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Cardiovascular Research 60 (2003) 26–39

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Infection and inflammation and the coagulation system ☆

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Received 4 November 2002; accepted 16 December 2002

Abstract

Severe infection and inflammation almost invariably lead to hemostatic abnormalities, ranging from insignificant laboratory changes to severe disseminated intravascular coagulation (DIC). Systemic inflammation results in activation of coagulation, due to tissue factor-mediated thrombin generation, downregulation of physiological anticoagulant mechanisms, and inhibition of fibrinolysis. Pro-inflammatory cytokines play a central role in the differential effects on the coagulation and fibrinolysis pathways. Vice-versa, activation of the coagulation system may importantly affect inflammatory responses by direct and indirect mechanisms. Apart from the general coagulation response to inflammation associated with severe infection, specific infections may cause distinct features, such as hemorrhagic fever or thrombotic microangiopathy. The relevance of the cross-talk between inflammation and coagulation is underlined by the promising results in the treatment of severe systemic infection with modulators of coagulation and inflammation.

Keywords: Cytokines; Hemostasis; Infection/inflammation

1. Introduction

The relevance of the interaction between coagulation and inflammation as a response to severe infection, in its most extreme form manifesting as disseminated intravascular coagulation (DIC) and multiple organ failure, is becoming increasingly clear [1,2]. In recent years, the various mechanisms that play an important role in this interaction have been elucidated and this knowledge has indeed been demonstrated to be applicable for the improvement of our understanding of the pathogenesis of severe infection or sepsis and, even more importantly, the clinical management of these patients [3]. In this article, the mechanisms that play a role in the interaction between inflammation and coagulation will be reviewed and specific features of infectious disease-mediated effects on the coagulation system will be highlighted. In addition, the cross-talk between activated coagulation and inflammatory mediators will be discussed.

2. Inflammation-induced coagulation modification

It has long been known that inflammation can lead to activation of the coagulation system. Acute inflammation, as a response to severe infection or trauma, results in a systemic activation of the coagulation system [3,4]. It was initially thought that this systemic activation of coagulation was a result of direct activation of the contact system of

☆ For this manuscript Dr. F. Calabrese acted as Guest Editor.

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Time for primary review 28 days.

0008-6363/03/$ – see front matter
doi:10.1016/S0008-6363(02)00857-X

coagulation by microorganisms or endotoxin. However, in the 1990s, it became apparent that cytokines played a mediatory role in the activation of coagulation and subsequent fibrin deposition and that the point of impact on the coagulation system was rather the tissue factor-factor VIIa ('extrinsic') pathway than the contact system ('intrinsic pathway') [5,6]. Furthermore, the significance of impaired physiological anticoagulant pathways became increasingly clear [7]. Lastly, it was shown that impaired fibrin removal by a suppressed fibrinolytic system contributed importantly to the microvascular deposition of fibrin.

Vascular endothelial cells play a central role in all mechanisms that contribute to inflammation-induced activation of coagulation. Endothelial cells respond to the cytokines expressed and released by activated leukocytes but can also release cytokines themselves [8]. Furthermore, endothelial cells are able to express adhesion molecules and growth factors that may not only promote the inflammatory response further but also affect the coagulation response (Fig. 1). However, it has recently become clear that, in addition to these mostly indirect effects of the endothelium, endothelial cells interfere directly with the initiation and regulation of fibrin formation and removal during severe infection [9,10].

3. Mechanisms of inflammation-induced coagulation and fibrinolysis activation

Inflammation-induced coagulation activation is characterized by widespread intravascular fibrin deposition, which appears to be a result of enhanced fibrin formation and impaired fibrin degradation [1,11]. As outlined in the following paragraphs and schematically represented in Fig. 1, enhanced fibrin formation is caused by tissue factor-mediated thrombin generation and simultaneously occurring depression of inhibitory mechanisms, such as the protein C and S system. The impairment of endogenous thrombolysis is mainly due to high circulating levels of PAI-1, the principal inhibitor of plasminogen activation. These derangements in coagulation and fibrinolysis are mediated by differential effects of various pro-inflammatory cytokines [5].

3.1. Tissue factor

The initiating factors comprise the molecule tissue factor and the plasma protein factor VII(a) [12]. Tissue factor is a membrane-bound 4.5-kDa protein, which is constitutively expressed on a number of cells throughout the body [13]. These cells are mostly in tissues not in direct contact with

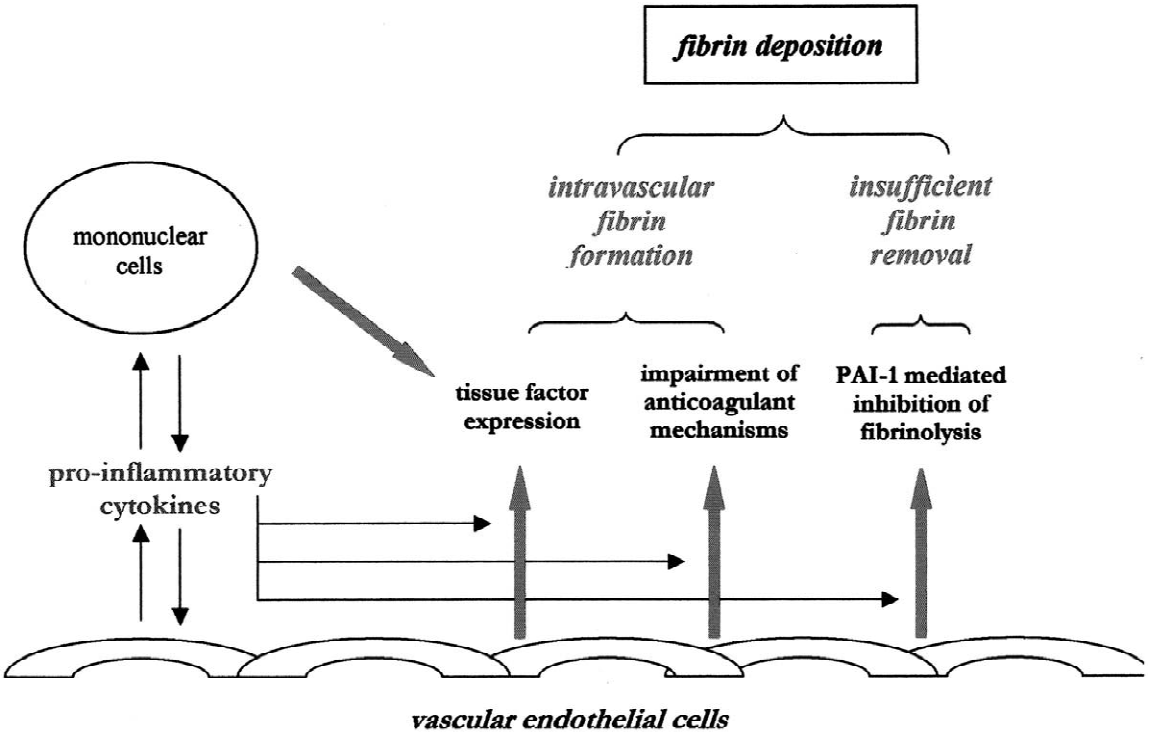

Fig. 1. Schematic representation of pathogenetic pathways in disseminated intravascular coagulation (DIC). During systemic inflammatory response syndromes, both perturbed endothelial cells and activated mononuclear cells may produce proinflammatory cytokines that mediate coagulation activation. Activation of coagulation is initiated by tissue factor expression on activated mononuclear cells and endothelial cells. In addition, downregulation of physiological anticoagulant mechanisms and inhibition of fibrinolysis by endothelial cells will further promote intravascular fibrin deposition. PAI-1 indicates plasminogen activator inhibitor, type 1.

blood, such as the adventitial layer of larger blood vessels. The subcutaneous tissue also contains substantial amounts of TF, and histologically TF appears to be present in all blood tissue barriers [14]. Upon expression at the cell surface, TF can interact with factor VII, either in its zymogen or activated form [15]. Upon complexing, factor VII is activated, and the TF-factor VIIa complex catalyzes the conversion of both factors IX and X [16,17]. Factors IXa and Xa enhance the activation of factors X and prothrombin, respectively (Fig. 1). In cells in contact with the blood circulation, TF is induced by the action of several compounds including cytokines, C reactive protein and advanced glycosilated endproducts [14]. Inducible TF is predominantly expressed by monocytes and macrophages. The expression of TF on monocytes is markedly stimulated by the presence of platelets and granulocytes in a P-selectin dependent reaction [18]. This effect may be the result of nuclear factor kappa B (NFkB) activation induced by binding of activated platelets to neutrophils and mononuclear cells. This cellular interaction also markedly enhances the production of IL-1b, IL-8, MCP-1, and TNFa [19].

Under in vitro conditions, different cytokines such as tumor necrosis factor a (TNFa), and interleukin (IL) 1, induce TF by vascular endothelial cells, but its relevance for in vivo coagulation is uncertain [14,20]. TF has also been localized on polymorphonuclear cells (PMN) in whole blood and ex vivo perfusion systems, suggesting an additional pool of 'blood borne' TF [21], but it is unlikely that PMN actually synthesize TF in detectable quantities [22]. Finally, TF and associated procoagulant activity in vitro has been detected on microvesicles derived from predominantly platelets and granulocytes in patients with meningococcal sepsis [23]. The localization on microvesicles may imply a highly efficient pool of procoagulant material assembling not only the initiating TF-factor VIIa complex but also the phospholipid surface facilitating the development of the tenase and prothrombinase complexes. In addition, a soluble form of TF is detectable in plasma from humans, and its levels are elevated in DIC [24]. The significance of soluble TF is unknown. It is likely the result of membrane cleavage by metalloproteinases produced under inflammatory conditions, and it circulates in free as well as complexed forms. Whether soluble TF is catalytically active and involved in inducing coagulation remains to be investigated. In addition to its effect on the haemostatic system, tissue factor also has cell signalling and mitogenic effects. These effects are probably mediated by the protease-activated receptors (PARs) 1 and 2. Other examples of such effects are described below in the paragraph describing the cross-talk between coagulation and inflammation.

In humans with infection and an activated coagulation system, monocytes expressing enhanced levels of TF and procoagulant activity have been demonstrated. The tissue expression of TF appears to be confined to certain organs and vascular beds, but it is uncertain whether its expression is genetically controlled in an organ specific way [9].

3.2. The contact system

Whether in humans with severe infection the contact route plays any role remains unknown. Negatively charged substances including phospholipids and glycosaminoglycans, are a theoretically relevant source of contact activation. Recent studies suggest that intact cell surfaces mediate the assembly of contact factor proteins that may initiate this route of activation [25]. The assembly of kininogens on a multiprotein receptor leads to controlled prekallikein activation. In the case of endothelial cells a complex between high molecular weight kininogen (HK) and prekallikrein or factor XI binds to a multiprotein receptor on these cells. This receptor consists of cytokeratin 1, uPAR and gC1qR [25]. Cytokeratin 1 was also demonstrated on platelets and granulocytes, which may allow the interaction of HK with these cells as well. On endothelial cells, PK activation leads to factor XII conversion amplifying PK activation. The activation of PK causes the cleavage of HK and liberation of bradykinin. Bradykinin induces tPA and this is one of the potential direct effects of the contact system on fibrinolysis [26,27,27]. Several lines of evidence support the important role of the contact system in activating fibrinolysis. Additional studies indicate that kininogens contain a peptide fragment that interferes with thrombin binding to platelets through preventing peptide liberation from PAR1 by thrombin [28]. Taken together, a profibrinolytic and anticoagulant function of the contact system is more likely than a procoagulant role in vivo.

Studies in patients with a clinical suspicion of DIC showed elevated levels of markers of activation of the contact system. Kaufmann et al. measured elevated concentrations of kininogen-a 1 anti-trypsin complexes [29], however studies with similar methods for coagulation activation markers have hardly ever detected elevated levels of contact activation [30]. A study in patients with meningococcal septicemia showed a negative correlation between plasma factor XII levels and factor XIIa-C1 inhibitor complexes [31]. Although this would suggest consumption of factor XII, and subsequent activation of factor XI downstream, a possible alternative explanation is a negative acute phase effect with reduced synthesis of factor XII, in conjunction with thrombin-mediated activation of factor XI [32].

Experimental studies suggested that blockade of the contact route in an *E. coli* sepsis model in primates with a monoclonal antibody against factor XIIa failed to protect animals from the coagulation derangement, but diminished development of lethal hypotension [33]. This latter study provided reasonable support for the current view that the contact activation pathway does not contribute to coagulation activation. The contact route may play important roles

in pro-inflammatory mechanisms related to vascular permeability, vascular proliferative processes (role of kininogen in smooth muscle cell proliferation) and stimulation of fibrinolysis [34].

3.3. Physiological anticoagulant pathways

Systemic activation of coagulation upon inflammation is counteracted by several mechanisms. First, coagulation inhibitors are present to slow down the coagulation mechanism (Fig. 2). Soluble inhibitors constitute antithrombin (AT), proteins C and S, and tissue factor pathway inhibitor (TFPI) [35]. Antithrombin inhibits coagulation proteases by 1:1 complex formation, which, in the case of thrombin–antithrombin complexes, can be detected by immunoassay in plasma from patients with DIC [24,36]. AT is thought to be one of the most important inhibitors of the activated coagulation system, and markedly lowered plasma levels are found in sepsis [37,38]. In the course of DIC the function of AT may be influenced in several ways. First, a reduced absolute amount of the inhibitor may occur due to reduced protein synthesis on the one hand and clearance on the other hand. Clearance may be either in the form of protease-inhibitor complex, or due to proteolytic cleavage by proteolytic enzymes including granulocyte elastase [39]. Second, the function of AT may be impaired due to the possibly reduced availability of glycosaminoglycans which may act as the physiological heparin-like cofactor of AT [40]. Under the influence of cytokines the synthesis of glycosaminoglycans by endothelial cells may be reduced, impairing the inhibitory potential of AT [41]. In animal models of experimental bacteremia, an antithrombin concentrate prolonged survival and reduced the severity of DIC [42]. This protective effect may have been distinct from the anticoagulant effect as such because in one of these models, an active site blocked factor Xa preparation reduced the severity of DIC, but did not protect the animals from dying due to sepsis [43]. The protective effect of antithrombin may have resulted from an anti-inflammatory effect. Infusion of recombinant antithrombin at very high concentrations (about 10-fold higher than the basal plasma concentration) markedly reduced mortality due to lethal *E. coli* infusion, but also caused a significant reduction in the plasma levels of IL-6 and IL-8 [44]. In spite of significantly higher levels of TNFa and lower levels of IL-10, the overall effect on DIC was apparently favourable because the reduction in fibrinogen level was less pronounced in the AT-treated animals. An unexpected finding was that tPA levels, after an initial small rise in both groups, were markedly lower in the *E. coli* challenged baboons given antithrombin. As a result there was no increment in the levels of plasmin–antiplasmin complexes (PAP), whereas the increase in PAI-1 levels was somewhat increased in AT-treated animals.

Since TNFa is a critical mediator of fibrinolysis in primates given a low-dose endotoxin [45], and given the elevated levels of TNF-α in baboons given antithrombin, a marked increase in tPA and PAP complexes would have been expected in the baboon model after AT infusion. The absence thereof suggests that TNF-α is not the single most important cytokine influencing fibrinolysis in sepsis. Potentially, the rise of PAI-1 sufficiently blunts fibrinolysis, but an alternative explanation may be a direct effect of antithrombin at tPA release by the endothelium, caused by AT binding to glycosaminoglycans at the endothelial surface. Given the net negative effect on fibrinolysis, the incomplete protection only achieved at very high doses of antithrombin, the therapeutic potential of antithrombin may be limited.

Protein C and its cofactor protein S form a second line of defense. Thrombin binds to the endothelial cell membrane associated molecule thrombomodulin, and this com-

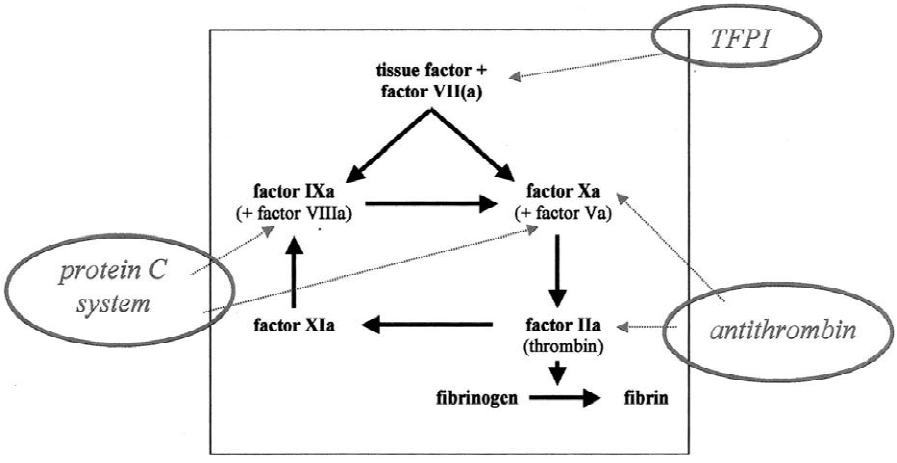

Fig. 2. Point of impact of the three major physiological anticoagulant pathways. Antithrombin is the most important inhibitor of thrombin and factor Xa, activated protein C is able to degrade the essential cofactors Va and VIIIa, and TFPI inhibits the tissue factor/factor VIIa complex. Abbreviation: TFPI, tissue factor pathway inhibitor.

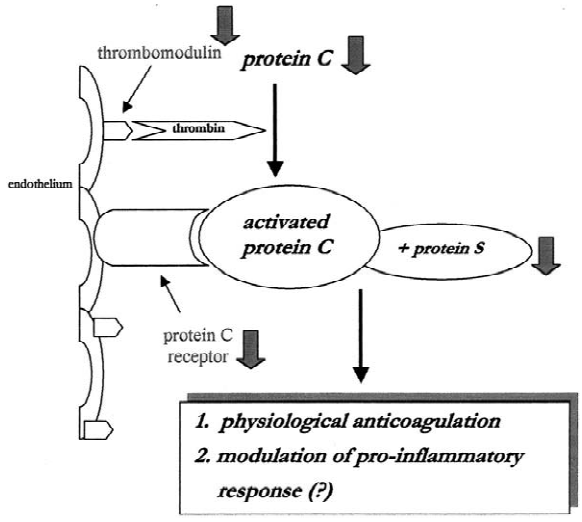

Fig. 3. Dysfunction of the protein C system in disseminated intravascular coagulation (DIC) is due to low levels of zymogen protein C, downregulation of thrombomodulin and the endothelial protein C receptor and low levels of free protein S due to acute phase-induced high levels of its binding protein (i.e. C4b-binding protein).

plex converts protein C to its active form, protein Ca (PCa) (Fig. 3) [46]. In addition, the thrombin–thrombomodulin complex accelerates the conversion of the thrombin activatable fibrinolytic inhibitor (TAFI) [47]. PCa inactivates factors Va and VIIIa by proteolytic cleavage, thus slowing down the coagulation cascade. Endothelial cells, primarily of large blood vessels, express an endothelial protein C receptor (EPCR), which augments the activation of protein C at the cell surface [48–50]. Activated protein C has anti-inflammatory effects on mononuclear cells and granulocytes, which may be distinct from its anticoagulant activity. Administration of PCa prevented thrombin-induced thromboembolism in mice, mainly through its antithrombotic effect [51]. An anti-inflammatory effect of PCa was demonstrated by Hancock et al. [52], showing reduced TNFα production in rats challenged with endotoxin. Activated PC reduced the severity of spinal cord injury in rats, probably due to inhibition of leukocyte activity [53]. In the latter model, soluble thrombomodulin had a similar anti-inflammatory effect, but this was also mediated by PCa. In a pulmonary inflammatory model induced by endotoxin, thrombomodulin also reduced vascular permeability through a protein C-dependent mechanism [54].

In contrast, defects in the protein C mechanism enhance the vulnerability to inflammatory reactions and the ensuing activation of coagulation. In patient studies, lowered levels of protein C and protein S are associated with increased mortality. Blockade of the activity of protein C by infusion of C4 binding protein turns a sublethal model of *E. coli* in baboons into a lethal model [55]. Blockade of EPCR by a neutralizing monoclonal antibody also increased mortality in the *E. coli* baboon model [56]. Vice versa, infusion of PC in the same model, protected against DIC and dying [57]. Thus, it appears that the protein C mechanism is of great importance in the host defense against sepsis and coagulation activation. In situations, associated with DIC and systemic inflammation, TNFa and interleukin-1 may significantly down-regulate the cellular expression of thrombomodulin, as suggested by cell culture experiments [58–60]. The formation of thrombin may induce the shedding of EPCR by the endothelium, due to activation of metalloproteinases by thrombin. It is presently unknown whether thrombomodulin is also cleaved by similar mechanisms.

A third inhibitory mechanism constitutes TFPI. This molecule, which exists in several pools either endothelial cell associated, or lipoprotein bound in plasma, inhibits the TF-factor VIIa complex by forming a quaternary complex in which factor Xa is the fourth component [61]. Clinical studies in septic patients have not provided clues as to its importance, because in the majority of patients the levels of TFPI are not diminished compared to normals [62]. This may be explained by the lack of downregulatory effects of inflammatory mediators on cultured endothelial cells [63]. The relevance of TFPI in sepsis-induced coagulation activation is illustrated by two lines of experimentation. First, depletion of TFPI sensitized rabbits to DIC induced with tissue factor infusion sensitizes the animals to develop a more severe DIC [64]. Second, TFPI infusion protected against the harmful effects of *E. coli* in primates. In this study, TFPI not only blocked DIC but all baboons challenged with lethal amounts of *E. coli* showed a marked improvement in vital functions and survived the experiment without apparent complications [65]. The beneficial effects of TFPI may in part be due to its capacity to bind endotoxin and to interfere with its interaction with CD14 [66], and to its attenuating effect on IL-6 generation. A recent study in volunteers confirmed the potential of TFPI to block the procoagulant pathway triggered by endotoxin [67]. However, in this study in healthy subjects, there was no reduction in cytokine levels, in contrast to the apparent anti-inflammatory effect of TFPI in the baboon study. There are several possible explanations for this discrepancy, which all relate to the marked differences between the endotoxin and *E. coli* sepsis models in which the effects of TFPI were investigated. In the beneficial effects of TFPI on survival, anti-inflammatory effects rather than antithrombotic activity may be prominent [65].

In general, the presence of intact function and normal plasma levels of inhibitors appears to be important in the defense against DIC. It should be noted however, that there are no strong indications that individuals with congenital deficiencies of inhibitors would suffer a greater chance of developing DIC, but this issue remains to be explored. In addition, the influence of inhibitors in modifying the interaction between coagulation and inflammation deserves further attention.

3.4. Fibrinolysis

In experimental models of severe infection, fibrinolysis is activated, demonstrated by an initial activation of plasminogen activation, followed by a marked impairment caused by the release in blood of plasminogen activator inhibitor, type 1 (PAI-1) [45,68,69]. The latter inhibitor strongly inhibits fibrinolysis causing a net procoagulant situation. The molecular basis is cytokine-mediated activation of vascular endothelial cells; TNFα and IL-1 decreased free tPA and increased PAI-1 production, TNFa increased total uPA production in endothelial cells [70,71]. Endotoxin and TNFα stimulated PAI-1 production in liver, kidney, lung and adrenals of mice. The net procoagulant state is illustrated by a late rise in fibrin breakdown fragments after *E. coli* challenge of baboons. Experimental data also indicate that the fibrinolytic mechanism is active in clearing fibrin from organs and circulation. Endotoxin-induced fibrin formation in kidneys and adrenals was most dependent on a decrease in urokinase type plasminogen activator (uPA) [72]. PAI-1 knockout mice challenged with endotoxin did not develop thrombi in the kidney in contrast to wild-type animals [71]. Endotoxin administration to mice with a functionally inactive thrombomodulin gene (TMProArg mutation) and defective protein C activator cofactor function caused fibrin plugs in the pulmonary circulation, while wild-type animals did not develop macroscopic fibrin [73]. This phenomenon proved to be temporary, with detectable thrombi at 4 h after endotoxin, and disappearance of clots at 24 h in animals sacrificed at that timepoint. These experiments demonstrate that fibrinolytic action is required to reduce the extent of intravascular fibrin formation.

Fibrinolytic activity is markedly regulated by PAI-1, the principal inhibitor of this system. Recent studies have shown that a functional mutation in the PAI-1 gene, the 4G/5G polymorphism, not only influenced the plasma levels of PAI-1, but was also linked to clinical outcome of meningococcal septicemia. Patients with the 4G/4G genotype had significantly higher PAI-1 concentrations in plasma and an increased risk of death [74]. Further investigations demonstrated that the PAI-1 polymorphism did not influence the risk of contracting meningitis as such, but probably increased the likelihood of developing septic shock from meningococcal infection [75]. These studies are the first evidence that genetically determined differences in the level of fibrinolysis influences the risk of developing complications of a Gram-negative infection. In other clinical studies in cohorts of patients with DIC, high plasma levels of PAI-1 were one of the best predictors of mortality [76,77]. These data suggest that activation of coagulation contributes to mortality in this situation, but as indicated earlier, the fact that PAI-1 is an acute phase protein, a higher plasma concentration may also be a marker of disease rather than a causal factor. Interestingly, platelet α-granules contain large quantities of PAI-1 and release PAI-1 upon their activation. Since platelets become activated in case of severe inflammation and infection, this may further increase the levels of PAI-1 and contribute to the fibrinolytic shut-off.

3.5. Platelets

In addition to the mechanisms described above, platelets may play a role in the pathogenesis of inflammation-induced coagulation activation as well. Endotoxin can directly activate platelets and many pro-inflammatory cytokines are capable of inducing platelet activation through the sphingosine pathway and platelet activating factor (PAF) and its receptor. The importance of platelet activation for the activation of coagulation in severe infection is probably related to the provision of a suitable phospholipid surface on the activated platelet membrane for assembly of complexes of activated coagulation factors, such as the prothrombinase complex, consisting of activated factors X and V, prothrombin and calcium. The presence of such a surface may catalyze several-fold the generation of thrombin and render the coagulation system less susceptible to fluid-phase protease inhibitors.

4. Cytokines and other mediatory factors

Activation of blood coagulation requires a number of cofactors. In particular, to develop coagulation activation, a number of interacting surfaces from cell remnants or intact cells, inflammatory mediators and coagulation proteins is required. The stimulus is presented by the underlying disease and can thus be diverse: a bacterial cell compound such as endotoxin, a protease induced by a malignant cell type, tissue damage exposing catalytic surfaces and constitutively expressed TF, a substance that might directly activate coagulation (fat, amniotic fluid) by unknown pathways, or others. Each of these triggers interact with other mediators: TF assembles on phospholipids, cells provide catalytic surfaces by exposure of phosphatidyl serine, cytokines interact with receptors and induce signalling pathways that induce TF and other proinflammatory components via the NFκB complex. To illustrate this situation, we will describe in greater detail the mechanisms that lead to endotoxin-induced activation of the coagulation mechanism. This model provides the best studied model of the complex network of interactions that involves activated coagulation in a DIC like fashion [78]. Endotoxin is the lipopolysaccharide compound from Gram-negative bacteria inducing the sepsis syndrome and DIC. Gram-negative bacteria liberate endotoxins from their membrane which interact with cell surfaces by different pathways. In blood, endotoxin directly binds to CD 14 at monocytes, and binds to endothelial cells after complexing with lipopolysaccharide binding protein (LBP) and the Toll-like receptor 4 complex [79]. By these interactions,

endotoxin induces a number of signalling pathways leading to the activation of the NFkB system, which starts the transcription of genes of proinflammatory cytokines, TF and others. These mechanisms are further reviewed elsewhere [80].

Except for a direct positive effect of endotoxin on TF synthesis, the formation of the cytokines IL-1, TNFα, and IL-6 stimulates TF formation [14]. Ex vivo, this was directly demonstrated by showing that the production of TF mRNA was rapidly induced in blood cells after injection of endotoxin to human volunteers [81]. In vitro, whole blood cells generate TF upon incubation with endotoxin or IL-1, TNFα and IL-6. In vitro, cultured human endothelial cells also induce TF after incubation with TNFα and IL-1, but their role in DIC remains unknown. Thus far, the only direct evidence for endothelial involvement in TF production has been the demonstration of TF expressing circulating endothelial cells in patients with sickle cell disease (a possible DIC associated condition) [82].

Studies in primates have revealed the molecular mechanism underlying endotoxin induced activation of coagulation [68]. After intravenous endotoxin challenge, a rapid production and liberation of proinflammatory cytokines is observed. Among those, TNFa [69] and IL-6 [83] are important for inducing fibrinolytic and procoagulant changes in the blood. Specifically, a rapid increase in the plasma levels of tissue plasminogen activator (tPA) and the prothrombin fragment F1+2 as well as thrombin–antithrombin complexes (TAT) occurs. Fibrinolytic activity indicated by plasmin–antiplasmin complexes is impaired by a subsequent rise in plasminogen activator inhibitor 1 (PAI-1), and a net procoagulant state results [5]. Studies in baboons challenged with lethal amounts of *E. coli* (a model of Gram-negative septicemia) have underscored the importance of cytokines in mediating the sepsis syndrome, and demonstrated the prolonged proinflammatory course in which the actual formation of fibrin is particularly evident at >24 h after *E. coli* challenge [84]. In fact most fibrin appears in blood, when thrombin generation is already declining, and evidence of endothelial cell activation becomes apparent indicated by elevated levels of soluble thrombomodulin. Both in the chimpanzee endotoxin model and the *E. coli* model, TF is essential for inducing coagulation activity, and inhibition of the TF pathway abolishes clotting activation [85]. IL-6 is an important mediator of procoagulant effects, while TNFa is particularly involved in the fibrinolytic response to endotoxin [86]. Vice versa, inhibition of TF by TFPI reduced IL-6 in blood in the baboon model [87], suggesting that a more extensive crosstalk between inflammatory mediators and coagulation exists (see further). These effects may be more obvious in the sepsis model, than in the endotoxin model [88]. Recent experiments in humans challenged with endotoxin did not reveal any effect of TFPI on IL-6 nor any of the other cytokines measured in blood [67]. Thus the strength of the trigger may determine the degree of involvement of the cytokine network and its interactions with the coagulation system.

Monocytes expressing TF directly bind factor VII(a), shed TF, or become associated with the damaged vessel wall, a process probably occurring when macrophages expressing TF invade the vessel wall and play a procoagulant role in atherosclerosis [89]. Circulating monocytes may trigger DIC, after interacting with platelets. Microvesicles may accelerate this process, and the complex interaction between cells, membrane fragments, soluble mediators and proteins may cause the full blown DIC syndrome. In the endotoxin model thrombin is an important feedback activator of the coagulation mechanism, because the thrombin-specific inhibitor hirudin reduces the procoagulant response in human volunteers [90]. The extent of catalysis and exhaustion of coagulation proteins and cellular elements is primarily determined by the strength of the triggers and the potential of the inhibitory mechanisms.

5. Cross-talk between coagulation and inflammation

Coagulation activation yields proteases that not only interact with coagulation protein zymogens, but also with specific cell receptors to induce signaling pathways. In particular, protease interactions that affect inflammatory processes may be important in the development of DIC. Factor Xa, thrombin and the tissue factor–factor VIIa complex have each been shown to elicit pro-inflammatory activities [91,92]. Fibrinogen/fibrin is important to the host defense mechanism, and probably has an additional role that is not directly related to clotting per se [93].

Thrombin has been shown to induce a variety of non-coagulant effects, some of which may influence DIC by affecting cytokine levels in blood. It induces production of monocyte chemotactic protein-1 (MCP-1) and IL-6 in fibroblasts, epithelial cells and mononuclear cells in vitro [94]. Endotoxin-stimulated whole blood produces significant IL-8, which has a procoagulant effect that is tissue factor- and thrombin-dependent [95]. Thrombin also induces IL-6 and IL-8 production in endothelial cells. These effects of thrombin on cell activation are probably mediated by protease-activated receptors (PARs) 1, 3 and 4 [96]. PARs are cellular receptors for activated proteases that may contribute to intracellular signaling. Several studies have indicated that the tissue factor–factor VIIa complex also activates cells by binding to PARs, and it appears that PAR2 in particular is involved. Binding of the catalytic tissue factor–factor VIIa complex elicits a variety of inflammatory effects in mononuclear cells that may influence their contribution to coagulation activity [97]. A direct indication of the relevance of these phenomena in vivo comes from a recent study showing that infusion of recombinant factor VIIa into healthy human volunteers causes a rise, albeit small, in plasma levels of IL-6 and

IL-8 [98]. Although the plasma concentrations of factor VIIa in this experiment were much higher than endogenous factor VIIa concentrations in patients with sepsis, it is possible that the mechanism by which VIIa causes cytokine induction (direct or factor Xa/thrombin-mediated) is of physiologic importance.

Another link between inflammation and coagulation is formed by the Protein C system. Activated Protein C has anti-inflammatory effects on mononuclear cells and granulocytes, which may be distinct from its anticoagulant activity [99]. The anti-inflammatory effect of activated Protein C was confirmed in vivo by the demonstration of reduced TNF-α production in rats challenged with endotoxin [52]. In addition, we have recently shown that mice with a one-allele targeted deletion of the Protein C gene (heterozygous Protein C-deficient mice) not only develop more severe DIC and increased fibrin deposition upon systemic endotoxemia, but also have significantly higher circulating levels of pro-inflammatory cytokines compared with wild-type littermates [100]. These experimental data are corroborated by the observations in the PROWESS trial that recombinant human Activated Protein C (drotrecogin alfa [activated]) accelerated the decrease in levels of IL-6 in patients with severe sepsis [101]. For high doses of antithrombin, similar interactions with the inflammatory cascades were seen [44]. Taken together, a number of coagulation proteases can induce pro-inflammatory mediators that have procoagulant effects, which may amplify the cascade that leads to DIC. Effects at the cellular level will be determined by the capacity of the coagulation inhibitors to inactivate these enzymes.

6. Relevance of the cross-talk between inflammation and coagulation for organ failure

There is ample evidence that inflammatory activation in concert with microvascular thrombosis contributes to multiple organ failure in patients with severe infection and DIC. Firstly, extensive data have been reported on post-mortem findings of patients with DIC [102,103]. 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 invariably due to fibrin deposition in small and mid-size vessels of various organs [104]. Importantly, the presence of these intravascular thrombi appears to be clearly and specifically related to the clinical dysfunction of the organ. 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 [42,87]. Lastly, clinical studies support the notion of coagulation as an important denominator of clinical outcome. DIC has been shown to be an independent predictor of mortality in patients with sepsis and severe trauma [1,105].

7. Coagulation and vascular complications in infectious diseases

Apart from the generalized response upon systemic inflammation as discussed above, specific infections may result in thrombohemorrhagic syndromes, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) or vasculitis. Symptoms and signs may be dominated by bleeding, thrombosis, or both [1,79,106]. Clinically overt infection-induced activation of coagulation may occur in 30–50% of patients with Gram-negative sepsis [107–110]. Contrary to widely held belief, this may appear as common in patients with Gram-positive sepsis as in those with Gram-negative sepsis [111]. Activation of the coagulation system has also been documented for non-bacterial pathogens, i.e. viruses [112,113], protozoa (Malaria) [114,115], fungi [116] and spirochetes [117].

7.1. Thrombosis and bleeding

Viral and bacterial infections may result in an enhanced risk for local thromboembolic disease, i.e. deep venous thrombosis or pulmonary embolism. In a thromboembolic prevention study of low-dose subcutaneous standard heparin for hospitalized patients with infectious diseases, morbidity due to thromboembolic disease was significantly reduced in the heparin group compared to the group receiving no prophylaxis. There was however no beneficial effect of prophylaxis on mortality due to thromboembolic complications [118]. In chronic viral diseases, such as CMV or HIV infection, the risk of thromboembolic complications is relatively low [119–121].

Viral hemorrhagic fever is complicated by DIC in the most severe cases [122–126]. DIC is not frequently encountered in other viral infections but has been reported in cases of infection with Rotavirus [127,128], Varicella, Rubella, Rubeola and Influenza [129–136]. TTP and HUS, triggered by a viral or bacterial infection [137,138], frequently lead to bleeding symptoms, but also platelet and fibrin thrombi may be generated in various organs, leading to prominent symptoms with organ dysfunction. In specific infections, such as viral hemorrhagic fever, bleeding complications are prominent [122,123]. In other viral and bacterial infections associated with TTP or HUS, bleeding also is often the prominent and presenting symptom (Table 1) [139–141].

Table 1
Viral hemorrhagic fevers

Virus	Geographic distribution	Source of infection
Dengue HF	Southeast Asia, Mid/South America, China	Mosquito
Chikungunya	Southeast Asia	Mosquito
Ebola	Zaire, Sudan	Unknown
Marburg HF	Zimbabwe, Kenya, Uganda	Unknown
Lassa fever	West Africa	Rodent
Yellow fever	South America, Africa	Mosquito
Omsk HF	Former Soviet Union	Tick
Hantaan	Central Europe, former Soviet Union, Asia	Rodent

7.2. Vasculitis

Bacterial and viral infections may result in a vasculitis-like syndrome with either bleeding manifestations or ischemic injury [142–144]. Vasculitis is a well-documented phenomenon in CMV infection [145,146], occurring predominantly in the vasculature of the gastrointestinal tract where it causes colitis [147,148], the central nervous system where it causes cerebral infarction [149,150], and the skin where it results in petechiae, purpura papules, localized ulcers or a diffuse maculopapular eruption [151]. HIV (human immunodeficiency virus) infection may be accompanied by vasculitis syndromes, e.g. polyarteriitis nodosa, Henoch Schönlein purpura and leukocytoclastic vasculitis [152–154]. Hepatitis B and C infection may cause polyarteritis-like vasculitis [155,156]. Parvovirus B19 has been suggested to be associated with vasculitis-like syndromes including Kawasaki disease, polyarteritis nodosa and Wegener's granulomatosis [157–159].

8. Specific features of the pathogenesis of coagulation disorders in infectious disease

Much of our knowledge on the mechanisms that play a role in infection-associated activation of coagulation comes from observations in clinical and experimental infectious disease. Essentially, and as outlined in detail in the preceding paragraphs, several pathways appear to play a role, including tissue factor-mediated generation of thrombin, impairment of physiological anticoagulant mechanisms, and a depression of the fibrinolytic system, due to elevated levels of PAI-1. In addition, specific features of coagulation activation in patients with infectious disease may be appreciated:

8.1. Endothelial cell activation

Endothelial cells may turn into a procoagulant state either by stimulation of cytokines in concert with circulating blood cells, such as lymphocytes or platelets, or by direct infection (viruses) of endothelial cells. The role of endothelial cells seems to be crucial in the development of shock and activation of coagulation [160,161]. Endothelial cell injury is a common feature of viral infection and can alter hemostasis in a direct or indirect manner. The endothelial cell can be directly infected by different viruses, e.g. HSV, Adenovirus, Parainfluenzavirus, Poliovirus, Echovirus, Measles virus, Mumps virus, Cytomegalovirus, HTLV-1 and HIV [162–165]. The ability to infect endothelial cells has also been demonstrated in hemorrhagic fevers due to Dengue virus, Marburg virus, Ebola virus, Hantaan virus and Lassa virus [166]. Such infections may result in a procoagulant state, mainly by inducing tissue factor expression on the endothelial surface, probably mediated by cytokines such as IL-1, TNFα, and IL-6 [167–170]. However not all viral infections affecting endothelial cells result in activation of coagulation, which may indicate that activation of endothelium is one factor in a multifactorial process.

The change in the endothelial cell from a resting to a procoagulant state may be associated with expression of endothelial surface adhesion molecules [171]. These molecules, i.e. ICAM, VCAM, E Selectin and von Willebrand factor (vWF), play an essential role in the binding of leukocytes, resulting in a local inflammatory response, endothelial cell damage and subsequent plasma leakage and shock [172,173]. The finding of increased plasma concentrations of these endothelial surface adhesion molecules is thought to be a reflection of the level of activation and perhaps damage of the endothelial cell [121].

8.2. Alterations in the protein C and S system

During sepsis, the protein C/S system is downregulated, as described earlier. Some viruses have the ability to induce specific changes in the coagulation inhibitory system. In the course of HIV infection, the protein C/S system may be impaired as a result of an acquired protein S deficiency, the pathogenesis of which is not yet clarified [174,175]. Increased plasma concentrations of the C4b-binding protein, an acute phase protein which binds protein S, may result in decreased levels of free protein S. Antiphospholipid antibodies, which may be present in HIV patients, might interfere with the protein C/protein S complex and diminish its activity [176]. In patients with Dengue hemorrhagic fever, we also found decreased levels of both protein C and protein S activity [177]. As in Dengue, HIV can affect the endothelial cell; hypothetically this could lead to decreased production of protein S.

8.3. Thrombocytopenia

Thrombocytopenia is seen in the course of many viral infections but is only occasionally serious enough to lead to hemostatic impairment and bleeding complications. It is assumed that thrombocytopenia is mainly immune-mediated [178]. Alternative mechanisms are decreased thrombopoiesis, increased platelet consumption, or a combina-

tion of both [179]. Direct interaction of the virus with platelets may lead to thrombocytopenia and/or thrombocytopathy [180,181]. Endothelial injury by the virus may lead to increased adherence and consumption of platelets [182].

Viral infections that have been associated with thrombocytopenia are Mumps, Rubella, Rubeola, Varicella, disseminated Herpes simplex, CMV, infectious mononucleosis, Hantaan virus infection, Dengue hemorrhagic fever, Crimean-congo hemorrhagic fever and Marburg hemorrhagic fever [183–188].

9. Inflammation-induced coagulation as a point of impact for (supportive) treatment of severe infection and sepsis

Based on the assumption that defective physiological anticoagulant mechanisms play a pivotal role in the pathogenesis of coagulation derangement in sepsis, restoration of these pathways may be a logical approach in the (supportive) treatment of patients with severe sepsis. In fact, for all three anticoagulant pathways, such treatment options are available or are at an advanced stage of development.

Restoration of the antithrombin pathway may be achieved by administration of antithrombin concentrates. The use of antithrombin concentrates in patients with DIC has been studied relatively intensively. Most of these trials concern patients with sepsis and/or septic shock. 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. In the more recent clinical trials, very high doses of antithrombin concentrate were used to attain supraphysiological plasma levels and the beneficial results in these trials seem to be more distinct [3,189]. Some trials showed a modest reduction in mortality in antithrombin-treated patients, however, the effect never reached statistical significance. A large-scale multicenter randomized controlled trial to directly address this issue has very recently been completed [190]. Preliminary results seem to indicate that there is no significant reduction in mortality of septic patients who were treated with antithrombin concentrate. A subgroup analysis indicates that patients not receiving concomitant heparin may experience some benefit of antithrombin treatment but this hypothesis remains to be confirmed in a prospective study.

A beneficial effect of recombinant human activated protein C was demonstrated in two randomized controlled trials. Firstly, in a dose-ranging clinical trial, 131 patients with sepsis were enrolled [101]. Included patients received activated protein C by continuous infusion at doses ranging from 12 to 30 μg/kg per h, or placebo. In these patients, a 40% reduction in the relative risk of mortality was shown, although this was not statistically significant (due to the size of the trial). Based on these encouraging results in the phase II trial, a large multicenter efficacy trial was carried out [101]. This trial was prematurely stopped at the second interim analysis because of a significant reduction in mortality in the activated protein C-treated patients. Mortality was 24.7% in the activated protein C group compared with 30.8% in the placebo group (relative risk reduction 19.4%, 95% confidence interval, 6.6–30.5).

The relative insufficiency of endogenous TFPI in sepsis and DIC may be overcome by the administration of pharmacological doses of recombinant TFPI. In a rat model of DIC, the infusion of recombinant tissue factor pathway inhibitor (TFPI) immediately after endotoxin administration significantly inhibited the consumption of coagulation factors and platelets [191]. Furthermore, a reduced number of fibrin thrombi was formed in liver, lungs, kidney and spleen. Similar effects were found in a rabbit model of DIC [64]. In human endotoxemia, recombinant TFPI was shown to dose-dependently reduce thrombin generation [67]. Clinical phase II trials on the use of TFPI in patients with sepsis have yielded promising results but a large multicenter phase III trial turned out to be negative, although published results are not yet available.

10. Conclusion

A bi-directional relationship between coagulation and inflammation appears to play a pivotal role in the mechanisms leading to organ failure in patients with severe infection or sepsis. 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. Pro-inflammatory cytokines are crucial in mediating these effects on endothelial cells, which themselves may also express cytokines, thereby amplifying the coagulative response. The interaction between inflammation and coagulation involves significant cross-talk between the systems. At all levels in the activated coagulation system in sepsis, natural anticoagulant pathways are defective and as a consequence incapable of containing the ongoing thrombin formation. Pharmacological restoration of these anticoagulant mechanisms may be a logical action in the (supportive) treatment of septic patients with coagulation abnormalities. Indeed, experimental and (initial) clinical studies show beneficial results of this type of treatment, not only confined to improvement of the coagulation status but also on clinically relevant outcome parameters such as organ function and survival.

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