Intensive Care Med (2004) 30:1032–1040
DOI 10.1007/s00134-004-2291-8

REVIEW

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Coagulation in sepsis

Received: 15 May 2002
Accepted: 23 March 2004
Published online: 18 May 2004

Competing interests: Dr SM Opal and Dr JL Vincent have been consultants for, and have received honorariums from, Aventis (antithrombin), Eli Lilly [drotrecogin alfa (activated)], and Chiron (tissue factor pathway inhibitor). Dr JL Vincent was the Principal Investigator of the European arm of the PROWESS study and principal investigator of the ENHANCE study (Eli Lilly) and an investigator for the TFPI study (Chiron). Dr Opal was the principal investigator for the Kybersept trial (Aventis), and was one of the North American investigators in the TFPI trial

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Abstract Coagulation abnormalities, ranging from a simple fall in platelet count to full-blown disseminated intravascular coagulation, are a common occurrence in critically ill patients and have been associated with increased mortality. In sepsis, activation of the extrinsic coagulation pathway by tissue factor induces increased coagulation, and simultaneous depression of the inhibitory mechanisms of coagulation, and suppression of the fibrinolytic system results in a procoagulant state that may lead to the formation of microvascular thrombi disturbing organ microcirculation and promoting the development of organ dysfunction. Many inflammatory mediators are involved in the activation of coagulation, but many coagulation proteins are themselves actively involved in the inflammatory process. In this article, we explore the complex relationship between inflammation and coagulation and how improved understanding of this interaction has led to the development of new therapeutic agents for patients with severe sepsis.

Introduction

As our knowledge of the pathophysiology of sepsis increases, new and exciting therapeutic approaches come to light. Although animal studies with innovative therapies for sepsis may show promise, not all succeed into clinical practice, as illustrated by the failure of randomized, placebo-controlled, clinical trials (RCTs). Recently, our focus has turned to coagulation abnormalities in sepsis and the links between coagulation and inflammation. This review briefly explores the role of the three natural anticoagulants, namely antithrombin (AT, formerly antithrombin III), activated protein C (APC), and tissue factor pathway inhibitor (TFPI) in sepsis. All three molecules have recently been the subject of large RCTs [1, 2, 2a].

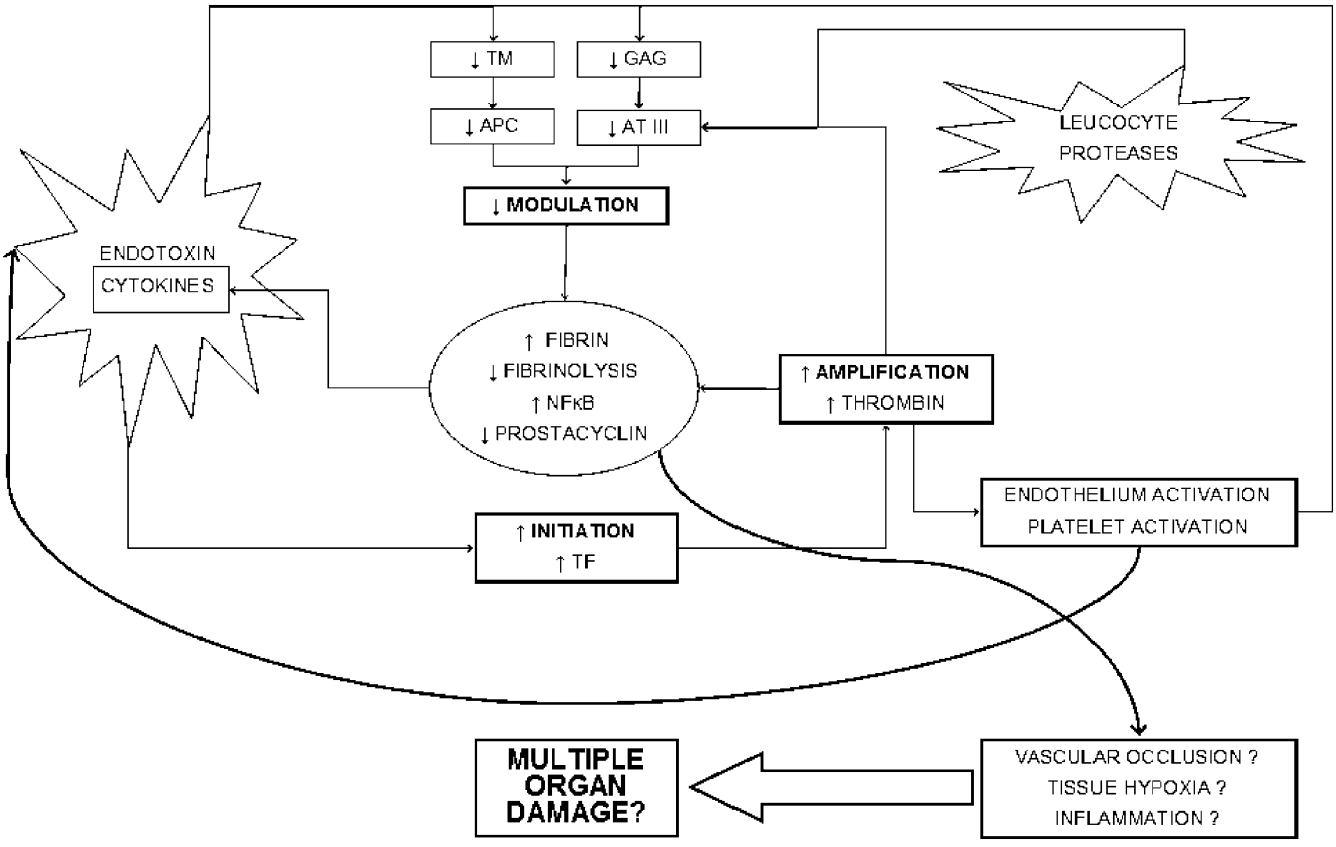

Fig. 2 The relationship between inflammation and coagulation

A different view of coagulation

Recently, our understanding of the coagulation system has changed from the classical 'cascade' model [3, 4] to a cell-based model of hemostasis [5], where tissue factor (TF), complexed with factor VIIa on membrane surfaces, is now known to be the major initiator of in vivo coagulation, followed by massive amplification of thrombin generation by the pro-thrombinase complex (factor Xa and factor Va) [6], an event that takes place on activated platelet membranes (Fig. 1).

Initiation and amplification of blood coagulation

Initiation of coagulation takes place when TF is exposed, such as by fibroblasts, when there is tissue damage or by cytokine-stimulated monocytes and endothelial cells [7], as in sepsis. While TF is the major initiator of coagulation, endotoxin, foreign bodies, and negatively charged particles may initiate coagulation via contact system activation. TF binds to factor VIIa, and this complex (TF:VIIa) may then activate factor X and factor IX [8]. Factor Xa, associated with factor Va, forms the prothrombinase complex, which subsequently turns prothrombin into thrombin. Factor IX activation amplifies the clotting reaction via interaction with the cofactor, factor VIII, which accelerates factor X activation. Thus, we see that initiation is dependent on the presence of both TF and factor VIIa.

Most of factor VII circulates as the zymogen (inactive form), leaving normal plasma with 1% of factor VIIa [9]. Once bound to TF, the zymogen is rapidly converted to factor VIIa via limited proteolysis [10]. Thus TF:VIIa may be formed in two different ways: first, TF may be complexed with factor VIIa already present in plasma; second, TF may bind factor VII with subsequent conversion to factor VIIa. Many proteases can activate the latter reaction, but it is unclear which is the most important in vivo, although factor Xa is considered the most likely candidate [11]. Importantly, although TF:VIIa can catalyze the reaction itself [12], this cannot occur outside a membrane surface, such as with soluble TF [13].

After initiation in TF-bearing cells, amplification of thrombin production takes place on the platelet surface. The essential steps in this phase are accomplished by the thrombin formed in the initiating phase, as it enhances platelet adhesion [14], and activates platelets [15] and factors V, VIII and XI [16]. Activated platelets also release factor IX [17]. Now the scene is set for massive thrombin formation. Factors V, VIII, IX, and X are pres-

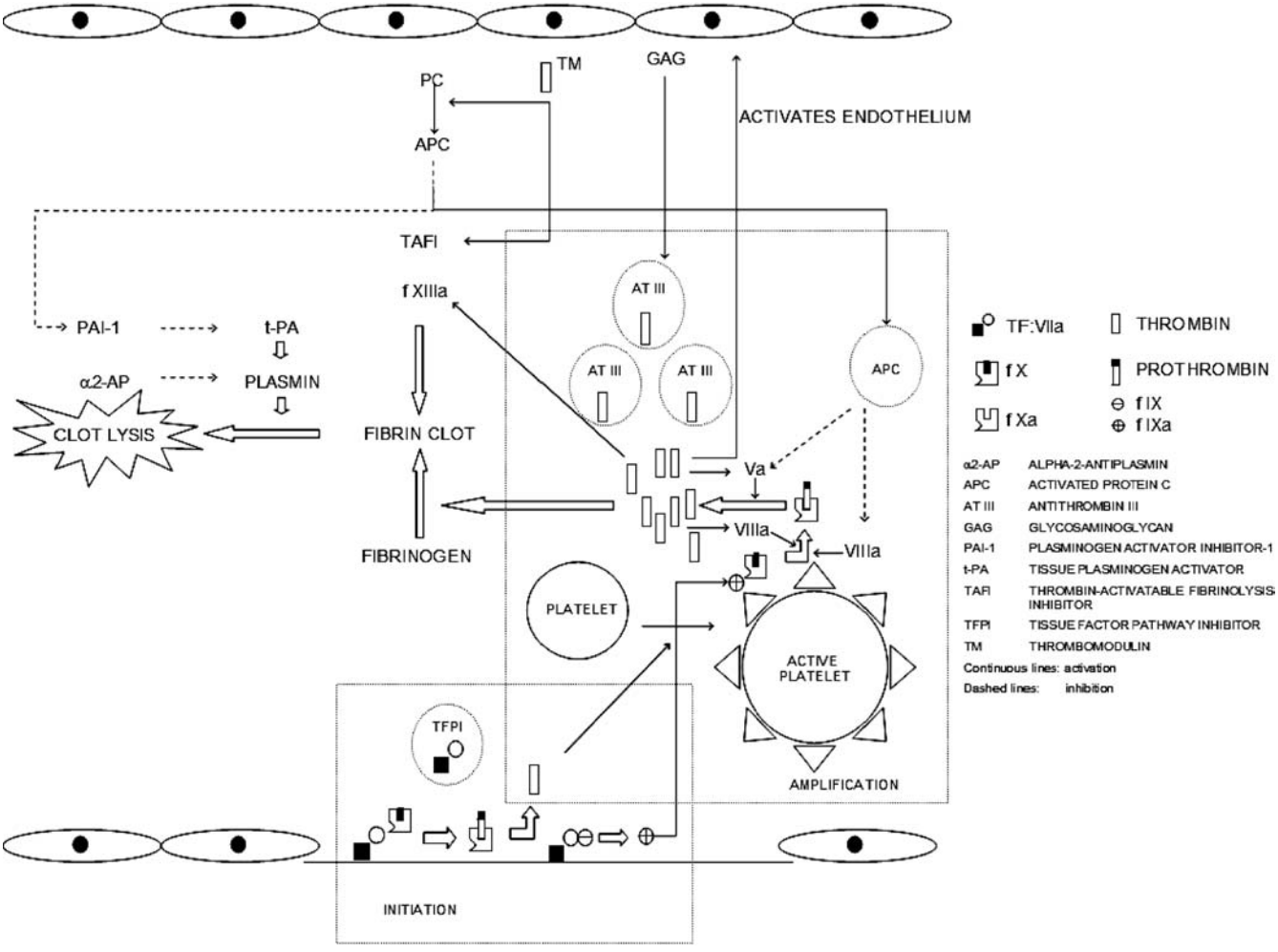

Fig. 1 Schematic representation of coagulation system initiation and activation

ent on the activated platelet surface, protected from inhibition by plasma proteases, and can produce thrombin that will turn fibrinogen into fibrin, and also exert positive feedback on its own pathway, by activating factors V, VIII, and XI.

Fibrin production and degradation

Thrombin will now cleave fibrinogen, producing fibrin [18]. Thrombin also activates factor XIII, which stabilizes the fibrin clot by creating cross-links that render it more resistant to plasmin-driven degradation [19]. Another important factor in the clot stabilization is the thrombin-activatable fibrinolysis inhibitor (TAFI). This protein also limits plasmin activity by removing from fibrin amino-acids that are essential for plasmin binding [20].

Degradation of the fibrin clot is performed by plasmin [21], which is generated from an inactive zymogen form, plasminogen, by the action of a series of proteases known as plasminogen activators [22], such as tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), which is the principal plasminogen activator in the extracellular space, e.g., within the alveoli [23]. This system may be inhibited at two points: first by the plasminogen-activator inhibitor-1 (PAI-1), which binds to t-PA and u-PA; and second by α_2-antiplasmin, which binds to plasmin.

Modulating coagulation

The process of initiating and amplifying coagulation is tightly regulated by the anticoagulant systems. The initiating phase is regulated mainly by TFPI, while the amplification phase is controlled both by AT and the APC pathway. TFPI inhibits the factor VIIa/TF complex before enough factor Xa is produced for hemostasis [24]; this reaction is greatly facilitated after TFPI has complexed with factor Xa [25]. Importantly, factor Xa cannot leave

the cell surface, as it is rapidly inhibited by TFPI or AT; thus only minute amounts of thrombin are formed during this period. This process seems to work as a 'yes' or 'no' reaction. That is, if the stimulus to produce thrombin is enough, TFPI, in physiologic amounts, will retard the process, but the same final concentration of thrombin will be formed as if there was no TFPI. On the other hand, if TFPI is present, but TF is absent or present in only limited amounts, no thrombin will be produced [6].

AT inhibits several procoagulant factors, such as thrombin, factors Xa, IXa, VIIa, and XIIa [26]. The anticoagulant action of AT is greatly enhanced by specific acid pentasaccharide moieties found on heparinoids, such as glycosaminoglycans (GAG), present on endothelial membranes or exogenous heparin [26]. Factor Xa is protected from inactivation by AT when in a membrane-associated complex with factor Va [27].

The APC pathway starts when thrombin binds to thrombomodulin (TM) on vascular endothelium surfaces [28]. This complex then activates the plasma zymogen, protein C [29], producing APC. This process of APC generation is enhanced by an endothelial cell protein receptor (EPCR) [30]. On endothelial surfaces, APC, interacting with protein S, catalyzes the inactivation of factors Va and VIIIa [29], thus stopping thrombin formation. It is interesting to notice that after binding to TM, thrombin not only loses fibrin formation properties, but also exerts negative feedback on its own pathway.

APC may have a role in fibrinolysis. First, by lowering thrombin production, fibrin production is also lowered; second, APC may form a tight complex with PAI-1 [31], protecting t-PA from inactivation. APC also is the principal inhibitor of TAFI since APC is a potent inhibitor of thrombin generation. Blockade of thrombin generation by APC prevents TAFI activation, thereby promoting fibrinolysis [32].

Links between coagulation and inflammation

The relationship between coagulation and inflammation is complex and, as yet, not completely understood (Fig. 2). It is known that blood clotting not only leads to fibrin deposition and platelet activation, but it also results in vascular cell activation, which contributes to leukocyte activation [33]. On the other hand, inflammation can induce TF expression in monocytes, via nuclear factor kappa-B (NF-κB) activation, thus initiating coagulation [7].

Examples of this interaction are readily seen. First, leukocytes are found at relatively high concentrations in venous thrombi, and leukocytes and activated platelets can form rosettes mediated by P-selectin expression on the surface of the activated platelet [34, 35]. These microscopic observations are probably elicited from the actions of thrombin, which can activate platelets and endothelium, increasing the surface expression of P-selectin [36, 37]. P-selectin is the primary initial mediator of leukocyte-endothelial cell rolling and is critical for leukocyte adhesion. Second, TF:VIIa and factor Xa have been shown to activate cells and generate responses similar to those mediated by thrombin [33]. Third, GAG and TM expression on cell surfaces are inhibited by inflammatory cytokines [38, 39, 40, 41] and lipopolysaccharide (LPS) [42], thus blocking the augmentation of AT action by GAG, and APC formation by TM.

Anti-inflammatory properties of natural anticoagulants

TFPI

TFPI has been shown to attenuate IL-6 and IL-8 release in an animal model [43]. However, in studies on volunteers receiving small doses of endotoxin, TFPI prevented the endotoxin-induced activation of coagulation, but had no effect on inflammatory mediators or on leukocyte and endothelial cell activation [44, 45]. TFPI can also compete with LPS binding protein (LBP) and bind LPS, resulting in attenuation of LPS stimulation. This LPS neutralizing activity is demonstrable in experimental settings, but its potential clinical significance remains uncertain [46].

Antithrombin

Binding of AT to GAG has been reported to induce prostacyclin formation in a time- and concentration-dependent manner [47, 48]. Prostacyclin can inhibit cytokine production and leukocyte activation, which might be responsible for the observed effects of AT in inhibiting leukocyte adhesion and changing vascular permeability [49, 50]. The in vitro production of tumor necrosis factor-alpha (TNF-α) and IL-1 is inhibited by AT, but only at high concentrations; lower concentrations may, in fact, enhance TNF-α synthesis [51]. The inhibition of thrombin may block the above-described pro-inflammatory actions of this molecule.

APC

In animal models, APC has been shown to block the production of TNF, both in the circulation [52] and in tissues [53, 54]. Inhibition of the APC pathway has also been shown to exacerbate cytokine responses [55, 56]. Like AT, APC blocks thrombin action by shutting down its pathway. APC may have other anti-inflammatory effects by blocking NF-kB in monocytes [57] as well as in endothelial cells [58], thus blocking a fundamental pathway for production of inflammatory cytokine and ex-

Table 1 Coagulation abnormalities in sepsis. Refer to text for references. (*PAP* plasmin-antiplasmin complexes)

	Sepsis	Septic shock	Non-survivors[a]
Initiation			
TF	↑	↑	-[b]
Factor VII	↓	↓	-
Amplification			
TAT	↑	↑	-
F 1+2	↑	↑	-
Fibrinolysis			
D-dimer	↑	↑	-
t-PA	↑	↑	Higher
Plasminogen	↓	↓	Lower
PAI-1	↑	↑	Higher
α_2-antiplasmin	Normal	Normal	Lower
PAP	↑	↑	-
TFPI			
Plasma	↑	No data	-
Membrane[c]	↓	No data	-
AT	↓	↓	Lower
APC	↓	↓	Lower
Protein S	Normal	↓	-

[a] When differences between survivors and non-survivors were observed
[b] Indicates that no data are available
[c] Laboratory data only

pression of adhesion molecules [59]. APC may also block genes that are upregulated in inflammation, such as those expressing adhesion molecules and oxidative enzymes, and increase the expression of antiapoptotic genes [58]. Thus, APC not only regulates the negative feedback on thrombin generation, but also has direct effects on the production of inflammation-related molecules.

What's going on in sepsis?

It has been known for a long time that sepsis is one of the causes of disseminated intravascular coagulation (DIC) [60], but only recently more subtle abnormalities in coagulation are being recognized. It is important to note that the ideas that follow are based on patients that have not as yet developed clinical DIC. These data are summarized in Table 1.

The activation of coagulation in sepsis can be demonstrated by the increased levels of TF [61], and the low levels of FVII, indicating consumption [62]. TFPI expression is only slightly augmented by endotoxin stimulation [63, 64], so that the enhanced production of TF may trigger coagulation.

Amplification systems are also activated, as reflected by high levels of prothrombin fragments 1 and 2 (F1+2) [61, 65], and thrombin-antithrombin (TAT) in sepsis [66, 67]. Thrombin is being generated and antagonized by AT, resulting in low blood AT levels in most patients [62, 65, 66, 67, 68, 69, 70]. In addition to consumption, destruction of AT by leukocyte proteases may also occur [71].

Protein C levels are also diminished in sepsis [62, 67, 68, 70, 72], especially in the non-survivors [72, 73, 74]. Low protein C levels may be due to consumption, and to reduced levels of TM in the cell surface [40, 41, 42], impairing its activation. Although TM levels in plasma (soluble TM) are usually elevated [75], they probably do not represent enhanced TM production or secretion, but rather cell damage [76] and decreased TM expression in vascular endothelium [42]. Importantly, this soluble form of TM is not capable of activating protein C efficiently.

Gando et al. [75] demonstrated that increases in soluble TM precede the decrease in fibrinogen and the prolongation of prothrombin time in septic patients. Hence, increased soluble TM levels may be related to decreased TM activity in cell surfaces, leading to decreased APC production.

TAFI is also activated (TAFIa) by the thrombin-TM complex [77], a reaction which is much faster than the activation of protein C. Thus at low TM concentrations, the production of TAFIa is higher than APC [78], and the milieu therefore becomes anti-fibrinolytic.

Thrombin activation is followed by fibrin formation, which is then degraded, as demonstrated by increased levels of D-dimer [79, 80]. This is performed by plasminogen activators, and increased levels of t-PA have been documented in sepsis [68, 69, 70], although its effects are counterbalanced by increased plasminogen-activator inhibitor-1 (PAI-1) levels [67, 68, 69, 81]. In fact, t-PA and PAI-1 levels are even higher in non-survivors [62, 68, 74], and tend to normalize in survivors [74]. Thus, it seems that fibrinolysis, although activated, is not sufficient to counteract fibrin formation. Increased fibrin formation associated with impaired fibrinolysis may contribute to both organ damage and mortality in sepsis [82].

Abnormal liver function, a common occurrence in patients with sepsis, can also influence coagulation, with decreased synthesis of coagulation proteins and reduced clearance of activated factors and enzyme:inhibitor complexes, quantitative and qualitative platelet defects, hyperfibrinolysis, and accelerated intravascular coagulation [83]. Liver disease is also commonly associated with vitamin K deficiency that can lead to a further reduction of plasma levels of factors II, VII, IX, and X, and proteins C and S, which require vitamin K as a co-factor for γ-carboxylation of glutamic acid residues in their amino-terminal region [83].

Although understanding of the basic mechanisms of coagulation and its derangements in sepsis is crucial for the development of new therapeutic strategies, the rational sequence of fibrin formation, leading to vascular occlusion and multiple organ dysfunction has not been clearly demonstrated so far. A more in-depth review on this area is beyond the scope of this paper and can be found elsewhere [84].

Administration of exogenous anticoagulants in sepsis

TFPI

A large RCT failed to show a reduced mortality in patients with severe sepsis 2a. A possible explanation for the failure of this trial is that the intervention comes too late, when coagulation has already been initiated, and amplification has developed, a phase in which TFPI may not have enough anticoagulant activity [6, 85], especially because factor Xa bound to platelets is relatively protected from TFPI activity [5]. As mentioned before, TFPI has not been shown to possess anti-inflammatory properties in human volunteers treated with endotoxin [44, 45].

AT

Another RCT including 2,314 patients, randomized to receive either placebo or AT over 4 days, led to nearly identical mortality rates in both groups (38.9% AT vs 38.7% placebo; *P*=ns) [2].

Several explanations can be found [86]. First, animal studies have shown that the anti-inflammatory properties of AT are exerted by binding to membrane surface GAG [47, 48], and the endothelium is deficient in GAG in sepsis [38, 39]. This seems to be the most important mechanism of AT advantage in animal models, because heparin, by competing for AT with GAG, reduces the relative advantage of ATIII [47, 48]. Indeed, the subgroup of patients that did not receive heparin seemed to have mortality reduction in the trial (15% relative risk reduction by AT therapy at 90 days follow up; $P<0.05$) [2]. Second, although AT can block thrombin activity, in the doses used it is not capable of reducing thrombin generation, a pathway that is only inhibited by higher doses of AT or by APC [87]. Therefore, continued thrombin generation may maintain the coagulation process as well as the associated inflammatory response.

APC

In another placebo-controlled trial, administration of exogenous recombinant human APC (drotrecogin-alfa [activated]) led to a 6.1% reduction in the absolute risk of death (19.4% relative risk reduction) in patients with severe sepsis over 28 days of follow up [1]. This recombinant human form of APC (rhAPC) was administered to 850 patients while 840 patients received placebo along with standard care for sepsis. The agent was given by continuous intravenous infusion for 4 days at 20 μg·kg·h. The therapy was remarkably well tolerated, except for excess bleeding, particularly following invasive procedures. The incidence of severe hemorrhage (defined as bleeding that necessitated >3 units of blood/day for two or more days or any significant intracranial bleeding) was 3.5% in the rhAPC group and 2.0% in the placebo group ($P=0.06$). There was no increased incidence of secondary infections, allergic reactions, or other side effects in the treatment group compared with placebo. Patients who received rhAPC also showed a decrease in D-dimer formation and IL-6, demonstrating both the anticoagulant and the anti-inflammatory properties of the drug.

APC is, so far, the only natural anticoagulant that has demonstrated direct activity in blocking thrombin formation, enhancing fibrinolysis, and diminishing the expression of inflammatory molecules. These combined actions offer a survival advantage in patients treated with APC. This drug, now known by the trade name Xigris, received regulatory approval by the Food and Drug Administration in the USA in November 2001. The drug is indicated for severe sepsis (i.e., APACHE II$\geq$25 or $\geq$2 organ dysfunctions).

Conclusion

The evolution in our understanding of the basic mechanisms underlying coagulation and sepsis, as well as the realization of the interaction between these systems, has led to the development of several potential new agents for the treatment of severe sepsis. Among these, drotrecogin alfa (activated) has been shown to improve outcome and is the first agent to be commercialized. This exciting development has added new impetus to the continuing search for other agents to expand our (rather limited) armamentarium.

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