Inflammation and coagulation

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In the pathogenesis of sepsis, inflammation and coagulation play a pivotal role. Increasing evidence points to an extensive cross-talk between these two systems, whereby inflammation leads to activation of coagulation, and coagulation also considerably affects inflammatory activity. Molecular pathways that contribute to inflammation-induced activation of coagulation have been precisely identified. Pro-inflammatory cytokines and other mediators are capable of activating the coagulation system and down-regulating important physiologic anticoagulant pathways. Activation of the coagulation system and ensuing thrombin generation is dependent on expression of tissue factor and the simultaneous down-regulation of endothelial-bound anticoagulant mechanisms and endogenous fibrinolysis. Conversely, activated coagulation proteases may affect specific cellular receptors on inflammatory cells and endothelial cells and thereby modulate the inflammatory response. (Crit Care Med 2010; 38[Suppl.]:S26–S34)

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The majority of critically ill patients with a systemic inflammatory response have coagulation abnormalities (1). These abnormalities range from subtle activation of coagulation that can only be detected by sensitive markers for coagulation factor activation to somewhat more robust coagulation activation that may be evident by a small decrease in platelet count and subclinical prolongation of global clotting times, to (in its most extreme form) fulminant disseminated intravascular coagulation (DIC), characterized by simultaneous widespread microvascular thrombosis and profuse bleeding from various sites (2). Patients with severe forms of DIC may present with manifest thromboembolic disease or clinically less apparent microvascular fibrin deposition that may contribute to the development of multiple organ dysfunction (3–7). There is abundant evidence that the activation of coagulation is mediated by inflammatory activity. Activation of coagulation and deposition of fibrin as a consequence of inflammation can be considered instrumental in containing inflammatory activity to the site of injury or infection, rendering this relationship physiologically efficient. However, inflammation-induced coagulation may also importantly contribute to disease, as illustrated by the coagulopathy that is associated with severe infection, such as sepsis, and also by the fact that thrombus formation on a ruptured atherosclerotic plaque, containing abundant inflammatory cells, is the pathologic substrate of acute arterial thrombotic events (8, 9). The main mediators of inflammation-induced activation of coagulation are proinflammatory cytokines. Several studies have shown, for example, the importance of interleukin (IL)-6 in the initiation of coagulation activation, and the role of tumor necrosis factor-α (TNF-α) and IL-1 in the regulation of physiologic anticoagulation (10–12). However, there is increasing evidence that extensive cross-talk between the systems of inflammation and coagulation exists, whereby inflammation leads to activation of coagulation, and coagulation also markedly affects inflammatory activity. This coagulation-driven modulation of inflammatory activity is driven by specific cell receptors on inflammatory cells and endothelial cells. In addition, systemic activation of coagulation and inflammation in critically ill patients can have some tissue-specific or organ-specific consequences pertinent to the development of multiorgan failure in the setting of severe sepsis (13).

Relevance of Inflammation-Induced Coagulation Abnormalities

There is evidence that activation of coagulation in concert with inflammatory activation can result in microvascular thrombosis and thereby contribute to multiple organ failure in patients with severe sepsis (14). First, there are several reports of postmortem findings in septic patients with coagulation abnormalities and DIC (15, 16). These autopsy findings include diffuse bleeding at various sites, hemorrhagic necrosis of tissue, microthrombi in small blood vessels, and thrombi in mid-size and larger arteries and veins. The demonstration of ischemia and necrosis was associated with fibrin deposition in small and mid-size vessels of various organs (17). Importantly, the presence of these intravascular thrombi appears to be clearly and specifically related to the development of organ dysfunction. Second, experimental animal studies of DIC show fibrin deposition in various organs. Experimental bacteremia or endotoxemia causes intravascular and extravascular fibrin deposition in kidneys, lungs, liver, brain, and various other organs. Amelioration of the hemostatic defect by various interventions in these experimental models appears to improve organ failure and mortality in some, but not all, cases (18–21). Interestingly, some studies indicate that amelioration of the systemic coagulation activation will have a profound beneficial effect on resolution of local fibrin deposition and improvement of organ failure (22, 23). Last, clinical studies support the notion of coagulation as an important determinant of clinical outcome. DIC has shown to be an independent predictor of organ failure and mortality (3, 24). In a consecutive series of patients with severe sepsis, the mortality of patients with DIC was 43%, compared with 27% in those

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without DIC. In this study, mortality was also directly related to the severity of the coagulopathy in septic patients (25).

Apart from microvascular thrombosis and organ dysfunction, coagulation abnormalities may also have other harmful consequences. For example, thrombocytopenia in patients with sepsis confers an increased risk of bleeding (26). In particular, critically ill patients with a platelet count of $<50 \times 10^9$/L have a four-fold to five-fold higher risk for bleeding compared to patients with a higher platelet count (27, 28). The risk of intracerebral bleeding in patients in the intensive care unit (ICU) is relatively low (0.3% to 0.5%), but in 88% of patients with this complication the platelet count is $<100 \times 10^9$/L (29). Regardless of the cause, thrombocytopenia is an independent predictor of ICU mortality in multivariate analyses, with a relative risk of 1.9 to 4.2 in various studies (27, 28, 30). In particular, a sustained thrombocytopenia more than 4 days after ICU admission or a decrease in platelet count of >50% during ICU stay is associated with a four-fold to six-fold increase in mortality (27, 31). The platelet count was shown to be a stronger predictor for ICU mortality than composite scoring systems, such as the Acute Physiology and Chronic Evaluation (APACHE) II score or the Multiple Organ Dysfunction Score (MODS). Also, low levels of coagulation factors in patients with sepsis, as reflected by prolonged global coagulation times, may be a risk factor for bleeding and mortality. A prothrombin time or partial thromboplastin time ratio >1.5 in critically ill patients was found to predict excessive bleeding and increased mortality (32, 33).

How Inflammation Causes Activation of Coagulation

The main mechanisms of the coagulation derangement during systemic inflammatory activity are tissue factor-mediated thrombin generation and an imbalance or dysfunction of the normal physiologic anticoagulant mechanisms, such as the antithrombin system and the protein C system (14, 34). In addition to enhanced fibrin formation, fibrin removal is impaired because of depression of the fibrinolytic system.

Initiation of Inflammation-Induced Activation of Coagulation

Tissue factor plays a central role in the initiation of inflammation-induced coagulation (35). Blocking tissue factor activity completely inhibits inflammation-induced thrombin generation in models of experimental endotoxemia or bacteremia (20, 36). The majority of cells constitutively expressing tissue factor are found in tissues not in direct contact with blood, such as the adventitial layer of larger blood vessels. However, tissue factor comes into contact with blood when the integrity of the vessel wall is disrupted or when endothelial cells and/or circulating blood cells start expressing tissue factor. The *in vivo* expression of tissue factor seems mostly dependent on IL-6, as demonstrated in studies showing that inhibition of IL-6 completely abrogates tissue factor-dependent thrombin generation in experimental endotoxemia, whereas specific inhibition of other proinflammatory cytokines had less or no effect (10, 37). Inflammatory cells in atherosclerotic plaques produce abundant tissue factor, and on plaque rupture there is extensive tissue factor exposure to blood (9). In severe sepsis, mononuclear cells, stimulated by proinflammatory cytokines, express tissue factor, which leads to systemic activation of coagulation (38). Even in experimental low-dose endotoxemia in healthy subjects, a 125-fold increase in tissue factor messenger RNA levels in blood monocytes can be detected (39). A potential alternative source of tissue factor may be endothelial cells, polymorphonuclear cells, and other cell types. It is hypothesized that tissue factor from these sources is shuttled between cells through microparticles derived from activated mononuclear cells (40). It is, however, unlikely that these cells actually synthesize tissue factor in substantial quantities (38, 41).

Propagation of Thrombin Generation and the Role of Platelets

On exposure to blood, tissue factor binds to factor VIIa. The complex of tissue factor–factor VIIa catalyzes the conversion of factor X to Xa, which will form the prothrombinase complex with factor Va, prothrombin (factor II), and calcium, thereby generating thrombin (factor IIa). One of the key functions of thrombin is to convert fibrinogen into fibrin. The tissue factor–factor VIIa complex can also activate factor IX, forming a tenase complex with activated factor IX and factor X, generating additional factor Xa, thereby forming an essential amplification loop. The assembly of the prothrombinase and tenase complex is markedly facilitated if a suitable phospholipid surface is available, ideally presented by activated platelets. In the setting of inflammation-induced activation of coagulation, platelets can be activated directly by endotoxin or by proinflammatory mediators, such as platelet activating factor. Thrombin itself is one of the strongest platelet activators *in vivo*.

Activation of platelets may also accelerate fibrin formation by another mechanism. The expression of tissue factor on monocytes is markedly stimulated by the presence of platelets and granulocytes in a P-selectin–dependent reaction (42). This effect may be the result of nuclear factor kappa B activation induced by binding of activated platelets to neutrophils and mononuclear cells (43). This cellular interaction also markedly enhances the production of IL-1b, IL-8, monocyte chemoattractant protein-1, and tumor necrosis factor (TNF)-α (44). The expression of P-selectin on the activated platelet membrane will mediate the adherence of platelets to endothelial cells and leukocytes.

Down-Regulation of Physiologic Anticoagulant Pathways During Inflammation

Procoagulant activity is regulated by three important anticoagulant pathways: antithrombin (AT), the protein C system, and tissue factor pathway inhibitor (TFPI). During inflammation-induced activation of coagulation, the function of all three pathways can be impaired (45) (Fig. 1).

The serine protease inhibitor antithrombin is the main inhibitor of thrombin and factor Xa. Without heparin, AT neutralizes coagulation enzymes in a slow, progressive manner (46). Heparin induces conformational changes in AT that result in at least a 1000-fold enhancement of AT activity. Thus, the clinical efficacy of heparin is attributed to its interaction with AT. Endogenous glycosaminoglycans, such as heparan sulfates, on the vessel wall also promote AT-mediated inhibition of thrombin and other coagulation enzymes. During severe inflammatory responses, AT levels are markedly decreased because of impaired synthesis (as a result of a negative acute phase response), degradation by elastase from activated neutrophils, and—quantitatively most importantly—consumption as a consequence of ongoing

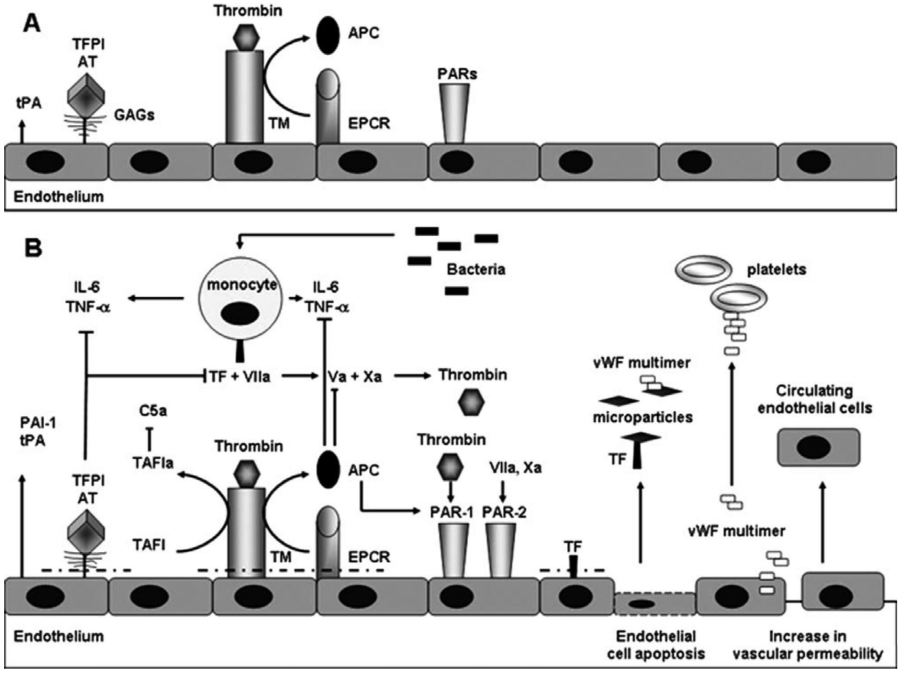

Figure 1. Endothelium-associated mediators of coagulation and inflammation (128). *A*, The normal situation in which the endothelium expresses thrombomodulin (*TM*) (activated by thrombin) and endothelial protein C receptor (*EPCR*), which generate activated protein C (*APC*). Other anticoagulant factors are tissue factor pathway inhibitor (*TFPI*) and antithrombin (*AT*) attached to the endothelial surface and from endothelium-released tissue-type plasminogen activator (tPA), which promotes fibrinolysis. *B*, Systemic activation of inflammation leads to cytokine release and endothelial perturbation, resulting in release of microparticles (*MP*), apoptosis, detachment of endothelial cells, and loss of barrier function. Coagulation is activated by induction of tissue factor (*TF*) on monocytes, MP, and endothelium, and by release of von Willebrand factor (*vWF*), which adds to platelet adhesion to the subendothelial surface. Production of glycosaminoglycans (*GAG*) is down-regulated, and the anticoagulant proteins TFPI, AT, EPCR, and TM are cleaved from the endothelial surface and are impaired in action. Fibrinolysis is impaired as a result of an increase in the main inhibitor of the plasminogen activator (PAI-1), which outweighs an increase in tPA, and complement activation is enhanced by loss of activation of thrombin-activatable fibrinolysis inhibitor (*TAFI*), which normally inhibits complement factors C3a and C5a and bradykinin activity. Anticoagulant proteins in turn modulate cytokine release: tissue factor-factor VIIa (*TF-FVIIa*), factor (*F*) Xa, and thrombin exert proinflammatory activity by cleaving mainly protease-activated receptor (*PAR*)-1 and PAR-2. APC cleaves PAR-1 in an EPCR-dependent manner and hereby modulates inflammation and apoptosis. *IL*, interleukin; *TNF*, tumor necrosis factor. Reprinted with permission from Schouten M, Wiersinga WJ, Levi M, et al: Inflammation, endothelium, and coagulation in sepsis. *J Leukoc Biol* 2008; 83:536–545.

thrombin generation (47). Proinflammatory cytokines can also cause reduced synthesis of glycosaminoglycans on the endothelial surface, which will also contribute to reduced AT function, because these glycosaminoglycans can act as physiologic heparin-like cofactors of AT (48).

Activated protein C (APC) appears to play a central role in the pathogenesis of sepsis and associated organ dysfunction (49). There is ample evidence that an insufficient functioning of the protein C pathway contributes to the derangement of coagulation in sepsis (50, 51). The circulating zymogen protein C is activated by the endothelial cell-bound thrombomodulin, once this is activated by thrombin (34). APC acts in concert with its cofactor protein S to proteolytically degrade the essential coagulation cofactors Va and VIIIa. In that manner it functions as an effective anticoagulant. The endothelial protein C receptor (EPCR) not only accelerates the activation of protein C several-fold but also serves as a receptor for APC, and binding of APC to this receptor may amplify its anticoagulant and anti-inflammatory effects (52) (Fig. 1). A recent study has demonstrated that exposure of cultured endothelial cells to APC results in the release of microparticles that contain EPCR (53), but the relevance of that observation for coagulation or inflammation is not yet clear.

In patients with severe inflammation, the protein C system is malfunctioning at virtually all levels. First, plasma levels of the zymogen protein C are low or very low, because of impaired synthesis, consumption, and degradation by proteolytic enzymes, such as neutrophil elastase (54–56). Furthermore, a significant down-regulation of thrombomodulin, caused by proinflammatory cytokines such as TNF-α and IL-1, has been demonstrated, resulting in diminished protein C activation (57, 58). Low levels of free protein S may further compromise an adequate function of the protein C system. In plasma, 60% of the cofactor protein S is complexed to a complement regulatory protein, C4b binding protein. Increased plasma levels of C4b binding protein as a consequence of the acute phase reaction in inflammatory diseases may result in a relative protein S deficiency, which further contributes to a procoagulant state during sepsis. Although it has been shown that the β-chain of C4b binding protein (which mainly governs the binding to protein S) is largely unaffected during the acute phase response (59), support for this hypothesis comes from studies showing that the infusion of C4b binding protein in combination with a sublethal dose of *Escherichia coli* into baboons resulted in a lethal response with severe organ damage attributable to DIC (60). Finally, but importantly, in sepsis the EPCR has shown to be down-regulated, which may further negatively affect the function of the protein C system (61). Apart from these effects, sepsis may cause a resistance toward APC by other mechanisms, which are partly dependent on a sharp increase in factor VIII levels (released from endothelial cells), but partly occur by yet unidentified mechanisms (62).

A third inhibitory mechanism of thrombin generation involves TFPI, the main inhibitor of the tissue factor–factor VIIa complex. The role of TFPI in the regulation of inflammation-induced coagulation activation is not completely clear. Experiments showing that administration of recombinant TFPI (and thereby achieving higher than physiologic plasma concentrations of TFPI) blocks inflammation-induced thrombin generation in humans, and the observation that pharmacologic doses of TFPI are capable of preventing mortality during systemic infection and inflammation suggests that high concentrations of TFPI are capable of importantly modulating tissue factor mediated coagulation (18, 63).

Effects of Inflammation on Fibrinolysis

Central regulators of plasminogen activators and inhibitors during inflammation

are TNF-α and IL-1β (64). Occurrence of these cytokines in the circulation leads to the release of plasminogen activators, particularly tissue-type plasminogen activator and urokinase-type plasminogen activator (u-PA), from storage sites in vascular endothelial cells. However, this increase in plasminogen activation and subsequent plasmin generation is counteracted by a delayed but sustained increase in plasminogen activator inhibitor type 1 (65). The resulting effect on fibrinolysis is a complete inhibition and, as a consequence, inadequate fibrin removal, contributing to microvascular thrombosis. Experiments in mice with targeted disruptions of genes encoding components of the plasminogen–plasmin system confirm that fibrinolysis plays a major role in inflammation. Mice with a deficiency of plasminogen activators have more extensive fibrin deposition in organs when challenged with endotoxin, whereas plasminogen activator inhibitor type 1 knockout mice, in contrast to wild-type controls, have no microvascular thrombosis on endotoxin administration (66).

How Coagulation Modulates Activation of Inflammation

Communication between inflammation and coagulation is bidirectional, such that coagulation can also modulate inflammatory activity. Coagulation proteases and protease inhibitors not only interact with coagulation protein zymogens but also interact with specific cell receptors to induce signaling pathways (Fig. 1). In particular, protease interactions that affect inflammatory processes may be important in critically ill patients. Coagulation of whole blood *in vitro* results in a detectable expression of IL-1β messenger RNA in blood cells (67), and thrombin markedly enhances endotoxin-induced IL-1 activity in culture supernatants of guinea pig macrophages (68). Similarly, clotting blood produces IL-8 *in vitro*. (69) Factor Xa, thrombin, and fibrin can also activate endothelial cells, eliciting the synthesis of IL-6 and/or IL-8 (70, 71). Thrombin, factor Xa, and fibrin can directly stimulate mononuclear cells and endothelial cells, inducing the synthesis of IL-6 or IL-8 (71). Furthermore, thrombin increases mRNA levels of IL-8, monocyte chemoattractant protein-1, and E-selectin in cultured endothelial cells, and potentiates TNF-α–induced E-selectin expression. *In vivo* evidence for a role of coagulation-protease stimulation of inflammation comes from experiments showing that the administration of recombinant factor VIIa to healthy human subjects causes a small but significant three-fold to four-fold increase in plasma levels of IL-6 and IL-8 (72).

Protease-Activated Cell Receptors

The pivotal mechanism by which coagulation proteases modulate inflammation is by binding to protease-activated receptors (PAR). Four types (PAR 1–4) have been identified, all belonging to the family of transmembrane domain, G-protein-coupled receptors (73). A typical feature of PAR is that they serve as their own ligand. Proteolytic cleavage by an activated coagulation factor leads to exposure of a neo-amino terminus, which activates the same receptor (and possibly adjacent receptors), initiating transmembrane signaling. PAR are localized in the vasculature on endothelial cells, mononuclear cells, platelets, fibroblasts, and smooth muscle cells (73). PAR-1, PAR-3, and PAR-4 are thrombin receptors, and PAR-1 can also serve as receptor for the tissue factor–factor VIIa complex and factor Xa. PAR-2 cannot bind thrombin but can be activated by the tissue factor–factor VIIa complex or factor Xa. Binding of thrombin to its cellular receptor may induce the production of several cytokines and growth factors. Binding of tissue factor–factor VIIa to PAR-2 also results in up-regulation of inflammatory responses (production of reactive oxygen species and expression of major histocompatability complex class II and cell adhesion molecules) in macrophages and was shown to affect neutrophil infiltration and proinflammatory cytokine (TNF-α, IL-1β) expression. The *in vivo* relevance of PAR has been confirmed in various experimental studies using PAR inhibitors or PAR-deficient mice (74–76).

Effects of AT, Thrombomodulin, APC, and EPCR on Inflammation

Antithrombin possesses anti-inflammatory properties, many of which are mediated by its actions in the coagulation cascade (77). Most importantly, thrombin inhibition by AT blunts activation of many inflammatory mediators. For example, thrombin activates platelets and endothelial cells, which in turn contribute to local inflammation (78). Activated platelets secrete inflammatory mediators such as IL-1, which stimulate leukocyte activity. In particular, recruitment and adhesion of neutrophils and monocytes to blood vessels within the microcirculation promote inflammation.

Increasing evidence suggests that AT possesses potent anti-inflammatory properties independent of its anticoagulation activity (78). Most of these effects have been demonstrated *in vitro* or *in vivo* at high concentrations. Nevertheless, these mechanisms may be important in clinical settings that are driven by a combined activation of inflammation and coagulation. Perhaps most importantly, AT induces prostacyclin release from endothelial cells (79–81). Prostacyclin inhibits platelet activation and aggregation, blocks neutrophil tethering to blood vessels, and decreases endothelial cell production of various cytokines and chemokines (82).

Additional anti-inflammatory actions of AT are mediated by direct interaction with leukocytes and lymphocytes. Antithrombin binds to receptors, such as syndecan-4, on the cell surfaces of neutrophils, monocytes, and lymphocytes, and blocks the interaction of these cells with endothelial cells (79, 80). Inhibition of leukocyte–endothelial cell interactions by AT may be mediated by prostacyclin release, down-regulation of P-selectin, or prevention of leukocyte activation (80, 81). Thus, AT directly hinders leukocyte migration and adhesion to endothelial cells, which in turn impacts the severity of capillary leakage and subsequent organ damage. Given the wide-ranging impact of AT on coagulation and inflammation, there are multiple potential clinical applications of AT in different clinical settings that encompass thrombotic states generally not associated with inflammation (e.g., pregnancy) and in coagulation-related disease states with powerful proinflammatory elements (e.g., sepsis).

There is compelling evidence that besides their role as an important regulator of coagulation activity components of the protein C system, they also have an important function in modulating inflammation (83, 84). APC plays an important role in attenuating the systemic inflammatory response in sepsis as demonstrated in experiments showing that blocking the protein C pathway in septic baboons exacerbated the inflammatory response. In contrast, administration of APC ameliorated the inflammatory activation on the intravenous infusion of *E. coli* (21). Similar experiments in ro-

dents showed identical results and demonstrated a beneficial effect on inflammatory effects in various tissues (85). Support for the notion that APC has anti-inflammatory properties comes from *in vitro* observations, demonstrating an APC binding site on monocytes, that may mediate downstream inflammatory processes (86, 87), and from experiments showing that APC can block nuclear factor kappa B nuclear translocation, which is a prerequisite for increases in proinflammatory cytokines and adhesion molecules (88). These *in vitro* findings are supported by *in vivo* studies in mice with targeted disruption of the protein C gene. In these mice with genetic deficiencies of protein C, endotoxemia was associated with a more marked increase in proinflammatory cytokines and other inflammatory responses as compared with wild-type mice (89, 90).

It is likely that the effects of APC on inflammation are mediated by the EPCR (83). Binding of APC to EPCR influences gene expression profiles of cells by inhibiting endotoxin-induced calcium fluxes in the cell and by blocking nuclear factor kappa B nuclear translocation (87, 88). The EPCR–APC complex itself can translocate from the plasma membrane into the cell nucleus, which may be another mechanism of modulation of gene expression, although the relative contribution of this nuclear translocation and cell surface signaling is unclear at present (50). Some studies also suggest that EPCR binding of APC can result in activation of PAR-1 and thereby affect cytokine responses (91). In contrast, other experiments demonstrated that a significant physiologic role for PAR-1 activation by APC was less likely (92). Like APC, EPCR itself may have anti-inflammatory properties. Soluble EPCR, the extracellular domain of the cell-associated EPCR shed from the cell surface by the action of an inducible metalloproteinase (93), can bind to proteinase 3, an elastase-like enzyme. The resulting complex binds to the adhesion integrin macrophage 1 antigen (94). Of considerable interest, the crystal structure of EPCR is remarkably similar to the structure of the major histocompatability complex class 1/CD1 family of proteins, the majority of which are involved in inflammation (95). Blocking the EPCR with a specific monoclonal antibody aggravates both the coagulation and the inflammatory response to *E. coli* infusion (61).

Apart from its effect on cytokine levels, APC has been shown to inhibit leukocyte chemotaxis and adhesion of leukocytes to activated endothelium (96, 97). This notion was confirmed in a hamster endotoxemia model at concentrations of recombinant human APC (rhAPC) that preclude a significant anticoagulant effect (98). Furthermore, in a human model of endotoxin-induced pulmonary inflammation, systemic administration of rhAPC resulted in significant local anti-inflammatory effects (99). A potential mechanism is that APC inhibits expression of platelet-derived growth factor in the lung (100). In addition, it has been shown that APC protects against the disruption of endothelial cell barrier in sepsis, probably by interfering with EPCR and PAR-1 on endothelial cells (101–103).

Finally, APC is capable of inhibiting endothelial cell apoptosis, which also seems to be mediated by a mechanism dependent on EPCR–PAR-1 (91, 104). Signaling through this pathway can affect Bcl-2 homolog protein, which can inhibit apoptosis, and further suppresses p53, which is a pro-apoptotic transcription factor (105, 106).

Fibrin and the Plasminogen–Plasmin System as Mediators of Inflammation

Fibrinogen and fibrin directly influence the production of proinflammatory cytokines and chemokines (including TNF-α, IL-1β, and monocyte chemoattractant protein-1) by mononuclear cells and endothelial cells (107). Fibrinogen-deficient mice show inhibition of macrophage adhesion and less thrombin-mediated cytokine production *in vivo*. The effects of fibrin(ogen) on mononuclear cells seem to be mediated by Toll-like receptor-4, which is also the receptor of endotoxin.

Fibrinolytic activators and inhibitors may have an effect on inflammatory cell recruitment and migration, thereby modulating the inflammatory response. In particular, u-PA and its receptor (u-PAR) are involved in these processes. The u-PAR mediates leukocyte adhesion to the vascular wall or extracellular matrix components (such as vitronectin), and the expression of u-PAR on leukocytes is strongly associated with their migratory and tissue-invasive potential. The underlying mechanism by which u-PAR and u-PA affect cell migration may be related to extracellular matrix degradation by proteases that are activated by u-PA bound to u-PAR (such as elastase, plasmin, and metalloproteinases). In addition, u-PAR exhibits protease-independent properties, involving transmembrane signal transduction after binding to various ligands, such as vitronectin and macrophage 1 antigen, which leads to cytokine and growth factor production (108). Plasminogen activator inhibitor type 1 can compete with u-PAR for binding to vitronectin and thereby inhibits cell adhesion and migration (109). Plasminogen activator inhibitor type 1 binding to vitronectin may also prevent integrin association with this extracellular matrix component, thereby further hindering cell adhesion and migration. The role of u-PAR in the regulation of inflammation is illustrated by experiments with u-PAR–deficient mice, which display a profoundly reduced neutrophil recruitment to the pulmonary compartment after induction of bacterial pneumonia (110). In these models the function of u-PAR as a chemotactic receptor was independent from its interaction with u-PA. In accordance, u-PA–deficient mice have normal neutrophil recruitment during bacterial pneumonia (110). Mediators of fibrinolysis can also affect cytokine synthesis. Plasminogen activator inhibitor type 1 inhibits endotoxin-induced TNF-α production by mononuclear cells *in vitro* (111). An anti-uPA monoclonal antibody attenuates TNF-α release by monocytic THP-1 cells incubated with endotoxin, whereas exogenous u-PA enhances endotoxin-induced TNF-α secretion by these cells, an effect that appears independent of plasmin activity (110). These facts make it clear that activators and inhibitors of the plasminogen–plasmin system impact on inflammatory responses, including cell adhesion and migration, and cytokine production.

Focus on Glycocalyx as the Interface Between Inflammation and Coagulation

The endothelium plays a central role in all major pathways involved in the cross-talk between inflammation and coagulation. As mentioned, endothelial cells may be a source of tissue factor and thereby involved in the initiation of coagulation activation. All physiologic anticoagulant systems and various adhesion molecules that modulate both inflammation and coagulation are connected to the endothelium. Recent research points to

an important role of the inner (luminal) layer of the endothelium, i.e., the glycocalyx, in the interaction between inflammation and coagulation. In sepsis, glycosaminoglycans are down-regulated as a result of proinflammatory cytokines, which can thereby impact on the function of antithrombin and TFPI, and also on leukocyte adhesion and transmigration. Besides glycosaminoglycans and highly sulfated polysaccharides, the glycocalyx consists of glycoproteins, hyaluronic acid, and membrane-associated proteins. The glycocalyx has been described to play a role not only in coagulation but also in other endothelial functions including maintaining vascular barrier function, nitric oxide-mediated vasodilation, and antioxidant functions, all processes that are known to be involved in sepsis (112, 113). It was shown recently that specific disruption of the glycocalyx results in thrombin generation and platelet adhesion within a few minutes (114, 115). Furthermore, loss of glycocalyx *in vivo* has been associated with subendothelial edema formation (116). The role of the glycocalyx in modulating endothelial function, including anticoagulation, and the role of the endothelium in modulating the glycocalyx in sepsis may indicate that this is an interesting point of impact for future therapy.

Clinical Implications

The keystone of the treatment of hemostatic abnormalities in patients with sepsis is to treat the underlying infection using appropriate antibiotics and source control. However, in many cases additional supportive treatment, aimed at circulatory and respiratory support and replacement of organ function, is required. Coagulation abnormalities may proceed, even after proper treatment has been initiated. In those cases, supportive measures to manage the coagulation disorder may be considered and may positively affect morbidity and mortality. The increase in the insight into the various mechanisms that play a role in the crosstalk between inflammation and coagulation abnormalities associated with sepsis has been accommodating in the development of such supportive management strategies.

In particular, in view of the deficient state of physiologic anticoagulant pathways in patients with sepsis, restoration of these inhibitors seems to be a rational approach (51). Because antithrombin is one of the most important physiologic inhibitors of coagulation, and because of successful preclinical results, a number of larger clinical trials have addressed the use of antithrombin III concentrates in patients with sepsis and/or DIC. Most of the randomized, controlled trials concern patients with sepsis, septic shock, or both. All trials show some beneficial effect in terms of improvement of laboratory parameters, shortening of the duration of DIC, or even improvement in organ function (117). However, a large-scale, multicenter, randomized, controlled trial to directly address this issue showed no significant reduction in mortality of patients with sepsis who were treated with antithrombin concentrate (118). Interestingly, *post hoc* subgroup analyses indicated some benefit in patients who did not receive concomitant heparin and who fulfilled the diagnostic criteria for DIC, but this observation needs prospective validation (119).

Based on the notion that depression of the protein C system may significantly contribute to the pathophysiology of DIC, supplementation of (activated) protein C might be beneficial (51). A beneficial effect of rhAPC was demonstrated in a phase III trial (PROWESS) in patients with sepsis, which was prematurely stopped because of efficacy in reducing mortality in these patients (120). All-cause mortality at 28 days after inclusion was 24.7% in the rhAPC group vs. 30.8% in the control group (19.4% relative risk reduction). The administration of rhAPC was demonstrated to cause an amelioration of coagulation abnormalities and rhAPC-treated patients had less organ failure (121). In view of the described effects that APC has on inflammation, part of the success may have been caused by a beneficial effect on inflammatory pathways. Interestingly, a *post hoc* analysis of this trial demonstrated that patients with a diagnosis of DIC, according to the DIC scoring system of the International Society on Thrombosis and Hemostasis, had a relatively greater benefit of rhAPC treatment than patients who did not have overt DIC (25). The relative risk reduction in mortality of patients with sepsis and DIC who received rhAPC was 38%, in comparison with a relative risk reduction of 18% in patients with sepsis who did not have DIC. This seems to underscore the importance of the coagulation derangement in the pathogenesis of sepsis and the point of impact that restoration of microvascular anticoagulant pathways may provide in the treatment of sepsis. The rhAPC has been licensed in most countries for treatment of patients with severe sepsis and two or more organ failures. It was shown not to be effective in patients with less severe sepsis (122).

The most frequently encountered adverse effect of rhAPC is bleeding. In the phase III study in patients with severe sepsis, the incidence of major bleeding (i.e., bleeding reported as a serious adverse event) during the infusion period was 2.4% in the rhAPC group compared with 1.0% in the control group ($p = .02$) (120). During the 28-day study period, the incidence of major bleeding was 3.5% in the rhAPC group and 2.0% in the placebo group ($p = .06$).

Based on the results of PROWESS, rhAPC has been approved for therapy by regulatory agencies in the United States, Europe, and other parts of the world. Its use in patients with severe sepsis and multiple organ failure, and in the absence of major risk factors for bleeding, has been advocated in guidelines for the treatment of sepsis (124). However, there is currently debate on the role of rhAPC in the treatment of sepsis (123). For example, a meta-analyses of published literature concluded that the basis for treatment with rhAPC, even in patients with high severity of disease, is weak if not insufficient (125, 126). A series of negative trials in specific populations of patients with severe sepsis has added to the skepticism regarding the use of rhAPC. Taken together, there is quite some uncertainty and doubt surrounding the exact place of rhAPC in patients with severe sepsis, and this equipoise has convinced the manufacturer of this agent to start a new placebo-controlled trial in patients with severe sepsis and septic shock in the coming months (127). Certainly, the result of this trial will be helpful to more precisely assess the effectiveness and safety of rhAPC in the treatment of patients with severe sepsis.

CONCLUSION

Systemic inflammation will invariably lead to activation of the coagulation system but, *vice versa*, components of the coagulation system may markedly modulate the inflammatory response. Increasing evidence points to extensive crosstalk between the two systems at various points, with tissue factor, thrombin,

components of the protein C pathway and fibrinolytic activators, and inhibitors playing pivotal roles. Increased insight into the molecular mechanisms that play a role in the close relationship between inflammation and coagulation may lead to the identification of new targets for therapies that can modify excessive activation or dysregulation of these systems.

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