more therapeutic targets to develop better preventive strategies will be revealed.

Conclusions

The past decade has seen remarkable advances in our understanding of the biochemical pathways that link coagulation and inflammation, and these are uncovering promising therapeutic strategies, which are at various stages of development. These include, for example, PAR1 antagonists for myocardial infarction and peripheral arterial disease; variants of APC for inflammatory and ischemic disorders; recombinant TM for sepsis and DIC; the complement inhibitor eculizumab for the thrombotic microangiopathies, atypical hemolytic uremic syndrome, and paroxysmal nocturnal hemoglobinuria. Although not yet proven, other interventions that target polyphosphate, NETs, and factor XIIa may yield drugs that simultaneously exhibit antithrombotic and anti-inflammatory properties, without disrupting normal hemostasis. Although great strides have been made recently to advance our knowledge of the cross talk between thrombosis and inflammation, there is still much to learn. The years ahead should yield novel targets and innovative strategies to simultaneously block prothrombotic events, including inflammation without a detrimental impact on hemostasis. Overall, in spite of many gaps in our knowledge, there is renewed excitement resulting from the enormous potential of targeting the interface between coagulation and inflammation.

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Disclosures

None.

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