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Disseminated Intravascular Coagulation in Sepsis*

Sacha Zeerleder, MD; C. Erik Hack, MD, PhD†; and Walter A. Wuillemin, MD; PhD

Disseminated intravascular coagulation is a frequent complication of sepsis. Coagulation activation, inhibition of fibrinolysis, and consumption of coagulation inhibitors lead to a procoagulant state resulting in inadequate fibrin removal and fibrin deposition in the microvasculature. As a consequence, microvascular thrombosis contributes to promotion of organ dysfunction. Recently, three randomized, double-blind, placebo-controlled trials investigated the efficacy of antithrombin, activated protein C (APC), and tissue factor pathway inhibitor, respectively, in sepsis patients. A significant reduction in mortality was demonstrated in the APC trial. In this article, we first discuss the physiology of coagulation and fibrinolysis activation. Then, the pathophysiology of coagulation activation, consumption of coagulation inhibitors, and the inhibition of fibrinolysis leading to a procoagulant state are described in more detail. Moreover, therapeutic concepts as well as the three randomized, double-blind, placebo-controlled studies are discussed.

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Key words: activated protein C; antithrombin; disseminated intravascular coagulation; multiple organ dysfunction syndrome; sepsis; tissue factor pathway inhibitor

Abbreviations: APC = activated protein C; AT = antithrombin; C1-Inh = C1 inhibitor; C4bBP = C4b-binding protein; DIC = disseminated intravascular coagulation; EPCR = endothelial protein C receptor; FM = fibrin monomers; IL = interleukin; INR = international normalized ratio; KyberSept = Study on High-Dose Antithrombin III in Severe Sepsis; MODS = multiple organ dysfunction syndrome; PAI-1 = plasminogen activator inhibitor type-1; PC = protein C; Plg = plasminogen; PROWESS = Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis; PS = protein S; rAPC = recombinant activated protein C; TAT = thrombin-antithrombin; TF = tissue factor; TFPI = tissue factor pathway inhibitor; TM = thrombomodulin; TNF = tumor necrosis factor; t-PA = tissue-type plasminogen activator

Sepsis is the leading cause of mortality in noncardiologic ICUs1 and is generally considered to result from excessive activation of the host’s inflammatory defense mechanisms. These mechanisms include the release of cytokines and the activation of plasma protein cascade systems such as the complement, contact-phase, coagulation, and fibrinolytic systems. The development of multiple organ dysfunction syndrome (MODS) is a frequent complication of sepsis and is associated with poor outcome. Although the pathogenesis of MODS is not well understood, coagulation activation is suggested to be involved.2-4

Disseminated intravascular coagulation (DIC) frequently complicates sepsis.5-7 Since the definition of DIC is difficult and different scoring systems have been used, a scoring system for overt DIC was proposed by the International Society of Thrombosis and Hemostasis.8 Using these criteria, overt DIC can be found in 25 to 50% in patients with sepsis and seems to be a strong predictor of mortality.9,10 DIC is an acquired syndrome characterized by the activation of intravascular coagulation culminating in intravascular fibrin formation and deposition in the microvasculature. Secondary fibrinolysis, or in later stages inhibition of fibrinolysis, accompanies coagulation activation.11,12 Although the initial trigger and the dynamics may vary, the clinical picture of severe sepsis or septic shock in latter stages is quite uniform. Fibrin deposition leads to a diffuse obstruction of the microvascular bed resulting in progressive organ dysfunction, such as the development of renal insufficiency and ARDS, hypotension, and circula-
tory failure. In some cases, diffuse skin necrosis up to limb gangrene may develop. Due to consumption of clotting factors and the interference of fibrin degradation products, diffuse bleeding may occur.

Since DIC is involved in the pathogenesis of sepsis and the development of MODS, inhibition of coagulation activation seems a valuable therapeutic option. At least three large multicenter trials13–15 that investigated the efficacy of coagulation inhibitors have been published. In one trial,13 administration of activated protein C (APC) in sepsis patients significantly decreased mortality, and outcome was significantly improved.

In this article, the pathophysiology of coagulation activation and the role of fibrinolysis will be discussed. Moreover, pathophysiologic concepts underlying the new treatment options in DIC are explained.

**Pathogenesis of DIC in Sepsis**

Systemic inflammation during sepsis leads to the generation of proinflammatory cytokines that, among other things, orchestrate coagulation and fibrinolytic activation. Both coagulation activation as well as down-regulation of fibrinolysis are principally regulated by tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6.16,17 Moreover, TNF-α influences coagulation activation via IL-6.18

The hallmark of the coagulation disorder in sepsis constitutes the imbalance between intravascular fibrin formation and its removal (Fig 1). Severely reduced anticoagulant capacity and inhibited fibrinolysis are opposed to a massive activation of coagulation, finally leading to overwhelming fibrin formation and consumption of clotting factors and inhibitors as well. Abundant intravascular fibrin formation leads to microvascular thrombosis, which causes widespread ischemic organ damage up to organ necrosis and clinically impresses as widespread skin necrosis and MODS.12

**Coagulation Activation in Sepsis**

Coagulation activation during sepsis is primarily driven by the tissue factor (TF) pathway. In sepsis models in animals, fibrin formation indeed was completely abrogated by blocking TF by antibodies or Factor VIIa by peptides or active site-inhibited Factor VIIa.19,20 Although in a primate model for severe sepsis the contact-phase system was found to be activated, it did not contribute to coagulation activation in sepsis. Inhibition of Factor XII activation in this model, however, prevented hypotension, indicating that activation of the contact-phase system is important, presumably via the formation of bradykinin, for the hemodynamic changes during sepsis.21 Expression of TF on monocytes and probably on endothelial cells triggers activation of coagulation in sepsis.22,23 An additional source of TF might be phospholipid particles originating from activated monocytes (Fig 2), which can be detected, eg, in plasma of patients with meningococcal sepsis.24,25 After binding to exposed TF, circulating Factor VII is activated. The TF/Factor VIIa complex then activates Factor X to Factor Xa, by which prothrombin is converted to thrombin. These tiny amounts of thrombin formed may activate Factor V and Factor VIII. Factor Va greatly enhances the capability of

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**Figure 1.** Coagulation imbalance during sepsis. The imbalance between coagulation activation and fibrinolysis and the decrease of anticoagulant mechanisms is schematically shown (adapted from Levi et al16).

**Figure 2.** Coagulation activation through TF pathway. Dotted lines indicate cells or microparticles expressing TF. Endothelial cells, monocytes, and their released microparticles may express TF, which finally results in thrombin generation. F = clotting factor.
Factor Xa to activate prothrombin. However, thrombin generation by the TF/Factor VIIa pathway is rapidly abrogated by TF pathway inhibitor (TFPI), a high-affinity inhibitor of TF/Factor VIIa/Factor Xa complex present in plasma and on endothelial cells. However, TF/Factor VIIa complex also activates Factor IX, which in concert with Factor VIIIa takes over the function of TF/Factor VIIa to activate Factor X, thereby propagating further thrombin generation. This amplification of Factor X activation by Factor IX and Factor VIII is important for coagulation in physiologic conditions, as is dramatically demonstrated by the clinical picture of hemophilia A and B, which results from deficiency of Factor VIII and Factor IX, respectively. Thrombin cleaves fibrinogen into fibrin monomers (FM) and activates Factor XIII, which then covalently crosslinks FM to form a stable clot. The thrombin generated by the TF/Factor VIIa pathway amplified by Factor IX and Factor VIII in some conditions is still insufficient to overcome fibrinolysis. To surmount this anticoagulant effect of fibrinolysis, activation of a second amplification loop, in addition to that of Factor VIII and Factor IX, is necessary. This second loop is triggered when the amount of thrombin generated becomes sufficient to activate Factor XI, which then generates Factor IXa, which then activates additional Factor X, thereby forming additional thrombin. This amplified Factor XI-dependent thrombin generation, among other things, will activate thrombin-activatable fibrinolysis inhibitor, which will cleave off binding sites for plasminogen on fibrin, thereby inhibiting fibrinolysis (Fig 3). Although Factor IXa/VIIIa and the Factor XIa amplification loop are considered to be important for coagulation activation in sepsis, the evidence for this is actually very poor. Although one study indeed indicates activation of Factor XI in sepsis patients, to our knowledge, specific interventions at this level have not been done.

**Anticoagulant Pathways in Sepsis**

Anticoagulant mechanisms deprive the activated coagulation system of thrombin. Thrombin is quickly inactivated by antithrombin (AT) by forming thrombin-antithrombin (TAT) complexes, which are rapidly cleared from circulation. Moreover, thrombomodulin (TM) expressed on endothelial cells binds thrombin and abrogates its procoagulant activity. The thrombin-TM complex activates protein C (PC). APC rapidly dissociates from the TM-thrombin com-

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**Figure 3.** Coagulation, anticoagulant mechanisms, and fibrinolysis in sepsis. Rectangular boxes indicate inactive zymogen. Ellipses indicate active enzymes. Circles indicate nonenzymatic cofactors. Rhombi indicate plasmatic inhibitors. a = activated coagulation factor; PCI = PC inhibitor; AT1 = α1-antitrypsin; C4BP = C4-binding protein; Pl = plasmin, AP = α2-antiplasmin; TAFI = thrombin activatable fibrinolysis inhibitor, Fbg = fibrinogen; X = fibrin degradation products. Arrows indicate “conversion to.” Dotted lines and circles indicate “inhibition.” See Figure 2 legend for expansion of abbreviations.
plex and inactivates Factor Va and Factor VIIIa, thereby decreasing thrombin generation.31 Free protein S (PS) potentiates the inhibitory capacity of APC against Factor Va and Factor VIIIa, respectively. In contrast, PS bound to C4b-binding protein (C4bBP) does not exhibit anticoagulant properties.31 Moreover, APC enhances fibrinolysis by neutralization of plasminogen activator inhibitor type-1 (PAI-1) [Fig 3].32

During sepsis, several anticoagulant mechanisms are severely compromised. Inactivation of AT by elastase released from activated neutrophils and consumption of AT due to the rapid clearance of TAT complexes decrease the availability of functional AT. Moreover, since AT is a negative acute-phase protein, hepatic de novo synthesis is decreased.33–36

The function of the APC system is also severely compromised during sepsis. Reduced TM expression on endothelial cells due to inflammatory mediators, such as TNF-α, has been claimed to explain the decreased APC activity.37,38 Indeed, expression of TM by the endothelium in purpuric lesions of children with meningococcal sepsis is decreased as compared to expression in control subjects.39 Moreover, decreased levels of free PS due to increasing concentrations of C4-binding protein may additionally impair APC function.40 Insufficient modulation of thrombin activity by TM and the resulting decreased inactivation of Factor VIIIa and Factor Va contribute to a severe procoagulant state, which promotes fibrin deposition in the microvasculature. In fact, elevated soluble TM and decreased levels of PC typically occur in meningococcal sepsis,39,41 and the decrease in APC activity correlates with the development of purpura-like skin lesions and poor outcome.41 Thus, together these studies suggest reduced activation of PC in sepsis due to decreased availability of TM and point to the crucial anticoagulant role of the APC system in the microvasculature.31 However, this concept is challenged by the observation that infusion of native, plasma-derived PC in children with severe meningococcal sepsis resulted in the formation of APC in vivo, and in a decrease of d-dimer levels similarly as observed on infusion of recombinant APC in adult patients with sepsis.42,43

Since coagulation activation during sepsis is mainly initiated through the extrinsic pathway, the TFPI has gained some interest.44 In circulation, TFPI originates from at least three pools; the majority (up to 85%) is bound via glycosaminoglycans to endothelial cells in the microvasculature, and a small fraction circulates either associated with lipoproteins or in platelets, respectively.45–48 Although normal or even elevated levels of TFPI can be found in DIC and sepsis, elevated TF levels can be measured in the plasma of these patients as well,49–52 suggesting a relative deficiency of TFPI to neutralize TF in patients with trauma, DIC, and sepsis, which finally results in unopposed thrombin generation.

As described above, on coagulation activation small amounts of thrombin formed may activate Factor XI, which then amplifies the Factor IXa/Factor VIIIa pathway by generating Factor IXa leading to an additional thrombin burst. C1 inhibitor (C1-Inh), a serine protease inhibitor, inhibits the activation of the classical and the mannan binding lectin pathway of the complement system and is a main inhibitor of the contact-phase proteases Factor XIIa and kallikrein. Additionally, C1-Inh also was found to be a main inhibitor of Factor XIa in vitro.53 Also, in vivo C1-Inh seems to be a main inhibitor of Factor XIa since a bolus infusion of Factor XIa resulted in significant formation of Factor XIa-C1-Inh complexes in chimpanzees.54 These data suggest that C1-Inh may have effect on thrombin generation in vivo. Indeed, in patients with hereditary or acquired angioedema, which result from deficiency of C1-Inh, significantly increased levels of Factor VIIa, TAT, and prothrombin fragments (F1 + 2) can be detected during attacks as compared to normal levels in remission.55,56

**Fibrinolysis in Sepsis**

The activation of the fibrinolytic system cumulates in the generation of plasmin, which degrades fibrin. Plasmin is formed by the conversion of plasminogen (Plg) by at least two types of activators: tissue-type plasminogen activator (t-PA) and urokinase-like plasminogen activator. Endothelial cells are the principle source of t-PA.57

In endotoxin models in nonhuman primates and healthy volunteers, coagulation activation was preceded by a rapid activation of fibrinolysis as reflected by an increase of t-PA levels and plasmin-α2-antiplasmoin complexes58–61 and TNF-α seems to be a key mediator in the activation of fibrinolysis.60,62,63 During sepsis, fibrinolysis is attenuated on two levels; first, α2-antiplasmin rapidly inactivates plasmin by forming plasmin-α2-antiplasmin complexes; second, both Plg activators can be inactivated by PAI-1 (Fig 3), which among other is stored in endothelial cells.64 Increasing PAI-1 levels likely shuts down fibrinolysis in sepsis models in animals and in the human endotoxin model.64 PAI-1 forms stable complexes with either t-PA or urokinase-like plasminogen activator, respectively. In vitro and in vivo studies demonstrated endotoxin and TNF-α to release PAI-1, which then abrogates fibrinolysis. Patients with severe sepsis have strongly elevated
PAI-1 levels, which predict unfavorable outcome.\textsuperscript{65–69} This relation between high PAI-1 levels and a poor outcome in sepsis may be causal since a functional polymorphism of the PAI-1 gene predisposing to high PAI-1 levels is also associated with an unfavorable outcome in children with meningococcal sepsis with severe coagulopathy.\textsuperscript{70}

**Anticoagulant and Antiinflammatory Effects: an Important Link**

During sepsis, severely reduced anticoagulant capacity and inhibited fibrinolysis are opposed to massive activation of coagulation leading to an overwhelming fibrin formation and to microvascular thrombosis, which causes widespread ischemic organ damage up to organ necrosis and may clinically impress as MODS and in severe cases with widespread skin necrosis. Therefore, restoration of anticoagulant capacity as well as fibrinolysis might be a promising target for therapy strategies.

Since consumption of the biological inhibitors of thrombin may contribute to the formation of thrombin during sepsis, one could speculate that administration of an inhibitor of thrombin formation or a direct inhibitor of the catalytic site of thrombin might be useful in this clinical condition. However, current insights indicate that this view on the efficacy of anticoagulant proteins in sepsis is too simple. A number of studies\textsuperscript{27,28} have shown that efficacy of clotting inhibitors in sepsis models not only depends on their anticoagulant properties but also on their antiinflammatory effects. For example, administration of a peptide that blocks the active site of Factor Xa, thereby preventing generation of thrombin, to septic baboons efficiently blocked the formation of thrombin and the development of DIC but had no effect on outcome.\textsuperscript{71} In contrast, administration of high doses of AT to septic baboons reduced thrombin formation and improved outcome.\textsuperscript{72}

A somewhat paradoxical finding in dogs challenged with endotoxin is that low-dose thrombin has a beneficial effect on survival probably by promoting the activation of PC.\textsuperscript{73–75} Administration of blocking antibodies against APC or infusion of C4bBP, which binds and neutralizes PS, to baboons challenged with nonlethal doses of *Escherichia coli* induced a lethal septic shock that was prevented by infusion of APC or blocking antibodies against C4bBP.\textsuperscript{40,76,77} Administration of APC in baboons lethally challenged with *E coli* attenuated the coagulatory response and improved survival. Interestingly, APC had antiinflammatory effects in this model, which occurred at lower APC levels than those needed for an anticoagulant effect,\textsuperscript{76,78} suggesting properties other than those on the coagulation system mediate the beneficial effects of APC. \textit{In vitro} experiments\textsuperscript{79,80} suggest recombinant APC (rAPC) to induce up-regulation of antiapoptotic genes in endothelial cells. A specific receptor for APC, the endothelial PC receptor (EPCR), may mediate the antiapoptotic effects and might therefore be important in sepsis, since baboons challenged with sublethal doses of \textit{E. coli} died whereas the control animals with intact EPCR survived.\textsuperscript{81,82} Moreover, APC seems to exert antiinflammatory effects by modulating monocyte activation most probably independent of EPCR.\textsuperscript{83}

Since coagulation activation in sepsis mainly occurs through the extrinsic pathway, inhibition of thrombin generation on the level of TF and/or Factor VIIa is another therapeutic option. Indeed, the coagulant response in primates after challenge with either bacteria or endotoxin was abrogated by treating the animals with antibodies against TF or Factor VIIa with a peptide-blocking Factor VIIa or with TFPI.\textsuperscript{19,20,60,84–86} Importantly, treatment with TFPI or with an antibody against TF prevented death.\textsuperscript{19,84,85} In contrast, blocking Factor Xa, in spite of abrogating thrombin generation, could not reduce mortality in this sepsis model,\textsuperscript{71} again demonstrating that clotting inhibitors should have anticoagulant as well as antiinflammatory properties to improve outcome in lethal sepsis models. To explain the antiinflammatory effects of some clotting inhibitors, TF has been claimed to modulate the inflammatory response in septic animals independently of its clotting function probably by modulating cytokine release.\textsuperscript{20,84,87} The antiagulant effect of TFPI has been confirmed in healthy humans challenged with endotoxin, but in contrast TFPI had no effect on the inflammatory response in this model.\textsuperscript{88,89} The discrepant effects of these findings are still not fully understood.

Another potential target to inhibit coagulation activation in sepsis might be to decrease thrombin amplification through the Factor IXa/Factor VIIIa pathway by activated Factor XIa. Indeed, there is evidence for Factor XI activation in children with severe meningococcal disease. Reduced levels of Factor XII, Factor XI, as well as prekallikrein and elevated complexes of C1-Inh-Factor Xa can be detected in these children.\textsuperscript{20} Since C1-Inh was demonstrated to be a main inhibitor of Factor XIIa, kallikrein, and Factor XIa \textit{in vivo} and \textit{in vitro}, it might be efficient in preventing DIC in sepsis.\textsuperscript{4} In baboons lethally challenged with \textit{E coli}, high-dose C1-Inh substitution significantly decreased complement activation through the classical pathway and significantly attenuated the cytokine response. Moreover, whereas Factor XII and prekallikrein consumption was prevented, no effect on fibrinogen consump-
tion or on TAT levels was observed in the treated animals.90 In fact, blocking Factor XIIa was demonstrated to improve hypotension in septic animals but did not prevent fibrin consumption.21 Interestingly, whereas postmortem analysis in the placebo-treated organs showed widespread microvascular thrombosis, no such changes were found in C1-Inh–treated animals.91 The same findings were reported in septic rabbits treated with C1-Inh.92 This finding is difficult to explain since one would also expect decreased TAT formation or fibrinogen consumption in that situation. Noteworthy, although the fibrinolytic response was equal in both groups, PAI-1 levels were significantly attenuated in the treated group.90

NEW THERAPEUTIC STRATEGIES IN DIC

Based on the pathophysiologic concepts and the striking anticoagulant and antiinflammatory properties of coagulation inhibitors in models for severe sepsis, administration of these inhibitors might be an attractive therapeutic approach for human sepsis. Multicenter placebo controlled trials13–15 on the efficacy of AT, APC, and TFPI to reduce mortality in human sepsis have been completed. Notably, each of these inhibitors reduced mortality in several animal models for severe sepsis. Moreover, a pilot study4,93 on C1-Inh in sepsis demonstrated a beneficial effect on organ dysfunction. The results of these trials are discussed.

Antithrombin in Sepsis

High-dose treatment with purified as well as recombinant AT protected baboons challenged with lethal doses of *E. coli* from death.72,94 Moreover, a significant reduction of the inflammatory response by AT was demonstrated.94 In humans, trials95–99 investigating the effect of AT in sepsis revealed promising results. In a phase II study,95 either placebo or AT (loading dose, 90 to 120 U/kg body weight followed by 90 to 120 U/kg body weight over 4 days) was administered to patients with septic shock and DIC. AT-treated patients showed a benefit in 28-day mortality as well as a reduced duration of DIC. In that study,95 high AT levels were achieved after 3 h on AT substitution. A 14-day high-dose, activity adapted, phase II study96,97 demonstrated AT at continuous AT levels >120% to be beneficial on organ dysfunction, to attenuate DIC as well as the inflammatory response. A nonsignificant reduction of mortality as well as a shortening of the stay on the ICU was achieved in a prospective randomized study on administration of AT (loading dose, 3,000 IU followed by 1,500 IU q12h for 5 days).98 Interestingly, only modest AT plasma concentrations on day 1 and day 6 (approximately 55 to 60% and >70%, respectively) after AT substitution were achieved.98 A trial including 120 critically ill patients did not show a benefit of AT substitution (loading dose, 4,000 IU followed by 2,000 IU q12h) on survival. However, subgroup analysis in septic shock patients demonstrated a significant reduction in mortality when treated with AT.99 In that study,99 AT levels at approximately 100% could be measured immediately and 24 h after AT administration, respectively. Although the three larger studies95,96,99 were comparable with regards to the AT levels on inclusion (40 to 55%), AT levels immediately after substitution differed apparently. Therefore, it can be concluded that high AT levels should be achieved quickly after starting AT therapy.95,98,99 This is in accordance to animal models, in which the benefit of AT substitution was restricted to animals in which AT levels were achieved very early in the septic process.72 Based on these promising results, a large, double-blind, placebo-controlled multicenter trial (High-Dose Antithrombin III in Severe Sepsis [KyberSept] trial)14 investigated the effect of AT in sepsis patients. Either AT or placebo were infused to 2,314 patients (loading dose, 6,000 IU followed by 6,000 IU/d over 4 days). Heparin in prophylactic doses (≤10,000 IU/d) as well as heparin flush for catheter patency were allowed in the study protocol. However, although considerable AT levels (180%) 24 h after AT administration had been achieved, no difference in mortality between the treatment group and the placebo group was found. Moreover, patients treated with AT had significantly more bleeding complications as compared to the placebo group.14 Subgroup analyses revealed some important clues. First, in the group of patients with AT activity levels >60%, a beneficial effect on 90-day mortality was found, which can be explained by the higher AT activity levels achieved by AT administration in these patients, as compared to patients starting with levels <60%. However, one could also argue that since low levels of AT predict a poor outcome, that patients starting with low levels have higher probability for fatal outcome. Second, in the subgroup analysis, patients without heparin in the AT group (n = 698) showed a clear trend toward absolute reduction of mortality with a relative mortality risk reduction of approximately 15% being significant on day 90. The interpretation of these results is difficult. A probable explanation for that finding might be that concomitant use of heparin decreases the ability of AT to bind to glycosaminoglycans on endothelial cells.100,101 That is supported by the findings in animal sepsis that administration of heparin in prophylactic doses might abolish the antiinflammatory effects of AT, such as inhibition of leukocyte-endo-
thelium interaction or improvement of microcirculation.\textsuperscript{102} Thus, heparin may have interfered with targeting of AT to the endothelial cells. Moreover, patients treated with AT receiving no heparin had fewer bleeding complications as compared to patients receiving heparin. These results emphasized that the effect of heparin in sepsis patients is not well and properly studied. Therefore, AT substitution should be recommended in patients with sepsis not receiving heparin should be investigated in future studies.

**APC in Sepsis**

Due to the crucial role of the APC pathway in the pathogenesis of sepsis, APC or PC substitution in sepsis seems to be a promising treatment. In animal models, APC administration turned out to be beneficial.\textsuperscript{76} A few cases and uncontrolled studies\textsuperscript{103–109} reported on PC substitution in patients with meningococcal sepsis, with promising results: PC administration resulted in attenuation of DIC, improvement of MODS, and reduced progression of the skin lesions and incidence of amputations. Interestingly, the mortality of the treated patients was lower than expected.\textsuperscript{110} Another study\textsuperscript{42} reported promising results on PC substitution in sepsis associated with purpura fulminans. A randomized, double-blinded, placebo-controlled study\textsuperscript{43} on PC substitution in meningococcal sepsis showed an increase of plasma APC and a resolution of coagulation abnormalities. Moreover, PC substitution turned out to be safe.\textsuperscript{43}

Treatment of patients with severe sepsis with rAPC has been studied in one phase 2 trial,\textsuperscript{111} a placebo-controlled, dose-finding trial comparing increasing doses of rAPC (12, 18, 24, and 30 μg/kg/h) and different duration of infusion (48 h and 96 h). rAPC and placebo were administered to 91 patients and 41 patients, respectively, and turned out to be safe and to reduce d-dimer and IL-6 levels in a dose-dependent way. According to these results, the continuous infusion of 24 μg/kg/h over 96 h was considered the optimum regimen for a phase 3 trial.\textsuperscript{111} The phase 3 trial,\textsuperscript{111} a randomized, double blind, placebo-controlled multicenter study (Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis [PROWESS]) demonstrated a significant improvement of survival on administration of rAPC (24 μg/kg/h over 96 h) to sepsis patients. Since a rAPC treatment led to significant reduction after the second interim analysis of 1,520 patients, the trial was stopped earlier than pretended. Of the 1,690 patients included, the 850 patients who had received rAPC showed a significant reduction in the 28-day mortality (absolute mortality reduction, –6.1%; relative risk reduction, 19.4%). However, the incidence of serious bleeding events was higher in the rAPC group than in the placebo group (3.5% vs 2.0%, p = 0.06) during the 28 days of the survey. Notably, this difference in the incidence of serious bleeding was observed only during the 4-day infusion period.\textsuperscript{13} Three of the treatment-associated deaths were due to severe thrombocytopenia. Moreover, a strong association between bleeding events and invasive procedures as well as a proportional increase of bleeding events with increasing organ dysfunction were noted. Notably, this was the first study to show a significant reduction of mortality by an intervention in human sepsis patients, although at the expense of a slightly increased risk of bleeding. Moreover, APC led to a significant attenuation of DIC and inflammation in these patients.\textsuperscript{13}

**TFPI in Sepsis**

Since coagulation activation in sepsis proceeds mainly through the TF/Factor VIIa pathway, TFPI substitution in sepsis seems reasonable. Indeed, in sepsis models in baboons, TFPI abrogated the coagulant response, attenuated the inflammatory response, and improved survival, even when administered up to several hours after the challenge.\textsuperscript{84,85} In addition, TFPI decreased mortality in a peritonitis model in rabbits.\textsuperscript{112} Although the anticoagulant response of TFPI was confirmed in humans challenged with endotoxin, no effect on the inflammatory response was found.\textsuperscript{88,89} A randomized, placebo-controlled dose-finding study\textsuperscript{113} investigated the effect of TFPI in 210 patients with severe sepsis. TFPI led to a decrease of the coagulant as well as the inflammatory responses. However, although not the primary end point, a trend toward reduction of the 28-day mortality was found and the treatment turned out to be safe with both doses used (0.025 mg/kg/h and 0.05 mg/kg/h over 96 h).\textsuperscript{113} Based on these results, a large randomized, double-blind, placebo-controlled multicenter trial (the Optimized Phase 3 Tifacogin in Multicenter International Sepsis Trial)\textsuperscript{15} was conducted to determine the efficacy of TFPI in sepsis. A population of 1,754 patients with an international normalized ratio (INR) ≥ 1.2 and 201 patients with an INR < 1.2 had been assigned to either receive TFPI (0.025 mg/kg/h over 96 h) or placebo, respectively. However, although TFPI significantly attenuated the coagulatory response in both groups (in patients with INRs ≥ 1.2 and < 1.2, respectively), no effect of TFPI therapy on mortality was found in the patients with an INR ≥ 1.2 in the final analysis. Even more, administration of TFPI was
associated with higher risk for bleeding complications in both groups. In contrast, in the patients with low INR, a trend toward a benefit in mortality was observed. It is difficult to explain the disappointing findings of this trial. In the KyberSept trial, heparin probably interfered with a beneficial effect of AT since mortality in the placebo group receiving heparin was lower than that in the placebo group with no heparin. However, in the TFPI trials, mortality rates in the treatment groups with or without heparin were comparable. Therefore, although heparin may displace TFPI from the glycosaminoglycans in the glycocalix of endothelial cells, this may not explain the failure of the TFPI trial in sepsis.

**C1-Inh in Sepsis**

Since amplification through activation of Factor Xll by C1-Inh might be beneficial. Although C1-Inh was demonstrated to decrease complement activation via the classical pathway to attenuate cytokine generation as well as to decrease the antifibrinolytic response, no effect on fibrin consumption or TAT generation was found. However, less microvascular thrombosis was found in the treated animals, in which C1-Inh was administered to septic patients demonstrated less need for vasopressor medications, possibly due to attenuation of complement and contact-phase activation. In a randomized, double-blind, placebo-controlled pilot study, C1-Inh was administered to patients with severe sepsis or septic shock. Organ dysfunction, especially renal function, significantly improved with C1-Inh treatment. Since only a limited number of patients had been included in that pilot study, no effect on mortality was found. C1-Inh lead to significant inhibition of the activation of the classical pathway of the complement system, whereas no effect on the contact phase system or on coagulation activation was found. The beneficial effect on organ dysfunction, especially renal function, in these patients was due to inhibition of neutrophil activation by C1-Inh, most probably by reducing mediators of neutrophil activation, such as C3a or IL-8.

**Concluding Remarks**

An overwhelming number of studies have shown activation of coagulation in sepsis. Evidence points to the TF-Factor VII pathway as being the principle route of activation in sepsis. Studies during the last decades in animal models have raised the hope that coagulation inhibitors that interfere with either thrombin generation or action and at the same time can attenuate inflammation may constitute efficient sepsis therapies in humans. However, results of multicenter trials evaluating the effects of three of these inhibitors in humans with sepsis have been disappointing: except for the APC trial (PROWESS), which showed an absolute risk reduction of 6% in mortality, no effect of TFPI and AT on mortality in severe sepsis was found. Although the discrepant outcomes of these trials are still not fully understood, these results again emphasize the limitations of animal models as a tool to investigate pathogenic mechanisms of human sepsis, since TFPI, AT, and APC have comparable effects in various animal models for this condition. Moreover, relationship between illness severity and treatment effect should be considered since the effect of antiinflammatory therapies in both animal and human sepsis appear to depend on the underlying mortality rate. These antiinflammatory agents generally lack efficacy at low mortality rates, and a benefit is often observed when mortality increases.

Interactions of the clotting inhibitors with heparin, even low-dose heparin, may have confounded the results of the KyberSept trial, the Optimized Phase 3 Tifacogin in Multicenter International Sepsis Trial, and the PROWESS trial. The effect of heparin on APC seem to be less as compared to the two other inhibitors. In contrast, there is strong evidence for interactions of heparin with AT and TFPI, respectively. Heparin acts as a cofactor of AT by enhancing its anticoagulant properties but at the same time interferes with the antiinflammatory properties of AT. TFPI has heparin-binding sites and was reported to be displaced by heparin from the endothelium. Interestingly, among the three inhibitors, APC is the only inhibitor that can bind to endothelial cells independently of glycosaminoglycans via the recently discovered EPCR.

Moreover, the three trials also emphasized our limited knowledge of the role of heparin in the treatment of sepsis. In all three studies, patients in the placebo group receiving prophylactic heparin had a lower overall 28-day mortality. Yet, interpretation of these results is difficult since administration of heparin was not randomized. Therefore, a randomized placebo-controlled trial to evaluate the efficacy of (low-dose) heparin in sepsis might help to understand the role of heparin in sepsis.

Although the pathophysiologic concepts of AT, TFPI, and APC are promising, only the latter was demonstrated to be efficient in human sepsis. APC was also recommended (Grade B recommendation) in the “Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock” (Delphi protocol). To obtain optimal benefit and to avoid bleeding complications, rAPC should only...
be administered to patients with an acute physiology and chronic health evaluation II score $\geq 25$, with sepsis-induced organ dysfunction, and with septic shock and sepsis-induced ARDS. To avoid bleeding effects on rAPC treatment, rAPC administration should be limited to patients with platelet counts $> 30,000/\mu L$ and not having conditions associated with an increased bleeding risk. However, the precise place of APC in the treatment of sepsis should be clarified in future studies.

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