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COAGULOPATHIES AND INFLAMMATORY DISEASES: ‘... GLIMPSE OF A SNARK’

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Abstract

Coagulopathies and inflammatory diseases, ostensibly, have distinct underlying molecular bases. Notwithstanding, both are host defense mechanisms to physical injury. In invertebrates, clotting can function directly in anti-pathogen defense. Molecules of the vertebrate clotting cascade have also been directly linked to the regulation of inflammation. We posit that thrombophilia may provide resistance against pathogens in vertebrates. The selective pressure of improved anti-pathogen defense may have retained mutations associated with a thrombophilic state in the human population and directly contributed to enhanced inflammation. Indeed, in some inflammatory diseases, at least a subset of patients can be identified as hypercoagulable. Therefore, anticoagulants such as warfarin or apixaban may have a therapeutic role in some inflammatory diseases.

Across the animal kingdom, survival requires processes that maintain organismal integrity and prevent pathogen invasion. When the external barrier of an organism is breached, there is an urgent requirement to prevent blood or hemolymph loss, as well as to deny entry to pathogens. These two functions are dealt with by the coagulation cascade and by the immune system, respectively. Are these two processes entirely distinct or are there functional crossovers between the two? Here, we explore the idea that the clotting cascade

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has immune defense functions (*the Snark*) in three fits – considerations of evolution, molecular mechanisms and human diseases.

COAGULATION AS AN ANCIENT HOST DEFENSE MECHANISM

“Just the place for a Snark! I have said it twice:

That alone should encourage the crew.

Just the place for a Snark! I have said it thrice:

What I tell you three times is true.” [1]

Following injury, the open or semi-closed circulatory system in invertebrates is plugged by an aggregation of cells or by coagulation [2]. For example, when the coelomic fluid of the sipunculan (marine worm) *Themiste petricola* is exposed to seawater, large granular leukocytes are activated and aggregate homotypically to form a macroscopic mass. In the sea urchin *Strongylocentrotus purpuratus*, a 75-kDa protein named amassin forms disulfide-linked multimers that cause the aggregation of coelomocytes.

Aggregation and clotting aside, most animal phyla have a separate, dedicated cellular system for sensing pathogens and launching an appropriate innate immune response. Specialized cells detect microorganisms or damage-associated molecular patterns. In many organisms, including vertebrates, this triggers an inflammatory response. Albeit that the mechanisms of coagulation and inflammation are often distinct, are these processes also interlinked? Coagulation in arthropods has been directly associated with defense against pathogens. In chelicerates, *Limulus polyphemus* or *Tachypleus tridentatus*, hemocytes detect lipopolysaccharide (LPS) from Gram-negative bacteria or β -1,3 glucan associated with fungi, and respond by releasing the serine proteases factor C and G, respectively, from their L granules [2]. The autocatalytic activation of factor C activates factor B, which in turn converts pro-clotting enzyme to clotting enzyme. Clotting enzyme then converts coagulogen into an insoluble coagulin gel. Various other agglutinins/lectins are released from L granules and invaders are trapped in this matrix.

Arthropods also have a distinct serine protease cascade that results in the conversion of the zymogen prophenoloxidase (PPO) into active phenoloxidase (PO). PO crosslinks the clot and transforms the soft clot formed by aggregation of degranulation products into a hard clot [3]. Apart from PO, some arthropods have a transglutaminase, a calcium-dependent enzyme that links side chains of lysine and glutamines *via* covalent bonds. In crustacea, for example in the crayfish *Pacifastacus leniusculus*, coagulation occurs by the crosslinking of a specific plasma clotting protein by a transglutaminase [3,4]. Similar processes have been reported in lepidopteran and dipteran insects [2,3]. Plasmatocytes, the professional phagocytes, spread across the hard clot. Additionally, PO oxidizes phenols to quinones, which are subsequently polymerized into melanin. Melanization is a distinct insect host defense response. Cytotoxic free radical by-products generated during melanization contribute to the killing of pathogens.

VERTEBRATE CLOTTING CASCADE CAN DIRECTLY COORDINATE INFLAMMATION

“Come, listen, my men, while I tell you again

The five unmistakable marks

By which you may know, wheresoever you go,

The warranted genuine Snarks.

Is the primordial pathogen defense function of coagulation extant in vertebrates? Since the local formation of thrombi, as well as inflammation, are part of the wounding response in vertebrates, the term *immunothrombosis* was coined [5]. While a direct functional link between pathogen sensing and coagulation has not been revealed, there are shared molecular mechanisms regulating coagulation and inflammation in vertebrates. Furthermore, some molecules that belong to the coagulation cascade have an active role in inflammation and host defense.

THROMBIN-PAR-1 AXIS

Injury in vertebrates is typically followed by a choreographed sequence of events wherein vessels constrict, a platelet plug is formed and then a stable blood clot seals the wound. The vertebrate coagulation cascade comprises serial proteolytic reactions that lead to the activation of thrombin [6–8]. This, in turn, cleaves fibrinogen into fibrin, the main constituent of a blood clot (Figure). Beyond coagulation, thrombin has been reported to regulate the inflammatory response (Figure).

Thrombin mediates cellular responses through the activation of a set of receptors present in platelets, endothelial cells, fibroblasts, and immune cells. The prototypical Protease-activated receptor (PAR), PAR-1, was identified by expression cloning while searching for receptors that function in thrombin-mediated platelet activation [9]. Pioneering efforts by Coughlin and his colleagues demonstrated that thrombin binds to and subsequently proteolytically cleaves the seven-pass transmembrane receptor PAR-1 at its N-terminus [10]. The cleavage of the amino-terminal domain reveals a tethered ligand that intramolecularly binds a receptor sequence within PAR-1. Three additional PARs have since been described. Thrombin activates PARs-1, 3 and 4. In contrast, PAR-2 is activated by multiple serine proteases including trypsin, tryptase, tissue factor/factor VIIa/factor Xa, but not thrombin.

Several studies have demonstrated that thrombin positively regulates inflammation. In a carrageenin-induced paw edema model of inflammation in rats, thrombin increased edema and mast cell degranulation [11]. In a model of glomerulonephritis, thrombin induced PAR-1-dependent inflammation [12]. In an infectious model with *Streptococcus pneumoniae*, antagonizing PAR-1 reduced neutrophil recruitment [13]. During lethal sepsis, dendritic cells (DCs) expressing PAR-1 amplified both inflammatory and thrombotic responses through the sphingosine-1-phosphate 3 (S1P3) signaling pathway [14].

Thrombin can also negatively regulate inflammation. In a mouse model of sepsis, selective agonists of PAR-1 increased vascular permeability and lung interstitial fluid accumulation,

two hallmarks of inflammation, at early time points [15]. Furthermore, early blockade of thrombin or PAR-1 improved overall survival, consistent with a pro-inflammatory role of PAR-1 [15]. When administered at later time points, however, PAR-1 agonists reduced vascular permeability and protected mice against disseminated intravascular coagulation [15]. Therefore, the action of thrombin can transition from an early vascular-disruptive function to a later role in preserving endothelial integrity.

aPC-EPCR Axis

In contrast to thrombin, anticoagulants such as activated Protein C (aPC) primarily function in negatively regulating inflammation. PC bioactivity requires γ -carboxylation in a vitamin K-dependent manner. When PC is γ -carboxylated in its N-terminal glutamic acid (Gla) domain it binds to the negatively charged phospholipid membrane in a calcium-dependent manner. PC also binds to the endothelial PC receptor (EPCR) [16] (Figure). EPCR was originally cloned as an endothelial cell-specific receptor that can bind to both PC and aPC [17]. It is a type I transmembrane protein with sequence and structural similarities to MHC class I/CD1 family proteins. EPCR crystal structure, with a tightly bound phosphatidylcholine, is strikingly similar to CD1d, a non-classical MHC class I glycoprotein. CD1d binds glycolipids in its antigen presenting groove and presents these glycolipids to T cells. Extraction of the phosphatidylcholine, or even its exchange for lysophosphatidylcholine or platelet activating factor (PAF), impairs EPCR's ability to bind PC [16].

PC is activated by thrombin-dependent cleavage. EPCR can augment the activation of PC. When thrombomodulin (TM; a glycoprotein present on the endothelial cell surface) binds to low concentrations of thrombin, it not only restricts fibrin formation and platelet activation by thrombin but also forms a TM-thrombin complex that cleaves EPCR-bound PC, giving rise to activated protein C (aPC). R binding to PC effectively reduces the K_m of PC activation. In turn, aPC detaches from the EPCR, and together with its cofactor Protein S (PROS1), inactivates clotting factors Va and VIIIa, regulating further thrombin generation (Figure) [18,19].

Mice embryos deficient in PC demonstrated noticeable thrombosis, consumptive coagulopathy and associated hemorrhage in the brain, consistent with the function of aPC in coagulation [20]. aPC also has a number of cytoprotective roles, including anti-inflammatory functions and protection of endothelial barrier integrity. aPC deficient mice also had focal necrosis in the liver. Neonates did not survive more than one day after birth [20]. Transgenic mice expressing low levels of PC survived, but these animals developed spontaneous inflammation [21]. *In vitro* treatment of LPS-stimulated human monocytes with aPC inhibited TNF α production [22]. Interestingly, responses associated with adhesion, phagocytosis, and killing of Gram-negative bacteria remained unaltered [22]. *In vivo*, aPC prevented TNF α -induced tight junction disruption in epithelial cells and favored healing of the intestinal mucosa after colitis [23]. During sepsis, macrophage activation leads to the release of histones that contribute to cytotoxicity, organ failure and death in a mouse model. aPC treatment *in vitro* and *in vivo* was able to cleave histones and reduce cell damage and lethality [24].

The cytoprotective and anti-inflammatory effects of aPC are mediated by aPC-dependent activation of PAR-1. aPC, much like thrombin, can cleave PAR-1. Unlike thrombin, aPC-dependent cleavage and activation of PAR-1 requires EPCR as a co-receptor [16]. Even though PAR-1 serves as a receptor for both thrombin and aPC, the signaling downstream aPC is distinct from that of thrombin. This conundrum can be somewhat explained by GPCR's ability to bias their signaling depending on: *1) The cleavage site and/or differential downstream signaling*: thrombin and aPC cleave PAR-1 at different amino acid sites. Thrombin cleavage at arginine 41 (Arg41) engages ERK1/2 activation, while aPC cleavage at Arg46 engages Akt signaling [25,26]. The generation of mice with single point mutations at either Arg41 or Arg46 of PAR-1 showed that the protective effects of aPC *in vivo* require non-canonical cleavage at Arg46 [27]. Activation of the SIP1 pathway has also been linked to the anti-inflammatory functions downstream of PAR-1-EPCR [28]. *2) Receptor localization within the membrane*: TM, EPCR, and PAR-1 constitutively reside in endothelial cell lipid rafts [29]. However, PAR-1 can also be compartmentalized in caveolae (caveolin 1-rich lipid rafts), which is critical for aPC (but not for thrombin)-selective signaling and endothelial barrier protection [30]. *3) The internalization rate*: thrombin activated PAR-1 is rapidly internalized as part of its signaling shut-off mechanism, while aPC activated PAR-1 is retained on the cell surface [31].

In an LPS model, EPCR expressing CD8⁺ DCs were required for the efficacy of the aPC-mediated anti-inflammatory effect [32]. Human intestinal microvascular endothelial cells treated with TNF α , as well as patients with inflammatory bowel diseases (IBD) have impaired EPCR and TM expression and consequently reduced aPC [33]. Th17 cells are a subset of T helper cells that can mediate inflammatory and/or autoimmune diseases. While a subset of *in vitro* differentiated Th17 cells are indeed pathogenic following adoptive transfer into mice, others are non-pathogenic [34]. Single-cell RNA-seq profiling of Th17 cells demonstrated that *Epcr* expression negatively correlates with the pathogenic Th17 signature [35,36]. Furthermore, EPCR acted as a negative regulator of Th17 pathogenicity in an experimental autoimmune encephalomyelitis (EAE) model [36]. A recent publication demonstrated EPCR is also part of a module of newly identified IL-27-dependent co-inhibitory receptors in T cells [37]. This program participates in different states of cell non-responsiveness, such as T cell tolerance and T cell exhaustion in cancer and chronic viral infection. However, whether there is a contribution from aPC signaling has not been addressed. Another interesting study has shown that $\gamma\delta$ T cells are able to bind EPCR during surveillance of infected endothelial or transformed cells [38].

Thrombin/aPC/PAR-1/EPCR also modulates other aspects of immune function, including the retention of long-term repopulating hematopoietic stem cells (LT-HSCs) in the bone marrow and their mobilization to the blood, which is required for protection from myelotoxic injuries such as radiation. aPC-EPCR signaling functions in the retention of LT-HSCs in the bone marrow by limiting nitric oxide (NO) production and promoting cell adhesion. In contrast, thrombin-PAR-1 signaling induces NO production and the shedding of EPCR, thereby mobilizing LT-HSCs to leave the bone marrow niche and enter circulation [39].

Studies showing that aPC prevents inflammation, coagulopathy and lethality in sepsis models, including in baboons, encouraged the development of a recombinant human aPC, drotrecogin alfa-activated (DAA; brand name Xigris) for the treatment of septic shock patients [40–42]. In 2001, DAA was approved for the treatment of sepsis based on results from the PROWESS trial, which demonstrated a 6.1% reduction in 28-day mortality [42]. However, subsequent trials including ADDRESS, PROWESS-SHOCK and APROCCHSS failed to replicate this result [43–46]. The 2012 Cochrane review found no improvement in survival and in fact showed an enhanced bleeding risk in patients with severe sepsis and septic shock who were given Xigris. Therefore, the use of DAA in sepsis is not currently recommended [47,48]. Xigris was discontinued by Eli Lilly. The precise reasons for the failure of this drug in randomized clinical trials remain controversial as observational trials have consistently shown a reduction in mortality [46].

PROS1-TAM RTK Axis

Another extensively studied anticoagulant protein is PROS1. PROS1 is also a vitamin K-dependent plasma glycoprotein. It is produced by hepatocytes, endothelial cells, vascular smooth muscle cells, megakaryocytes, macrophages, DCs, T cells and osteoblasts. PROS 1 is present in circulation. However, this protein has a Gla domain that mediates calcium-dependent binding to phosphatidylserine (PtdSer) on the surface of apoptotic cells or on live cells that are known to transiently expose PtdSer, such as activated T cells [49,50]. This might generate higher local concentrations of PROS1 than in the blood.

In 1980, Walker reported that the rate of factor Va inactivation by aPC could be enhanced by the addition of plasma [51]. This led to the identification of PROS1 as a cofactor of PC [51] (Figure). PROS1 also has direct, aPC-independent anticoagulant functions, autonomously inhibiting coagulation by binding the prothrombinase complex (factor Xa and Va) [52,53]. In humans, approximately 40% of PROS1 circulates free, and approximately 60% is bound to C4b-binding protein. In mice, PROS1 only exists in its free form. Total ablation of PROS1 in mice results in embryonic lethality; PROS1 knockout embryos phenocopy PC deficient mice, with intracranial hemorrhage and fibrin deposition in the liver [54,55]. Mice with heterozygous deficiency are viable, have reduced PROS1 levels and aPC cofactor activity in plasma, and develop thromboembolism upon challenge [54,55].

PROS1 also has direct anti-inflammatory roles (Figure). Apart from the Gla domain described above, PROS1 has four EGF-like repeats and two laminin G repeats that comprise the sex hormone binding globulin (SHBG) domain [56]. The latter region mediates binding to the TAM subfamily of receptor tyrosine kinases (RTKs). The identification of PROS1 as a ligand of TAM RTKs was made by Stitt *et al.*, who observed that PROS1 in serum and in the conditioned media of endothelial cells induced phosphorylation of TYRO3 [57]. Subsequently, PROS1 was shown to also activate MERTK [58,59]. The two main biological functions of TAM RTKs are phagocytosis of apoptotic cells and negative regulation of the inflammatory response [60]. MERTK in macrophages inhibits LPS-induced TNF α production [61]. Mechanistically, after an inflammatory stimulus, TLRs in DCs induce TAM RTK expression through IFNAR/STAT1 signaling. Activation of TAM RTKs shuts down

inflammation *via* the same IFNAR/STAT1 pathway by inducing *Socs1* and *Socs3* genes and suppressing inflammatory cytokine production [56].

Early apoptotic cells expose PtdSer from the inner to the outer leaflets of their cell membranes. The Gla domain of PROS1 binds to PtdSer moieties triggering phagocytic responses through MERTK [50,62,63]. The rapid elimination of apoptotic cells contributes to the regulation of the inflammatory response. Interestingly, binding of PROS1 to PtdSer promotes auto-oxidation of cysteine residues, resulting in PROS1 dimers and oligomers that preferentially bind to MERTK. This might enhance the uptake of apoptotic cells and prevent constitutive activation in circulating cells that have permanent contact with high amounts of PROS1 [64]. Genetic ablation of *Axl* and *Mertk* resulted in an accumulation of apoptotic cells and uncontrolled inflammation in a mouse model of colitis [65]. After inflammation, macrophages clear apoptotic cells and induce pro-resolution responses. The presence of apoptotic cells together with tissue repair cytokines is, however, required for the induction of the repair response in macrophages [66]. Recently, it has been shown that macrophages upregulate PROS1 during the resolution of inflammation [67]. This is required for efficient engulfment of apoptotic cells and for the production of resolving enzymes [67].

The use of conditional knockout of *Pros1* has allowed the characterization of different cellular sources of this protein [54]. IL-4 induces *Pros1* expression in T cells [49,68,69]. After activation, T cells also expose PtdSer and presumably, membrane-bound PROS1 on T cells engages TAM RTKs on activated DCs. PROS1-TAM RTK signaling at the interface of the adaptive and innate immune system downregulates inflammatory cytokine production in DCs *via* pathways downstream of TAM RTKs [49,69].

COAGULOPATHIES AND INFLAMMATORY DISEASES: COMORBIDITIES

“For, although common Snarks do no manner of harm,

Yet, I feel it my duty to say,

Some are Boojums-” [1]

Many human diseases show an intriguing association between coagulation disorders and pathological inflammation. These clinical features may simply be independent variables. Additionally, chronic inflammation can lead to thromboembolism. Inflammation regulates thrombotic responses. Conversely, thromboembolism is known to lead to a general pro-inflammatory state. Crosstalk between these two molecular pathways has been extensively reviewed.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies and chronic inflammation. However, SLE is also associated with a procoagulant state. The risk of deep vein thrombosis (DVT) and pulmonary embolism (PE) is significantly higher in SLE patients [72,73]. SLE patients sometimes have lupus anticoagulants and/or are diagnosed with Antiphospholipid syndrome is [74]. Lupus anticoagulants are antibodies that react against anionic phospholipids and, in spite of their

name, function as prothrombotic agents *in vivo* as they cause increased and inappropriate blood clotting. The name derives from the paradoxically increased clotting time observed *in vitro*. antiphospholipid syndrome is associated with the presence of lupus anticoagulant, anti-cardiolipin autoantibodies or anti- β 2-glycoprotein 1 autoantibodies. However, thrombophilic defects in SLE are much more frequent than the presence of antiphospholipid antibodies [73]. Two mutations (factor V Leiden and prothrombin G20210A) were associated with an increased risk of venous thromboembolic events (VTE) in SLE [73]. In another study, aPC resistance in SLE was driven by acquired free PROS1 deficiency [75]. Significant reduction in free PROS1 was observed in a sub-cohort of SLE patients whose clinical manifestations include serositis, neurologic, hematologic, and immunologic disorders [76]. Reduced levels of free PROS1 in SLE likely result from increases in C4b-binding protein, a complement protein that forms a complex with PROS1 [77].

INFLAMMATORY BOWEL DISEASES

IBD are chronic inflammatory disorders of the gastrointestinal tract that include ulcerative colitis (UC) and Crohn's disease (CD). IBD is also associated with systemic thromboembolic events [78,79]. The incidence of systemic thromboembolic complications in IBD has been known historically, and described to range between 1.3% [80] and 6.4% [81]. When compared to the general population, patients with IBD have a threefold higher risk of developing DVT and PE [82]. Mortality rates were significantly increased in thrombotic patients [83]. Postmortem studies revealed an incidence of venous thrombosis of up to 39% in UC patients [84]. In fact, thromboembolic complications were estimated to be the third leading cause of death in UC patients [84]. While it is difficult to ascribe causality, it is interesting to note that mutations that affect coagulation have been found in IBD patients. Plasminogen activator inhibitor type 1 (PAI-1) impairs clot dissolution (fibrinolysis) and is increased in the plasma of IBD patients [85]. The promoter of *PAI-1* has a single guanosine insertion/deletion 4G/5G polymorphism that restricts binding of a repressor protein. The *4G allele thus induces higher transcriptional levels of *PAI-1*, and has been associated with a subset of IBD patients [86]. Furthermore, PROS1 is reduced in patients with active IBD in comparison to healthy controls [49,87–90].

ASTHMA

Asthma is a reactive airway disease characterized by bronchial hyperresponsiveness to a variety of stimuli and chronic airway inflammation leading to airway obstruction. Patients with asthma have increased concentrations of thrombin, thrombin-antithrombin complex (TAT), and tissue factor in their sputum compared to healthy individuals. The correlation between the levels of thrombin and TAT and the degree of bronchial hyperresponsiveness [91], suggests that local coagulation participates in the airway wall thickening during chronic asthma. Similarly, increased pulmonary tissue factor in severe asthma patients correlates with eosinophil numbers [92]. Plasma from asthmatic patients also showed increased thrombin generation and impaired fibrinolysis compared to control samples [93]. Severe asthma can be associated with exaggerated intra-alveolar fibrin deposits [94]. An axis of coagulation and inflammation has been implicated in asthma [95] and inhaled heparin has been considered for its treatment [96,97].

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease that targets the soft tissue of certain joints, usually the small joints of the hands and feet. Local and systemic activation of coagulation and fibrinolysis take place in the synovial fluid and plasma of RA patients [98]. RA patients have an increased risk of VTE compared to non-RA patients [99–101]. Lower levels of free PROS1 may account for increased thrombosis in RA [102]. A recent study demonstrated that PROS1 reduces inflammatory arthritis in mice and in human synovial three-dimensional micromass cultures [103]. Additionally, transglutaminase factor XIII was demonstrated to promote local inflammation and tissue degradation in a mouse model of collagen-induced RA [104]. A previous study by Flick *et al.* demonstrated that prothrombin anticoagulant mutants or administration of anticoagulants suppress fibrin formation and inflammation in a RA mouse model [105]. Interestingly, the presence of autoantibodies against fibrinogen has been observed in patients with RA [106,107]. The role of coagulation in arthritis and its potential treatment through the targeting of PARs have been recently reviewed [108,109].

We posit that the association of coagulopathies with inflammatory diseases, at least in a subset of patients with inflammatory diseases, reflects the evolutionarily conserved role of coagulation in the regulation of inflammation and anti-pathogen defense. This idea raises the interesting possibility of comprehensive management of inflammation and coagulation in diseases such as SLE, IBD and RA. For example, the Padua Prediction algorithm currently used by clinicians to determine whether or not DVT/PE prophylaxis should be used in hospitalized patients does not take into account a causal role of thrombophilia in inflammation (although acute infection and trauma/surgery are included as predictors) [110]. Albeit anticoagulants are used prophylactically when indicated, the therapeutic use of anticoagulants, for example during flares, may help improve the management of IBD and other inflammatory diseases. Admittedly, the risk of major bleeding such as GI bleeding and intracranial hemorrhaging will be enhanced by the use of anticoagulants, and the cost-benefit assessment remains to be fully ascertained.

CONCLUSION AND FUTURE DIRECTIONS

For the Snark was a Boojum, you see.

Why are mutations and/or polymorphisms associated with a procoagulant state found in the human population despite the significant risk to life and health? We postulate that a procoagulant state is congruent with inflammation and perhaps with improved defense against pathogens. Specific disease resistance associated with coagulopathies have not been yet described. Notwithstanding, selective pressures such as resistance to malarial parasites can fix traits associated with diseases in humans such as sickle cell trait. We propose that this hypothesis merits experimental testing.

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Highlights

- Molecules of the coagulation cascade directly regulate inflammation and anti-pathogen defense.
- Multiple inflammatory disorders show an intriguing association with coagulopathies.
- Loss-of-function mutations in genes encoding anticoagulants might have been retained for improved host defense.

Figure. Procoagulant and anticoagulant molecules, and the regulation of pathogen defense.

After an injury, the activation of the coagulation cascade is triggered to prevent blood loss. In the figure, the cascade of proteolytical reactions has been reduced to its key steps for simplicity. When factor V gets activated, together with activated factor X (not shown), it forms the prothrombinase complex that cleaves the zymogen prothrombin into active thrombin. Thrombin, in turn, converts soluble fibrinogen into fibrin, a polymer that seals the wound. Furthermore, thrombin can directly act through PAR-1 (a protease-activated receptor expressed on the surface of platelets, endothelial cells, fibroblasts, and immune cells) to promote inflammation. Both clotting and PAR-1 signaling lead to anti-pathogen responses, or if uncontrolled, to inflammatory disorders (green sharp-ended arrows). Thrombin can also form a complex together with Thrombomodulin. This complex converts Protein C (PC) into activated PC (aPC). aPC is an anticoagulant molecule, that in combination with its cofactor Protein S (PROS1), inactivates factors V and VIII. Moreover, aPC and PROS1 also signal

through Endothelial PC Receptor (EPCR)-PAR-1 or TYRO3, AXL and MERTK receptor tyrosine kinases (TAM RTKs) respectively, to drive anti-inflammatory responses. The anticoagulant function, as well as the anti-inflammatory signaling, can dampen the immune response (red blunt-ended arrows).

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