The coagulant response in sepsis and inflammation

M. Levi
Department of Medicine and Vascular Medicine, Academic Medical Center, University of Amsterdam, The Netherlands

Keywords
Coagulation, sepsis, inflammation

Summary
Critically ill patients often have systemic activation of both inflammation and coagulation. 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 have major consequences for 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. 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 tissue factor pathway inhibitor. Simultaneously, endothelial-bound anticoagulant mechanisms, in particular the protein C system and the antithrombin system, are shut-off by pro-inflammatory cytokines. Modulation of inflammatory activity by activation of coagulation also occurs by various mechanisms. Activated coagulation proteases, such as the tissue factor-factor VIIa complex, factor Xa and thrombin can bind to protease-activated receptors on various cells and the ensuing intracellular signaling leads to increased production of pro-inflammatory cytokines and chemokines. Physiological anticoagulants can modulate inflammatory activity as well. Increasing knowledge on the various mechanisms underlying activation of inflammation and coagulation may lead to better (adjunctive) management strategies in critically ill patients.

Schlüsselwörter
Koagulation, Sepsis, Entzündung

Zusammenfassung
Bei intensivpflichtigen Patienten kommt es oft zu einer systemischen Aktivierung von Entzündung und Koagulation. Zunehmend finden sich Hinweise auf eine weitreichende Wechselwirkung zwischen beiden Systemen, wonach die Entzündung nicht nur zu einer Aktivierung der Koagulation führt, sondern die Koagulation auch erheblich die Entzündungsaktivität beeinflusst. Die komplexe Beziehung zwischen Entzündung und Koagulation könnte bedeutende Konsequenzen für die Pathogenese von mikrovaskulärer Störung und anschließendem Multiorganversagen haben, die als Folge einer schweren Infektion und der damit verbundenen systemischen Entzündungsantwort auftreten. Molekulare Signalwege, die zu einer entzündungsbedingten Aktivierung der Koagulation beitragen, wurden bereits exakt identifiziert. Die Aktivierung des Gerinnungssystems und die daraus folgende Bildung von Thrombin hängt von einer durch Interleukin-6 induzierten Expression des Gewebefaktors auf aktivierten mononukleären und endothelialen Zellen ab. Der Gewebefaktorinhibitor wirkt diesem Mechanismus nur unzureichend entgegen. Gleichzeitig werden endothelial gebundene Antikoagulationsmechanismen, insbesondere das Protein-C-System und das Antithrombin-System, durch proinflammatorische Zytokine abgeschaltet. Für die Regulierung der Entzündungsaktivität durch Aktivierung der Koagulation sind ebenfalls verschiedene Mechanismen verantwortlich. Aktivierte Gerinnungsproteasen wie der Gewebefaktor-Faktor-VIIa-Komplex, Faktor Xa und Thrombin können an proteaseaktivierte Rezeptoren auf verschiedenen Zellen binden und die anschließende intrazelluläre Signalübertragung führt zu einer verstärkten Bildung proinflammatorischer Zytokine und Chemokine. Physiologische Antikoagulanzien können die Entzündungsaktivität ebenfalls regulieren. Ein besseres Verständnis für die verschiedenen Mechanismen, die der Aktivierung von Entzündung und Koagulation zugrunde liegen, könnte zur Entwicklung besserer (zusätzlicher) Behandlungsstrategien für intensivpflichtige Patienten führen.

Correspondence to:
Marcel Levi, MD
Dept. of Medicine (F-4)
Academic Medical Center, University of Amsterdam
Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands
Tel. +31/20/566 21 71, Fax +31/20/691 96 58
E-mail: m.m.levi@amc.uva.nl

Virtually all situations that lead to a systemic inflammatory response are associated with some degree of activation of coagulation (1). This may range from subtle activation of coagulation that can only be detected by sensitive markers for coagulation factor activation to somewhat more robust coagulation activation that may be evident by a small decrease in platelet count and subclinical prolongation of global clotting times to (in its most extreme form) fulminant disseminated intravascular coagulation (DIC), characterized by simultaneous widespread microvascular thrombosis and profuse bleeding from various sites (2). Patients with severe forms of DIC may present with manifest thrombo-embolic disease or clinically less apparent microvascular fibrin deposition that may contribute to the development of multiple organ dysfunction (3). There is abundant evidence that the activation of coagulation is mediated by inflammatory activity. Activation of coagulation and deposition of fibrin as a

consequence of inflammation can be considered instrumental in containing inflammatory activity to the site of injury or infection, rendering this relationship physiologically efficient.

However, inflammation-induced coagulation may also importantly contribute to disease, as illustrated by the coagulopathy that is associated with severe infection, such as sepsis, and also by the fact that thrombus formation on a ruptured atherosclerotic plaque, containing abundant inflammatory cells, is the pathological substrate of acute arterial thrombotic events (4, 5). The main mediators of inflammation-induced activation of coagulation are pro-inflammatory cytokines. Several studies have shown, for example, the importance of interleukin (IL)-6 in the initiation of coagulation activation, and the role of tumor necrosis factor-α (TNF-α) and IL-1 in the regulation of physiological anticoagulation (6–8). However, there is increasing evidence that extensive cross-talk between the systems of inflammation and coagulation exists, whereby inflammation not only leads to activation of coagulation, but coagulation also markedly affects inflammatory activity. This coagulation-driven modulation of inflammatory activity is driven by specific cell receptors on inflammatory cells and endothelial cells. In addition, systemic activation of coagulation and inflammation in critically ill patients can have some tissue- or organ-specific consequences pertinent to the development of multi-organ failure in the setting of severe sepsis (9).

Inflammation-induced activation of coagulation

The main mechanisms of the coagulation derangement during systemic inflammatory activity are tissue factor-mediated thrombin generation and an imbalance or dysfunction of the normal physiologic anticoagulant mechanisms (▶Fig.), such as the antithrombin system and the protein C system (10, 11). In addition to enhanced fibrin formation, fibrin removal is impaired due to depression of the fibrinolytic system.
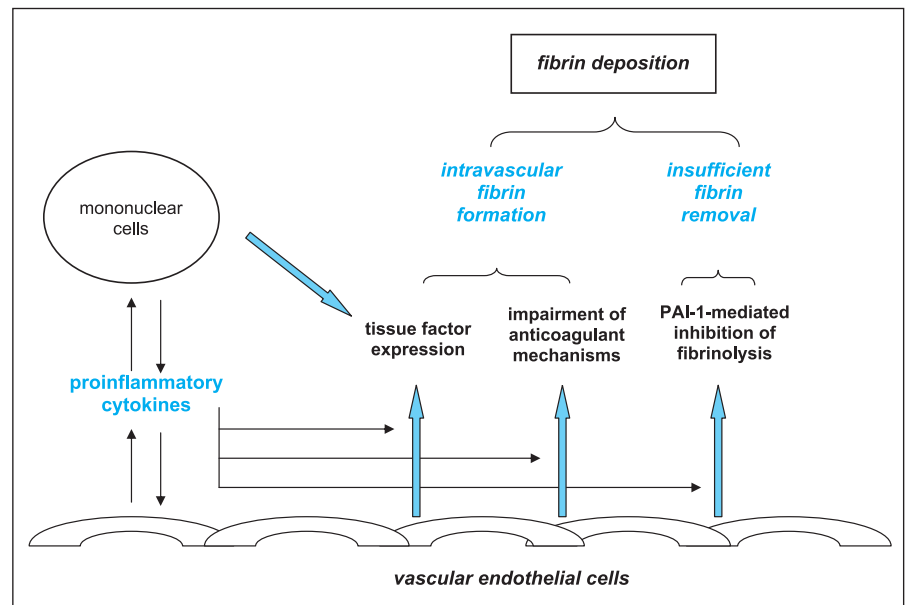

Fig. Pathogenetic pathways in inflammation-induced activation of coagulation: During systemic inflammatory response syndromes, both perturbed endothelial cells and activated mononuclear cells may produce pro-inflammatory cytokines that mediate coagulation activation. Activation of coagulation is initiated 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.

Tissue factor plays a central role in the initiation of inflammation-induced coagulation (12). Blocking tissue factor activity completely inhibits inflammation-induced thrombin generation in models of experimental endotoxaemia or bacteraemia (13, 14). 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 endotoxaemia, whereas specific inhibition of other pro-inflammatory cytokines had less or no effect (6, 15). Inflammatory cells in atherosclerotic plaques produce abundant tissue factor and upon plaque rupture there is extensive tissue factor exposure to blood (5). In severe sepsis, mononuclear cells, stimulated by pro-inflammatory cytokines, express tissue factor, which leads to systemic activation of coagulation (16). Even in experimental low-dose endotoxaemia in healthy subjects, a 125-fold increase in tissue factor mRNA levels in blood monocytes can be detected (17). 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 (18). It is, however, unlikely that these cells actually synthesize tissue factor in substantial quantities (16, 19).

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 pro-thrombinase 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 es-

sential 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. This effect may be the result of nuclear factor kappa B (NF-kB) activation induced by binding of activated platelets to neutrophils and mononuclear cells (20). This cellular interaction also markedly enhances the production of IL-1b, Il-8, MCP-1, and TNF-α (21). The expression of P-selectin on the activated platelet membrane will mediate the adherence of platelets to endothelial cells and leukocytes.

Two-way relationship between inflammation and coagulation

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. In particular, protease interactions that affect inflammatory processes may be important in critically ill patients. Coagulation of whole blood in vitro results in a detectable expression of IL-1 mRNA in blood cells (22), and thrombin markedly enhances endotoxin-induced IL-1 activity in culture supernatants of guinea pig macrophages (23). Similarly, clotting blood produces IL-8 in vitro (24). Factor Xa, thrombin and fibrin can also activate endothelial cells, eliciting the synthesis of IL-6 and/or IL-8 (25,26). Thrombin, factor Xa, and fibrin can directly stimulate mononuclear cells and endothelial cells, inducing

the synthesis of IL-6 or IL-8 (26). Furthermore, thrombin increases mRNA levels of IL-8, monocyte chemoattractant protein (MCP)-1, and E-selectin in cultured endothelial cells, and potentiates TNF-α-induced E-selectin expression. In vivo evidence for a role of coagulation-protease stimulation of inflammation comes from experiments showing that the administration of recombinant factor VIIa to healthy human subjects causes a small but significant three- to four-fold rise in plasma levels of IL-6 and IL-8 (27).

The pivotal mechanism by which coagulation proteases modulate inflammation is by binding to protease activated receptors or PAR's. Four types (PAR 1–4) have been identified, all belonging to the family of transmembrane domain, G-protein-coupled receptors (28). 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-aminoterminus, which activates the same receptor (and possibly adjacent receptors), initiating transmembrane signaling. PAR's are localized in the vasculature on endothelial cells, mononuclear cells, platelets, fibroblasts, and smooth muscle cells (28). PAR's 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 up-regulation 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 PAR's has been confirmed in various experimental studies using PAR inhibitors or PAR-deficient mice (29–31).

Anticoagulant pathways

Procoagulant activity is regulated by three important anticoagulant pathways:

- protein C system,
- antithrombin (AT) and
- tissue factor pathway inhibitor (TFPI).

During inflammation-induced activation of coagulation, the function of all three pathways can be impaired (32).

Activated protein C (APC) appears to play a central role in the pathogenesis of sepsis and associated organ dysfunction. There is ample evidence that an insufficient functioning of the protein C pathway contributes to the derangement of coagulation in sepsis (33, 34). The circulating zymogen protein C is activated by the endothelial cell-bound thrombomodulin once this is activated by thrombin (11). APC acts in concert with its co-factor protein S to proteolytically degrade the essential coagulation co-factors Va and VIIIa; and in that manner functions as an effective anticoagulant. The endothelial protein C receptor (EPCR) not only accelerates the activation of protein C several-fold, but also serves as a receptor for APC, and binding of APC to this receptor may amplify its anticoagulant and anti-inflammatory effects (35). A recent study has demonstrated that exposure of cultured endothelial cells to APC results in the release of microparticles that contain EPCR (36), but the relevance of that observation for coagulation or inflammation is not yet clear.

In patients with severe inflammation, the protein C system is malfunctioning at virtually all levels. First, plasma levels of the zymogen protein C are low or very low, due to impaired synthesis, consumption, and degradation by proteolytic enzymes, such as neutrophil elastase (37, 38). 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 (39, 40). Low levels of free protein S may further compromise an adequate function of the protein C system. Finally, but importantly, in sepsis the EPCR has shown to be downregulated, which may further negatively affect the function of the protein C system (41). Apart from these effects, sepsis may cause a resistance toward APC by other mechanisms, which are partly dependent on a sharp increase in factor VIII levels (released

from endothelial cells), but partly occur by yet unidentified mechanisms (42).

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 (43). Heparin induces conformational changes in AT that result in at least a 1000-fold enhancement of AT activity. Thus, the clinical efficacy of heparin is attributed to its interaction with AT. Endogenous glycosaminoglycans, such as heparan sulfates, on the vessel wall also promote AT-mediated inhibition of thrombin and other coagulation enzymes. During severe inflammatory responses, AT levels are markedly decreased 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 (44). 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 (45).

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 (46, 47).

All anticoagulant pathways are involved in modulation of inflammation as well. 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* (48). Similar experiments in rodents showed identical results and demonstrated a beneficial effect on inflammatory effects in various tissues (49). 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 (50), 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 (51). 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, endotoxaemia was associated with a more marked increase in pro-inflammatory cytokines and other inflammatory responses as compared with wild-type mice (52). It is likely that the effects of APC on inflammation are mediated by the EPCR (53). 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 (51, 54). The EPCR-APC complex itself can translocate from the plasma membrane into the cell nucleus, which may be another mechanism of modulation of gene expression, although the relative contribution of this nuclear translocation and cell surface signaling is unclear at present (33). Some studies also suggest that EPCR binding of APC can result in activation of PAR-1 and thereby affect cytokine responses (55). However, other experiments demonstrated that a significant physiological role for PAR-1 activation by APC was less likely (56). Apart from its effect on cytokine levels, APC has been shown to inhibit leucocyte chemotaxis and adhesion of leukocytes to activated endothelium (57, 58). Finally, APC is capable of inhibiting endothelial cell apoptosis, which also seems to be mediated by an EPCR-PAR-1-dependent mechanism (55, 59).

Antithrombin possesses antiinflammatory properties, many of which are mediated by its actions in the coagulation cascade (60). Most importantly, thrombin inhibition by AT blunts activation of many

inflammatory mediators. However, increasing evidence suggests that AT possesses potent anti-inflammatory properties independent of its anticoagulation activity (61). 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 (62). Prostacyclin inhibits platelet activation and aggregation, blocks neutrophil tethering to blood vessels, and decreases endothelial cell production of various cytokines and chemokines (63). Additional antiinflammatory 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. 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.

From bench to bedside

While the most appropriate management strategy in patients with systemic inflammatory syndromes is aimed at the underlying cause, in many cases additional supportive treatment is required. Concerning the severity of the activation of coagulation simple scoring systems have been developed and validated, which allow to make a diagnosis of DIC, that does not only has prognostic significance, but may also guide patient selection for complex treatment interventions (64, 65). The increase in the insight into the various mechanisms that play a role in the crosstalk between inflammation and coagulation abnormalities associated with systemic inflammation has indeed been accommodating in the development of supportive management strategies.

Based on the notion that depression of the protein C system may significantly contribute to the pathophysiology of DIC, supplementation of (activated) protein C might be beneficial (34). A positive effect of rhAPC was demonstrated in a phase III trial (PROWESS) in patients with sepsis, that was prematurely stopped because of efficacy in reducing mortality in these patients (66). All-cause mortality at 28 days after inclusion was 24.7% in the rhAPC group versus 30.8% in the control group (19.4% relative risk reduction). The administration of rhAPC was demonstrated to cause an amelioration of coagulation abnormalities and rhAPC-treated patients had less organ failure (67). In view of the above described effects that APC has on inflammation, part of the success may have been caused by a beneficial effect on inflammatory pathways. Interestingly, a post-hoc analysis of this trial demonstrated that patients with a diagnosis of DIC, according to the DIC scoring system of the ISTH, had a relatively greater benefit of rhAPC treatment than patients that did not have overt DIC (68). RhAPC was shown not to be effective in patients with sepsis with less severe sepsis.(69) Based on the results of PROWESS, rhAPC has been approved for therapy by regulatory agencies in the US, Europe, and other parts of the world. Indeed, its use in patients with severe sepsis and multiple organ failure, and in the absence of major risk factors for bleeding, has been advocated in guidelines for the treatment of sepsis (70). However, there is currently debate on the role of rhAPC in the treatment of sepsis (71). For example, a meta-analyses of published literature concluded that the basis for treatment with rhAPC, even in patients with a high disease severity, is weak if not insufficient (72, 73). A series of negative trials in specific populations of patients with severe sepsis has added to the skepticism regarding the use of rhAPC.

Taken together, there is quite some uncertainty and doubt surrounding the exact place of rhAPC in patients with severe sepsis and this equipoise has convinced the manufacturer of this agent to start a new placebo-controlled trial in patients with severe sepsis and septic shock in the coming months (74).

Since antithrombin is one of the most important physiological inhibitors of coagulation, and owing to successful preclinical results, a number of larger clinical trials have addressed the use of antithrombin III concentrates in patients with sepsis and/or DIC. Most of the randomized controlled trials concern patients with sepsis, septic shock, or both. All trials show some beneficial effect in terms of improvement of laboratory parameters, shortening of the duration of DIC, or even improvement in organ function (75). However, a large-scale, multicenter, randomized controlled trial to directly address this issue showed no significant reduction in mortality of patients with sepsis who were treated with antithrombin concentrate (76). Interestingly, post-hoc subgroup analyses indicated some benefit in patients who did not receive concomitant heparin and who fulfilled the diagnostic criteria for DIC, but this observation needs prospective validation (77).

Conclusion

Systemic inflammation will invariably lead to activation of the coagulation system but, vice versa components of the coagulation system may markedly modulate the inflammatory response. Increasing evidence points to extensive cross-talk between the two systems at various points, with pivotal roles of tissue factor, thrombin, components of the protein C pathway and antithrombin. Increased insight into the molecular mechanisms that play a role in the close relationship between inflammation and coagulation may lead to the identification of new targets for therapies that can modify excessive activation or dysregulation of these systems.

References

1. Levi M, Opal SM. Coagulation abnormalities in critically ill patients. Crit Care 2006; 10: 222.
2. Levi M. Disseminated intravascular coagulation. Crit Care Med 2007; 35: 2191–2195.
3. Levi M, ten Cate H. Disseminated intravascular coagulation. N Engl J Med 1999; 341: 586–592.
4. Opal SM, Esmon CT. Bench-to-bedside review: functional relationships between coagulation and the innate immune response and their respective roles in the pathogenesis of sepsis. Crit Care 2003; 7: 23–38.
5. Libby P, Aikawa M. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med 2002; 8: 1257–1262.
6. Van der Poll T, Levi M, Hack CE et al. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. J Exp Med 1994; 179: 1253–1259.
7. Van Deventer SJ, Buller HR, ten Cate JW, Aarden LA, Hack CE, Sturk A. Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. Blood 1990; 76: 2520–2526.
8. Boermeester MA, van Leeuwen P, Coyle SM et al. Interleukin-1 blockade attenuates mediator release and dysregulation of the hemostatic mechanism during human sepsis. Arch Surg 1995; 130: 739–748.
9. Aird WC. Vascular bed-specific hemostasis: role of endothelium in sepsis pathogenesis. Crit Care Med 2001; 29: S28-S34.
10. Levi M, Keller TT, van Gorp E, ten Cate H. Infection and inflammation and the coagulation system. Cardiovasc Res 2003; 60: 26–39.
11. Esmon CT. The regulation of natural anticoagulant pathways. Science 1987; 235: 1348–1352.
12. Levi M, van der Poll T, ten Cate H. Tissue factor in infection and severe inflammation. Semin Thromb Hemost 2006; 32: 33–39.
13. Taylor FBJ, Chang A, Ruf W et al. Lethal E. coli septic shock is prevented by blocking tissue factor with monoclonal antibody. Circ Shock 1991; 33: 127–134.
14. Levi M, ten Cate H, Bauer KA et al. Inhibition of endotoxin-induced activation of coagulation and fibrinolysis by pentoxifylline or by a monoclonal anti-tissue factor antibody in chimpanzees. J Clin Invest 1994; 93: 114–120.
15. Levi M, van der Poll T, ten Cate H, van Deventer SJ. The cytokine-mediated imbalance between coagulant and anticoagulant mechanisms in sepsis and endotoxaemia. Eur J Clin Invest 1997; 27: 3–9.
16. Osterud B, Rao LV, Olsen JO. Induction of tissue factor expression in whole blood – lack of evidence for the presence of tissue factor expression on granulocytes. Thromb Haemost 2000; 83: 861–867.
17. Franco RF, de Jonge E, Dekkers PE et al. The in vivo kinetics of tissue factor messenger RNA expression during human endotoxemia: relationship with activation of coagulation. Blood 2000; 96: 554–559.
18. Rauch U, Bonderman D, Bohrmann B et al. Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. Blood 2000; 96: 170–175.
19. Osterud B, Bjorklid E. Sources of tissue factor. Semin Thromb Hemost 2006; 32: 11–23.
20. Furie B, Furie BC. Role of platelet P-selectin and microparticle PSGL-1 in thrombus formation. Trends Mol Med 2004; 10: 171–178.
21. Neumann FJ, Marx N, Gawaz M et al. Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. Circulation 1997; 95: 2387–2394.
22. Mileno MD, Margolis NH, Clark BD et al Coagulation of whole blood stimulates interleukin-1 beta gene expression. J Infect Dis 1995; 172: 308–311.
23. Jones A, Geczy CL. Thrombin and factor Xa enhance the production of interleukin-1. Immunology 1990; 71: 236–241.

24. Johnson K, Choi Y, DeGroot E et al. Potential mechanisms for a proinflammatory vascular cytokine response to coagulation activation. J Immunol 1998; 160: 5130–5135.

25. Sower LE, Froelich CJ, Carney DH et al. Thrombin induces IL-6 production in fibroblasts and epithelial cells. Evidence for the involvement of the seven-transmembrane domain (STD) receptor for alpha-thrombin. J Immunol 1995; 155: 895–901.

26. Van der Poll T, de Jonge E, Levi M. Regulatory role of cytokines in disseminated intravascular coagulation. Semin Thromb Hemost 2001; 27: 639–651.

27. De Jonge E, Friederich PW, Levi M, van der Poll T. Activation of coagulation by administration of recombinant factor VIIa elcicits interleukin-6 and interleukin-8 release in healthy humen subjects. Clin Diagn Lab Immunol 2003; 10: 495–497.

28. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000; 407: 258–264.

29. Camerer E, Cornelissen I, Kataoka H et al. Roles of protease-activated receptors in a mouse model of endotoxemia. Blood 2006; 107: 3912–3921.

30. Slofstra SH, Bijlsma MF, Groot AP et al. Protease-activated receptor-4 inhibition protects from multiorgan failure in a murine model of systemic inflammation. Blood 2007; 110: 3176–3182.

31. Sevastos J, Kennedy SE, Davis DR et al. Tissue factor deficiency and PAR-1 deficiency are protective against renal ischemia reperfusion injury. Blood 2007; 109: 577–583.

32. Levi M, van der Poll T. The role of natural anti-coagulants in the pathogenesis and management of systemic activation of coagulation and inflammation in critically ill patients. Semin Thromb Hemost 2008; 34: 459–468.

33. Esmon CT. Role of coagulation inhibitors in inflammation. Thromb Haemost 2001; 86: 51–56.

34. Levi M, de Jonge E, van der Poll T. Rationale for restoration of physiological anticoagulant pathways in patients with sepsis and disseminated intravascular coagulation. Crit Care Med 2001; 29 (7 Suppl): S90-S94.

35. Esmon CT. The endothelial cell protein C receptor. Thromb Haemost 2000; 83: 639–643.

36. Perez-Casal M, Downey C, Fukudome K et al. Activated protein C induces the release of microparticle-associated endothelial protein C receptor. Blood 2005; 105: 1515–1522.

37. Mesters RM, Helterbrand J, Utterback BG et al. Prognostic value of protein C concentrations in neutropenic patients at high risk of severe septic complications. Crit Care Med 2000; 28: 2209–2216.

38. Vary TC, Kimball SR. Regulation of hepatic protein synthesis in chronic inflammation and sepsis. Am J Physiol 1992; 262: C445-C452.

39. Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 1986; 163: 740–745.

40. Faust SN, Levin M, Harrison OB et al. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. N Engl J Med 2001; 345: 408–416.

41. Taylor FBJ, Stearns-Kurosawa DJ, Kurosawa S et al. The endothelial cell protein C receptor aids in host defense against Escherichia coli sepsis. Blood 2000; 95: 1680–1686.

42. De Pont AC, Bakhtiari K, Hutten BA et al. Endotoxaemia induces resistance to activated protein C in healthy humans. Br J Haematol 2006; 134: 213–219.

43. Levi M. Antithrombin in sepsis revisited. Crit Care 2005; 9: 624–625.

44. Levi M, van der Poll T, Buller HR. Bidirectional relation between inflammation and coagulation. Circulation 2004; 109: 2698–2704.

45. Kobayashi M, Shimada K, Ozawa T. Human recombinant interleukin-1 beta- and tumor necrosis factor alpha-mediated suppression of heparin-like compounds on cultured porcine aortic endothelial cells. J Cell Physiol 1990; 144: 383–390.

46. De Jonge E, Dekkers PE, Creasey AA et al. Tissue factor pathway inhibitor (TFPI) dose-dependently inhibits coagulation activation without influencing the fibrinolytic and cytokine response during human endotoxemia. Blood 2000; 95: 1124–1129.

47. Creasey AA, Chang AC, Feigen L et al. Tissue factor pathway inhibitor reduces mortality from Escherichia coli septic shock. J Clin Invest 1993; 91: 2850–2856.

48. Taylor FBJ, Chang A, Esmon CT et al. Protein C prevents the coagulopathic and lethal effects of Escherichia coli infusion in the baboon. J Clin Invest 1987; 79: 918–925.

49. Murakami K, Okajima K, Uchiba M et al. Activated protein C attenuates endotoxin-induced pulmonary vascular injury by inhibiting activated leukocytes in rats. Blood 1996; 87: 642–647.

50. Hancock WW, Tsuchida A, Hau H et al. The anticoagulants protein C and protein S display potent antiinflammatory and immunosuppressive effects relevant to transplant biology and therapy. Transplantation Proceedings 1992; 24: 2302–2303.

51. White B, Schmidt M, Murphy C et al. Activated protein C inhibits lipopolysaccharide-induced nuclear translocation of nuclear factor kappaB (NF-kappaB) and tumour necrosis factor alpha (TNF-alpha) production in the THP-1 monocytic cell line. Br J Haematol 2000; 110: 130–134.

52. Levi M, Dorffler-Melly J, Reitsma PH et al. Aggravation of endotoxin-induced disseminated intravascular coagulation and cytokine activation in heterozygous protein C deficient mice. Blood 2003; 101: 4823–4827.

53. Esmon CT. New mechanisms for vascular control of inflammation mediated by natural anticoagulant proteins. J Exp Med 2002; 196: 561–564.

54. Hancock WW, Grey ST, Hau L et al. Binding of activated protein C to a specific receptor on human mononuclear phagocytes inhibits intracellular calcium signaling and monocyte-dependent proliferative responses. Transplantation 1995; 60: 1525–1532.

55. Riewald M, Petrovan RJ, Donner A et al. Activation of endothelial cell protease activated receptor 1 by the protein C pathway. Science 2002; 296: 1880–1882.

56. Ludeman MJ, Kataoka H, Srinivasan Y et al. PAR1 cleavage and signaling in response to activated protein C and thrombin. J Biol Chem 2005; 280: 13122–13128.

57. Feistritzer C, Sturn DH, Kaneider NC et al. Endothelial protein C receptor-dependent inhibition of human eosinophil chemotaxis by protein C. J Allergy Clin Immunol 2003; 112: 375–381.

58. Sturn DH, Kaneider NC, Feistritzer C et al. Expression and function of the endothelial protein C receptor in human neutrophils. Blood 2003; 102: 1499–1505.

59. Cheng T, Liu D, Griffin JH et al. Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. Nat Med 2003; 9: 338–342.

60. Roemisch J, Gray E, Hoffmann JN, Wiedermann CJ. Antithrombin: a new look at the actions of a serine protease inhibitor. Blood Coagul Fibrinolysis 2002; 13: 657–670.

61. Opal SM. Interactions between coagulation and inflammation. Scand J Infect Dis 2003; 35: 545–554.

62. Mizutani A, Okajima K, Uchiba M et al. Antithrombin reduces ischemia/reperfusion-induced renal injury in rats by inhibiting leukocyte activation through promotion of prostacyclin production. Blood 2003; 101: 3029–3036.

63. Uchiba M, Okajima K, Murakami K. Effects of various doses of antithrombin III on endotoxin-induced endothelial cell injury and coagulation abnormalities in rats. Thromb Res 1998; 89: 233–241.

64. Levi M. The diagnosis of disseminated intravascular coagulation made easy. Neth J Med 2007; 65: 366–367.

65. Bakhtiari K, Meijers JC, de Jonge E, Levi M. Prospective validation of the international society of thrombosis and maemostasis scoring system for disseminated intravascular coagulation. Crit Care Med 2004; 32: 2416–2421.

66. Bernard GR, Vincent JL, Laterre PF et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001; 344: 699–709.

67. Vincent JL, Angus DC, Artigas A et al. Effects of drotrecogin alfa (activated) on organ dysfunction in the PROWESS trial. Crit Care Med 2003; 31: 834–840.

68. Dhainaut JF, Yan SB, Joyce DE et al. Treatment effects of drotrecogin alfa (activated) in patients with severe sepsis with or without overt disseminated intravascular coagulation. J Thromb Haemost 2004; 2: 1924–1933.

69. Abraham E, Laterre PF, Garg R et al. Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. N Engl J Med 2005; 353: 1332–1341.

70. Dellinger RP, Levy MM, Carlet JM et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008; 36: 296–327.

71. Levi M. Activated protein C in sepsis: a critical review. Curr Opin Hematol 2008; 15: 481–486.

72. Wiedermann CJ, Kaneider NC. A meta-analysis of controlled trials of recombinant human activated protein C therapy in patients with sepsis. BMC Emerg Med 2005; 5: 7–17.

73. Marti-Carvajal A, Salanti G, Cardona AF. Human recombinant activated protein C for severe sepsis. Cochrane Database Syst Rev 2007; CD004388.

74. Barie PS. „All in" for a huge pot: the PROWESS-SHOCK trial for refractory septic shock. Surg Infect (Larchmt) 2007; 8: 491–494.

75. Levi M, ten Cate H, van der Poll T. Disseminated intravascular coagulation: State of the art. Thromb Haemost 1999; 82: 695–705.

76. Warren BL, Eid A, Singer P et al. Caring for the critically ill patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial. JAMA 2001; 286: 1869–1878.

77. Kienast J, Juers M, Wiedermann CJ et al. Treatment effects of high-dose antithrombin without concomitant heparin in patients with severe sepsis with or without disseminated intravascular coagulation. J Thromb Haemost 2006; 4: 90–97.