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REVIEW

# Systemic versus localized coagulation activation contributing to organ failure in critically ill patients

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**Abstract** 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 not only leads to activation of coagulation but coagulation also considerably affects inflammatory activity. The intricate relationship between inflammation and coagulation may not only be relevant for vascular atherothrombotic disease in general but has in certain clinical settings considerable consequences, for example in the pathogenesis of microvascular failure and subsequent multiple organ failure, as a result of severe infection and the associated systemic inflammatory response. 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 physiological anticoagulant pathways. Activation of the coagulation system and ensuing thrombin generation is dependent on an interleukin-6-induced expression of tissue factor on activated mononuclear cells and endothelial cells and is insufficiently counteracted by physiological anticoagulant mechanisms and endogenous fibrinolysis. Interestingly, apart from the overall systemic responses, a differential local response in various vascular beds related to specific organs may occur.

**Keywords** Inflammation · Coagulation

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## Introduction

Most critically ill patients have an activated coagulation system. This activation of coagulation is measurable with highly sensitive assays for molecular markers of activated coagulation proteases, their activation peptides, or protease–protease inhibitor complexes [1]. In many patients, this activation may go undetected, although in the majority of them some abnormality in routine coagulation tests such as a drop in platelet count or a minor prolongation of global coagulation tests may occur. Most clinicians do not regard these abnormalities very relevant. In more severe forms of coagulation activation, however, it is now clear that the ensuing formation of intravascular fibrin may contribute to the pathogenesis of multiple organ failure, in particular in patients with a systemic inflammatory response, for example due to severe infection or trauma [2]. Indeed, in the majority of patients with disseminated intravascular coagulation, fibrin thrombi can be found in many organs (Table 1) [3, 4].

The pathogenesis of the systemic activation of coagulation and microvascular fibrin formation has become more clear in recent years [5]. The trigger for the activation of the coagulation system is mediated by several pro-inflammatory cytokines, expressed and re-

**Table 1** Organ involvement by (micro)thrombi in patients with disseminated intravascular coagulation

| Organ | Mean percentage of patients with (micro)thrombi at autopsy |
|---|---|
| Kidney | 70.4 |
| Lung | 70.0 |
| Brain | 41.1 |
| Heart | 40.4 |
| Liver | 39.6 |
| Spleen | 39.6 |
| Adrenals | 37.1 |
| Pancreas | 24.1 |
| Gut | 20.7 |

leased by mononuclear cells and endothelial cells. Thrombin generation proceeds via the (extrinsic) tissue factor/factor VIIa route and simultaneously occurring depression of inhibitory mechanisms, such as antithrombin III and the protein C and S system. Also, impaired fibrin degradation, due to high circulating levels of PAI-1, contributes to enhanced intravascular fibrin deposition.

## Clinical importance of the interaction between inflammation and coagulation

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 [6]. Firstly, there are several reports of post-mortem findings in septic patients with coagulation abnormalities and DIC [7, 8]. These autopsy findings include diffuse bleeding at various sites, hemorraghic 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 [9]. Importantly, the presence of these intravascular thrombi appears to be clearly and specifically related to the development of organ dysfunction. Secondly, experimental animal studies of DIC show fibrin deposition in various organs. Experimental bacteremia or endotoxemia causes intra- 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, in some but not all cases, mortality [10–13]. 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 [14, 15]. Lastly, 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 [2, 16]. In a consecutive series of patients with severe sepsis, the mortality of patients with DIC was 43%, as compared with 27% in those without DIC. In this study, mortality was also directly related to the severity of the coagulopathy in septic patients [17].

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 [18]. Indeed, in particular critically ill patients with a platelet count of $<50\times10^9$/l have a 4- to 5-fold higher risk for bleeding as compared to patients with a higher platelet count [19, 20]. The risk of intracerebral bleeding in patients in the intensive care unit (ICU) is relatively low (0.3–0.5%), but in 88% of patients with this complication the platelet count is less than $100\times10^9$/l [21]. 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 [19, 22, 23]. In particular, a sustained thrombocytopenia during more than 4 days after ICU admission or a drop in platelet count of >50% during ICU stay is associated with a 4- to 6-fold increase in mortality [19, 24]. 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 PT or aPTT ratio >1.5 in critically ill patients was found to predict excessive bleeding and increased mortality [25, 26].

## Initiation and propagation of inflammation-induced activation of coagulation

Tissue factor plays a central role in the initiation of inflammation-induced coagulation [27]. Blocking tissue factor activity completely inhibits inflammation-induced thrombin generation in models of experimental endotoxemia or bacteremia [12, 28]. The vast 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 pro-inflammatory cytokines had less or no effect [29, 30]. Inflammatory cells in atherosclerotic plaques produce abundant tissue factor and upon plaque rupture there is extensive tissue factor exposure to blood [31]. In severe sepsis, mononuclear cells, stimulated by pro-inflammatory cytokines, express tissue factor, which leads to systemic activation of coagulation [32]. Even in experimental low-dose endotoxemia in healthy subjects, a 125-fold increase in tissue factor mRNA levels in blood monocytes can be detected [33]. 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 [34]. It is, however, unlikely that these cells actually synthesize tissue factor in substantial quantities [32, 35].

Upon 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 pro-inflammatory 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 TF on monocytes is markedly stimulated by the presence of platelets and granulocytes in a P-selectin-dependent reaction [36]. This effect may be the result of nuclear factor kappa B (NF-κB) activation induced by binding of activated platelets to neutrophils and mononuclear cells [37]. This cellular interaction also markedly enhances the production of Il-1b, Il-8, MCP-1, and TNF-α [38]. The expression of P-selectin on the activated platelet membrane will mediate the adherence of platelets to endothelial cells and leukocytes [39].

## Downregulation of physiological anticoagulant and fibrinolytic 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 [40] (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 [41]. Heparin induces conformational changes in AT that result in at least a 1,000-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 owing to 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 thrombin generation [42]. Pro-inflammatory cytokines can also cause reduced synthesis of glycosaminoglycans on the endothelial surface, which will also contribute to reduced AT function, since these glycosaminoglycans can act as physiological heparin-like cofactors of AT [43].

Activated protein C (APC) appears to play a central role in the pathogenesis of sepsis and associated organ dysfunction [44]. There is ample evidence that an insufficient functioning of the protein C pathway contributes to the derangement of coagulation in sepsis [45, 46]. 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 due to impaired synthesis, consumption, and degradation by proteolytic enzymes, such as neutrophil elastase [47–49]. Furthermore, a significant downregulation of thrombomodulin, caused by pro-inflammatory cytokines such as TNF-α and IL-1, has been demonstrated, resulting in diminished protein C activation [50, 51]. Low levels of free protein S may further compromise an adequate function of the protein C system. In plasma, 60% of the co-factor protein S is complexed to a complement regulatory protein, C4b binding protein (C4bBP). Increased plasma levels of C4bBP 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 C4bBP (which mainly governs the binding to protein S) is largely unaffected during the acute phase response [52], support for this hypothesis comes from studies showing that the infusion of C4bBP in combination with a sublethal dose of *Escherichia coli* into baboons resulted in a lethal response with severe organ damage due to DIC [53]. Finally, but importantly, in sepsis the EPCR has shown to be downregulated, which may further negatively affect the function of the protein C system [54]. Apart from these

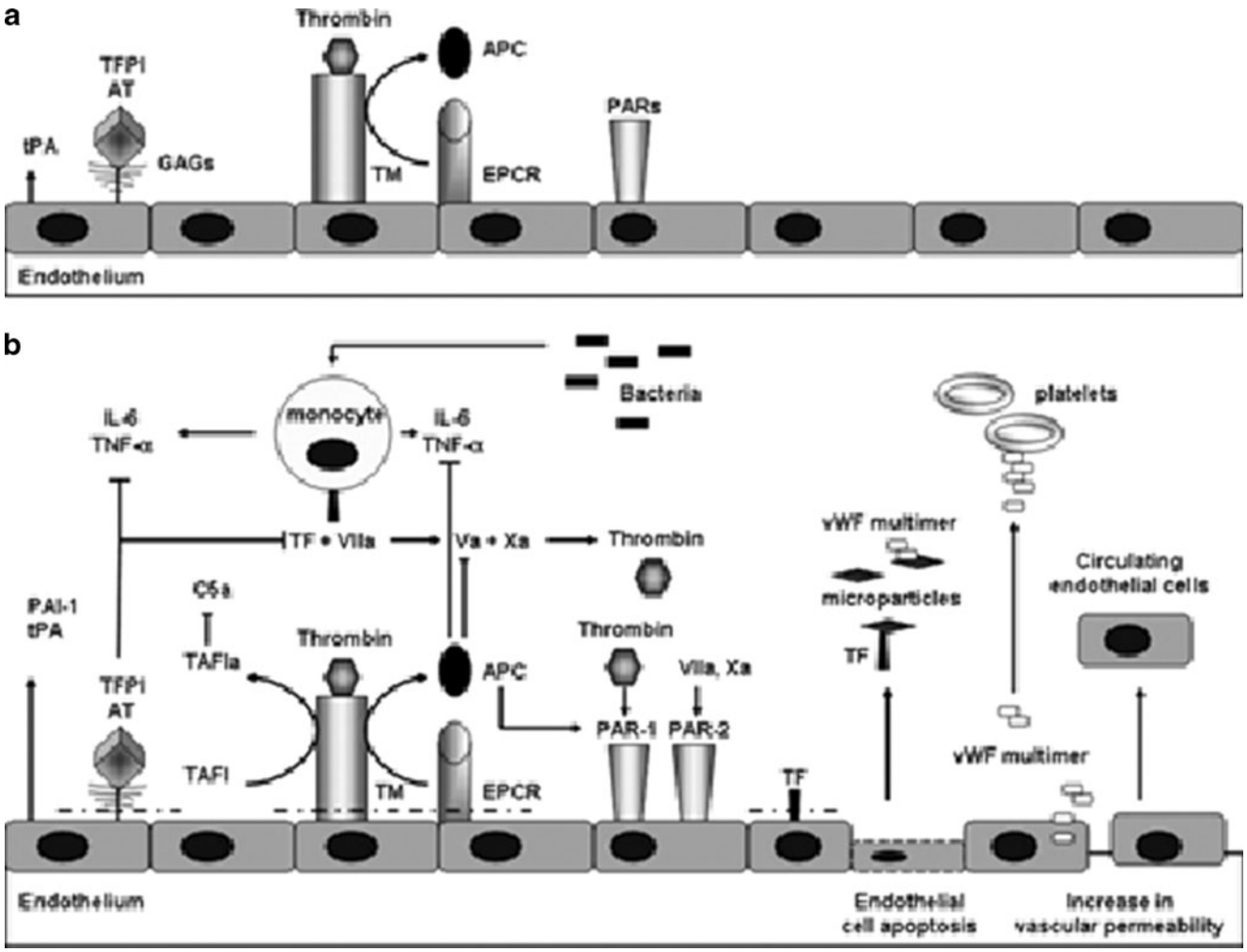


**Fig. 1** Endothelium-associated mediators of coagulation and inflammation. Panel (**a**) depicts the normal situation in which the endothelium expresses thrombomodulin (*TM*) (activated by thrombin) and endothelial PC receptor (*EPCR*), which generate activated PC (*APC*). Other anticoagulant factors 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 (MPs), apoptosis, detachment of endothelial cells, and loss of barrier function. Coagulation is activated by induction of tissue factor (*TF*) on monocytes, MPs, and endothelium and by release of von Willebrand factor (*vWF*), which adds to platelet adhesion to the subendothelial surface. Production of glycosaminoglycans (GAGs) is downregulated, 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 a rise in the main inhibitor of the PA (*PAI-1*), which outweighs a rise in t-PA, and complement activation is enhanced by loss of activation of thrombin-activatable fibrinolysis inhibitor (*TAFI*), which normally inhibits complement factor 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 pro-inflammatory 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 [27]

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 [55].

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 physiological plasma concentrations of TFPI) blocks inflammation-induced thrombin generation in humans, and the observation that pharmacological 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 [10, 56].

Central regulators of plasminogen activators and inhibitors during inflammation are TNF-α and IL-1β [57]. Occurrence of these cytokines in the circulation leads to the release of plasminogen activators, in particular tissue-type plasminogen activator (t-PA) 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 (PAI-1) [58]. 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 PAI-1 knockout mice, in contrast to wild-type controls, have no microvascular thrombosis upon endotoxin administration [59].

## Modulation of inflammation by coagulation *in vivo*

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 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. *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 3- to 4-fold rise in plasma levels of IL-6 and IL-8 [60].

A pivotal mechanism by which coagulation proteases modulate inflammation is by binding to protease activated receptors or PARs. Four types (PAR 1–4) have been identified, all belonging to the family of transmembrane domain, G-protein-coupled receptors [61]. A typical feature of PARs 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. PARs are localized in the vasculature on endothelial cells, mononuclear cells, platelets, fibroblasts, and smooth muscle cells [61]. PARs 1, 3, and 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 upregulation of inflammatory responses (production of reactive oxygen species and expression of MHC class II and cell adhesion molecules) in macrophages and was shown to affect neutrophil infiltration and pro-inflammatory cytokine (TNF-α, IL-1β) expression. The *in vivo* relevance of PARs has been confirmed in various experimental studies using PAR inhibitors or PAR-deficient mice [62–64].

## Effects of anticoagulant molecules on inflammation

Antithrombin possesses anti-inflammatory properties, many of which are mediated by its actions in the coagulation cascade [65]. 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 [66]. 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 [66]. 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 [67–69]. Prostacyclin inhibits platelet activation and aggregation, blocks neutrophil tethering to blood vessels, and decreases endothelial cell production of various cytokines and chemokines [70]. 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 [27]. Inhibition of leukocyte–endothelial cell interactions by AT may be mediated by prostacyclin release, downregulation of P-selectin, or prevention of leukocyte activation. 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 pro-inflammatory 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 also have an important function in modulating inflammation [71, 72]. 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 upon the intravenous infusion of *E. coli* [13]. Similar experiments in rodents showed identical results and demonstrated a beneficial effect on inflammatory effects in various tissues [73]. 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 [74, 75] and from experiments showing that APC can block NF-κB nuclear translocation, which is a prerequisite for increases in pro-inflammatory cytokines and adhesion molecules [76]. 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 pro-inflammatory cytokines and other inflammatory responses as compared with wild-type mice [77, 78].

It is likely that the effects of APC on inflammation are mediated by the EPCR [71]. Binding of APC to EPCR influences gene expression profiles of cells by inhibiting endotoxin-induced calcium fluxes in the cell and by blocking NF-κB nuclear translocation [75, 76]. Blocking the EPCR with a specific monoclonal antibody aggravates both the coagulation and the inflammatory response to *E. coli* infusion [54].

Apart from its effect on cytokine levels, APC has been shown to inhibit leukocyte chemotaxis and adhesion of leukocytes to activated endothelium [79, 80]. This notion was confirmed in a hamster endotoxemia model at concentrations of recombinant human APC (rhAPC) that preclude a significant anticoagulant effect [81]. Moreover, in a human model of endotoxin-induced pulmonary inflammation, systemic administration of rhAPC resulted in significant local anti-inflammatory effects [82]. A potential mechanism is that APC inhibits expression of platelet-derived growth factor in the lung [83]. 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 [84–86].

## Systemic versus localized responses

Although the mechanisms mentioned above have been demonstrated to occur *in vivo* as a general response upon pro-inflammatory stimuli, it is likely that marked differences in the procoagulant response as well as the underlying pathogenetic pathway may exist between cells and tissues [87]. This may be caused by differences in cell-specific gene expression, environmental factors, and organ-specific differences. First, localization of coagulation activity may relate to a cell-specific gene expression. For example, inflammatory mediators enhance PAI-1 gene expression in a complex and tissue-specific way [88]. Recent studies have demonstrated that the von Willebrand factor promoter contains cell-specific elements, and similar response elements may be involved in protein synthesis in cells in general [89]. Second, the tissue environment may determine whether specific gene transcription occurs [90]. It is not completely clear why specific sites and organs are at greater risk of developing microvascular thrombosis and also local differences in the consequences of (micro) thrombosis are still poorly understood. Environmental factors underlying the inflammatory response are thought to play a role in this differential coagulative response as well. In mice with disturbances in the plasminogen–plasmin system subjected to hypoxia, the formation of fibrin is induced and is particularly evident in the lungs [59]. In contrast, these same mice respond to endotoxemia with fibrin deposition in the microvasculature of the kidney in particular. Similarly, mice with a functional thrombomodulin deficiency had a marked increase in pulmonary fibrin deposition after hypoxic challenge [91]. In addition, when mice with a functional defect in the thrombomodulin gene were challenged with endotoxin in sublethal amounts, fibrin formation was apparent in the lungs, but not in any other organ studied. In the latter model, fibrin was only temporarily present, and had disappeared after 24 h [92]. These models illustrate the assumption that fibrin formation is a localized phenomenon rather than a generalized process.

Lastly, various organ systems may markedly differ in their endothelial cell response towards inflammation and injury. In general, endothelial cells play a central role in the coagulation response upon systemic inflammation [42]. The endothelium plays a central role in all major pathways involved in the pathogenesis of hemostatic derangement during severe inflammation. Endothelial cells appear to be directly involved in the initiation and regulation of thrombin generation and the inhibition of fibrin removal. Endothelial cells may express tissue factor, which is the main initiator of coagulation. In addition, physiological anticoagulant pathways, such as antithrombin, the protein C system, or tissue factor pathway inhibitor (TFPI), are mostly located on endothelial cells and endothelial cell dysfunction is directly related to impaired regulation of coagulation. Also, endothelial cells are the main storage site of plasminogen activators and inhibitors and can acutely

release these factors, thereby importantly mediating fibrinolytic activity or inhibition. Pro-inflammatory cytokines are crucial in mediating these effects on endothelial cells, which themselves may also express cytokines, thereby amplifying the coagulative response [30]. Although not completely clear, various organs may differ in all these endothelial cell-related factors influencing local coagulation activation and fibrin deposition.

## Organ-specific responses by endothelial cells

In their excellent overview, Rosenberg and Aird postulate that endothelial cells integrate different extracellular signals and cellular responses in different regions of the vascular bed [89]. Various exogenous stimuli, such as shear stress, inflammatory mediators, and growth factors, exert their action on endothelial cells, and the response of the endothelial cells to transduce the signal may vary between various tissues and even from endothelial cell to endothelial cell within a tissue. As a result, the pro- or anticoagulant response of endothelial cells may differ between organs. Experiments in mice with a targeted deletion of the anticoagulant part of the thrombomodulin gene show abundant fibrin formation in lungs, heart, and spleen [93]. Mice with homozygous deficiencies of plasminogen activators form clots in liver, heart, and lungs, but not in brain or kidneys [94]. Plasminogen activator inhibitor-deficient mice have fibrin deposition predominantly in kidneys [88]. Rosenberg and Aird state that various mechanisms play a role in the individual response of endothelial cells. Cell-to-cell communication may have important effects, as evidenced by the fact that PAI-1 expression in endothelial cells is upregulated if the culture is incubated with medium from aorta or umbilical vein cell culture but, in contrast, is downregulated by addition of conditioned medium from vascular smooth muscle cells [95, 96]. In addition, cell signaling pathways may vary between endothelial cell subtypes. For example, hemodynamic changes may have opposite reactions in nitric oxide mRNA expression in endothelial cells from the aorta or from the pulmonary artery [97]. Another example is provided by the experiment showing that endothelial cells from renal and cerebrovascular vessels have decreased prostacyclin production and more apoptosis when exposed to plasma of patients with thrombocytopenic thrombotic purpura, whereas endothelial cells derived from lungs and liver do not respond to this same stimulus [98]. Lastly, also at the level of transcription a differential phenotype of endothelial cells between various organs can be demonstrated. Coagulation proteins, such as von Willebrand factor, have been shown to respond to various promoters by expressing this factor in different tissues, for example exclusively in heart and skeletal muscle or in brain [90].

## Coagulation activation and the kidney

Coagulation is important in two groups of renal disorders in man. In one group, the kidney is the major site of disease, and localized thrombosis and fibrin formation is superimposed on demonstrable immunological and or endothelial damage. These disorders will not be discussed here. In the second group, renal lesions associated with fibrin formation are involved as a consequence of systemic intravascular coagulation or DIC [99]. In the latter group, acute renal failure (ARF) is the usual associated renal presentation, occurring in the course of sepsis, major surgery, severe trauma, and hypovolemic and cardiogenic shock. The pathogenesis of ARF in these conditions is caused by hypoperfusion resulting in ischemia–reperfusion injury. The decrease in oxygen saturation and hormonal dysregulation causes acute tubular necrosis [100]. At least in septic shock, it has been suggested that microthrombi contribute to ARF [101, 102]. The older literature strongly suggests that intravascular coagulation causes immediate changes that are detrimental to renal function. Electron microscopical studies have shown that coagulation causes mesangial swelling and an increase in vacuoles, organelles free ribosomes, and mitochondria [103]. These changes were associated with phagocytosis of fibrin and secretion of basement membrane-like material. The glomerular lesions occurring in the course of DIC may resemble those seen in acute glomerulonephritis, with platelets and fibrin deposits intraluminally, swollen endothelium, subendothelial deposits of fibrin cleavage fragments, and cellular proliferative effects. When these processes continue, complete occlusion of glomerular capillaries and hyalinization of glomeruli may follow [99]. The pathophysiology of renal failure in shock is also thought to be influenced by vasoactive substances, and renal damage was markedly reduced by adrenergic blockade in a model of hemorrhagic shock or endotoxin shock [104]. Catecholamine infusion in experimental animals causes shock and DIC. Heparin reduces the effects of catecholamine-induced shock and endotoxin-related complications in animal models. It thus appears that the combination of hypoperfusion-related ischemia–reperfusion injury and vasoactive reactions are of major influence on the occurrence of ARF in shock. The finding of fibrin deposits suggests that DIC contributes to organ damage, and the observed improvement under heparin treatment supports this concept. The trigger to thrombosis is probably locally induced by the ischemia–reperfusion responses of hypoxia-inducible factor-mediated TF expression [105]. In addition, systemic stimuli such as endotoxin cause cytokine-mediated upregulation of TFmRNA in the kidney, while local fibrinolytic defense mechanisms are also activated (u-PA and t-PA, without concurrent upregulation of PAI-1). Furthermore, experimental studies have demon-

strated that specific blockade of the factor VII–TF complex reduced fibrin in the kidney [106]. Infusion of hirudin caused a dose-dependent decrease in mortality and also reduced the amount of fibrin deposition in the kidney. An important role of the protein C system in preventing glomerular thrombosis may be inferred from the abundant presence of thrombomodulin expression on endothelial cells in the glomerulus [107]. In inflammatory glomerular disease, such as acute membranoproliferative or lupus glomerulonephritis, an increase in thrombomodulin expression has been implicated [108]. In contrast, in ischemia–reperfusion injury in kidneys, thrombomodulin has been markedly downregulated. Administration of soluble thrombomodulin to rats with renal ischemia–reperfusion injury prevented massive glomerular thrombosis and kidney dysfunction [109]. In another experimental study of renal ischemia and reperfusion, administration of activated protein C prevented histological changes and the decrease in renal blood flow, and preserved kidney function, whereas treatment with active site-blocked factor Xa, heparin, and inactivated protein C were less effective [110]. It therefore appears that inhibition of coagulation also reduces the amount of fibrin in the kidney. This may imply an improvement of renal function; however, there have been no controlled trials in which the beneficial effect of anticoagulant treatment in patients with DIC and ARF was investigated.

## Coagulation activation and the lung

The lungs are among the most frequently affected organs during severe infection and sepsis [111, 112]. Lung injury in this situation is characterized by increased permeability of the alveolar–capillary membrane, diffuse alveolar damage, and the accumulation of pulmonary edema, containing a high concentration of proteases and other proteins. Pathological examination of the injured lung demonstrates epithelial cell injury represented by extensive necrosis of pneumocytes, swelling of endothelial cells with the widening of intercellular junctions, and the formation of hyaline membranes, for an important part composed of fibrin in alveolar ducts and airspaces. At later stages, massive infiltration of neutrophils and other inflammatory cells will occur and fibrin thrombi can be seen in the alveolar capillaries and smaller pulmonary arteries [113]. The abundant presence of intravascular and extravascular fibrin appears to be a specific hallmark of acute lung injury following sepsis and is much more outspoken than the fibrin deposition in other organs. Based on this observation, many authors have hypothesized that fibrin deposition plays an important role in the pathogenesis of acute lung injury in sepsis, a concept that is further supported by large clinical studies in patients with sepsis demonstrating the association between lung injury and coagulation abnormalities [114]. Furthermore, the extensive local fibrin deposition may suggest that local activation of coagulation or perturbation of local physiological regulatory systems could be involved in this. Interestingly, it has been shown that effective blocking of the coagulopathy in experimental sepsis attenuates lung injury and local inflammatory activity, which may point at pivotal cross-talk between the (local) mechanisms of coagulation and inflammation [14, 15].

In BAL fluids from patients with ARDS, it has been demonstrated that there is activation of coagulation and inhibition of fibrinolysis [115–117]. Almost immediately after onset of ARDS, an increased but transient procoagulant activity can be detected in BAL fluid. At the same time, fibrinolytic activity is strongly inhibited and is kept at a low level up to 14 days. Experimental and clinical studies have shown that fibrin deposition is due to tissue factor-mediated thrombin generation and suppressed fibrinolysis [118]. The most important determinants of these local disturbances are TF and PAI-1; high levels of soluble TF can be measured in BAL fluid from patients with ARDS, while increased production of PAI-1 is the most consistent finding reported as being related to suppressed fibrinolytic activity. Recently, lower levels of pulmonary PC levels were correlated with a higher degree of lung injury and worse outcome in patients with acute lung injury [119]. Similar to acute lung injury and ARDS, pneumonia is characterized by a shift in the alveolar hemostatic balance. In BAL fluid from patients with severe pneumonia, a markedly increased procoagulant activity was detected. Concordantly, fibrinolytic activity was depressed in BAL fluids, which is related to high concentrations of PAI-1 in the lungs. Patients at risk for ventilator-associated pneumonia show similar changes in pulmonary fibrin turnover [120]. Similarly, in mechanically ventilated patients who developed pneumonia, an increase in coagulation products was detected in lung lavage fluids. Interestingly, the diagnosis of pneumonia was preceded by a strong increase in PAI-1 levels in the lungs, with a resulting decrease in fibrinolytic activity. Similar to the inflammatory responses in patients with unilateral pneumonia patients, there is overt activation of coagulation and depressed fibrinolytic activity due to PAI-1 upregulation [121]. Recently, it has been demonstrated that the protein C system is also suppressed at the site of infection, contributing to the procoagulant effects of pulmonary infection [121].

## Coagulation activation and the intestinal tract

Acute intestinal ischemia and reperfusion may result in impaired intestinal structure and function, in experimental models characterized by intestinal cell swelling and protein

leakage and impaired intestinal absorptive capacity. In addition, intra- and extravascular fibrin deposits may be present due to activation of mesenteric coagulation and inhibition of fibrinolysis [122]. Upon 20 to 40 min of occlusion of the superior mesenteric artery and subsequent reperfusion, portal vein plasma levels of thrombin–antithrombin levels increased, indicating local thrombin generation. This increase in portal coagulation activity is associated with a marked fall in protein C activity levels. Simultaneously, markers for fibrinolysis in portal plasma showed a complete inhibition due to an increase in levels of plasminogen activator inhibitor, type 1 (PAI-1). This activation of coagulation upon ischemia–reperfusion could be almost completely blocked by systemic administration of activated protein C, whereas heparin and antithrombin were less effective (Schoots et al., submitted for publication). Interestingly, amelioration of ischemia–reperfusion-induced intestinal intra- and extravascular fibrin deposition by administration of activated protein C caused a significant improvement in intestinal function.

## Coagulation activation and the liver

The liver is the major site of synthesis of almost all coagulation factors. In addition, Kupfer cells of the liver are most important bacterial scavengers, and neutralize bacterial products and pro-inflammatory cytokines. Impaired synthesis of physiological anticoagulant proteins antithrombin and protein C, and low levels of free protein S due to acute phase upregulation of C4b-binding protein (the carrier of protein S) are well-known consequences of impaired liver function [123]. However, failure of the coagulation system is not only a consequence of liver failure but may also contribute to the pathogenesis of liver failure in systemic inflammatory states. Under these circumstances, endothelial cells of the liver show a marked upregulation of tissue factor, leading to local thrombin generation and fibrinogen to fibrin conversion [124]. A marked cross-talk between coagulation and inflammation is also strongly present in liver tissue, as protease-activated receptors (PARs) are abundantly present and activated coagulation proteases may not only lead to fibrin formation but also to increased inflammation, and in case of liver tissue, ultimately to tissue fibrosis [125]. Indeed, it has been shown that anticoagulant treatment can prevent ischemia–reperfusion injury in an experimental model in rat livers [126].

## Conclusion

The response of the coagulation system upon systemic inflammation may considerably vary between cells, tissues, and organs. This may explain, in part, the variable clinical presentation of multiple organ failure in patients with a systemic inflammatory response upon sepsis or trauma. Many coagulation pathways in various organs may act according to parallel routes, but marked differences exist in the emphasis of a specific mechanism in a specific organ system. Detailed knowledge on the site-specific activation and regulation of coagulation may provide more insight in better management strategies in case of specific organ failures in the setting of a systemic inflammatory response.



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