# Natural Coagulation Inhibitors and Inflammation

Eberhard F. MAMMEN

School of Medicine, Wayne State University, Detroit, MI, USA

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In the last few years is has become evident that hemostasis and inflammation are closely related processes. It is recognized that the activation of hemostasis fosters, to a certain extent, inflammation and that inflammation, in turn, activates the hemostasis system. These interrelationships are best illustrated in sepsis, were microorganisms or their release products, most notably cytokines, causing inflammation, activate the hemostasis system. This process starts early in the inflammatory response. Already at the time of systemic inflammatory response syndrome (SIRS) there is evidence of an accelerated activation of the hemostasis system[1]. Levels of molecular markers of in vivo hemostasis activation begin to rise and increase further as inflammation progress. This process may ultimately lead to disseminated intravascular coagulation (DIC), also known as consumptive coagulopathy or thrombohemorrhagic syndrome[2,3]. Initially DIC is clinically unrecognized because it is compensated i.e. the increased consumption of coagulation componets is compensated for by an increased production. Routinely performed laboratory tests may still be normal, although elevated fibrinogen and D-dimer levels as well as decreased platelet counts may serve as early indicators[4]. If the inflammatory process is not aggressively controlled, DIC may decompensate. This is clinically recognized by a diffuse and profuse bleeding tendency, and laboratory data reveal consumption of many coagulation factors and platelets, leading ultimately to a complete breakdown of the hemostasis system, i.e. the bleeding tendency[2]. While hemorrhages are readily recognized, the thrombotic component of DIC is difficult to assess until signs of the multiple organ dysfunction syndrome (MODS) become evident[5]. There is considerable data to suggest that MODS is closely related to diffuse fibrin depositions in the microvasculature[6]. This would imply that the fibrinolytic system is malfunctioning. Early in sepsis the fibrinolytic system is also activated, but as the process progresses, the fibrinolytic system becomes inhibited by the release of plasminogen activator inhibitor 1 (PAI-1), the main regulator of fibrinolytic activation[7,8].

It is well recognized that tissue factor (TF) is the main stimulus for the activation of the clottings system in sepsis and DIC[9,10]. TF can be released from numerous cell types, such as endothelial cells, other tissue cells, macrophages and monocytes, to name a few.

The release from monocytes and macrophages appears to be of special importance in sepsis and DIC[10]. It has been shown that endotoxins express potent TF activity from monocytes[11]. The same was demonstra-

ted for several cytokines, especially tumor necrosis factor (TNF) and several interleukins (IL), such as IL-6, IL-8, IL-1b and others[10,12].

These interrelationships clearly raise the question, whether the natural inhibitors of the clotting system, tissue factor pathway inhibitor (TFPI), antithrombin (AT), and the protein C (PC)/protein S (PS) system only regulate clotting or whether they also influence the inflammatory response?

### Tissue Factor Pathway Inhibitor (TFPI)

TFPI regulates the initiation of the activation of the clotting system by TF through inhibition of the activity of the TF/F VIIa/F Xa complex[13]. TFPI is produced by endothelial cells. Up to this time, no congenital dysfunction of TFPI leading to thrombophilia has been described. Even patients with DIC and with sepsis seem to have normal or even above normal levels of TFPI, although few reports have reported low levels[14]. This seems to indicate, that TFPI is not consumed durgn DIC.

Concerning the relationship between TFPI and inflammation, little is known at this time, except that it inhibits the proinflammatory properties of F VIIa and that it decreases IL-6 levels[12]. In septic animal models the infusion of recombinant TFPI was found to reduce mortality[15]. This prompted clinical trials in septic patients with apparently promising results. Phase III studies are presently conducted.

### Antithrombin (AT)

At is a serine protease inhibitor which neutralizes all coagulation-related enzymes, especially thrombin and F Xa[16]. It is a plasma protein that is synthesized by liver parenchymal cells. It serves as the substrate for heparins and other glycosaminoglycans. These form complexes with AT. As a consequence of this complex formation, AT changes its molecular configuration and becomes about 1000-fold more active in neutralizing the enzymes[17]. This explains the anticoagulant action of heparins. The main physiological function of AT is likely limited to its interaction with glycosaminoglycans located on the endothelial cell surfaces.

AT defects are closely linked to thromboembolic diseases and patients with congenital AT defects have a high incidence of thrombosis, predominantly venous[18]. There are numerous acquired AT deficiencies described[16]. These may be due to impaired production or due to increased consumption. AT deficiency in DIC is a typical example of increased consumption. Moreover, as intravascular clotting accelerates, AT will attempt to control the generation of enzymes. This will lead to AT consumption which, in turn, will lead to more clotting[2]. This negative feedback process will ultimately cause a complete breakdown of the hemostasis system. This is of great importance in septic and trauma patients and plasma levels of AT predict patients demise with a high degree of accuracy[19]. Since AT is such a critical regulator of clotting, replacement in patients with deficiency states with concentrates is an appealing approach to treatment (see later).

In the last few years it has become evident that AT has, besides its well-known anticoagulant properties, antiinflammatory features[20]. It was observed that binding of AT to glycosaminoglycans located on endothelial cells surfaces released prostaglandin I2 (PGI2) or prostacyclin. This release is blocked in the presence of heparin[21]. Also heparin/AT complexes would inhibit the release of PGI2 is not only a strong inhibitor of platelet function, it also exerts antiinflammatory properties[22]. AT was also found to be involved in regulating the release of protease/serpin complexes, lysosomal proteases (PMN-elastase, cathepsin B) and soluble intercellular adhesion molecules in septic and trauma patients[12,23]. In addition, AT was shown to reduce leukocyte adhesion to endothelium, a prerequisite for the development of MODS in septic patients[24,25]. It also seems to have a vascular protective effect[26]. All of these observations were made on animal models of sepsis as well as on septic patients.

Since AT has anticoagulant and antiinflammatory properties, studies were conducted on the efficacy of AT supplementation in DIC and in sepsis with DIC. As previously reviewed[27]. At concentrates, administered to a variety of animal models of sepsis, not only improved the features of DIC, but also prevented MODS and mortality in virtually every model studied. Two important observations were, however, made: Outcome was better, when treatment was started early during the septic process, and better results were obtained when, during treatment, plasma levels of AT were above normal range instead of normal ranges[27,28].

The human experience with AT supplementation in septic patients with DIC is less favorable as shown in several studies[27,29-31]. Case reports of patients with nonseptic DIC were generally favorable, but no major, double-blind and placebo-controlled trials have been conducted. In contrast, several reports, double-blind and placebo-controlled describe the experience with AT replacement in patients in septic shock. None of them demonstrated significant differences between groups when all-cause mortality was the endpoint. There was, however, in all studies a trend toward improvement, and other parameters (laboratory values, time in the ICU) showed differences. This lack of significance could have several reasons: The number of patients studies in each group was small, most patients enrolled were in septic shock rather than in earlier stages, and plasma AT levels were beraly in normal range. Based on experiences with animal models, these are all unfavorable conditions.

Recently, however, the results of a large trial (over 2300 patients) were published where high doses of AT concentrate were administered to patients with severe sepsis and in septic shock[32]. The study was multicenter, double-blind and placebo-controlled. Unfortunately on significant difference was fund in 28-day mortality between the two patient groups. Some of the patients received concomitantly heparin which caused a greater bleeding tendency in the AT-treated group. Interestingly, a subgroup analysis of patients treated with heparin or not receiving heparin showed improved outcome in those not treated. As pointed out before, heparin blocks the release of PGI2 from endothelial cells and it is conceivable that this could have impacted the outcome. This outcome once again illustrates that heparin should not be administered when AT concentrate treatment is considered. This had long been sated[27,33].

### Protein C (PC) and Protein S (PS) System

The PC/PS/thrombomodulin system repsents the third regulation device for the generation of thrombin. PC is a plasma protein that is also synthesized in liver parenchymal cells; it is a vitamin K-dependent factor. It is a proenzyme that needs to be converted into its enzymatic form[34]. This conversion in facilitated by thrombin, but requires the binding of thrombin to an endothelial cell surfaces bound receptor, called thrombomodulin. When thrombin binds to this receptor, it looses its procoagulant and other clot-promoting properties and assumes a clot-inhibiting feature. Protein Ca or activated protein C (APC) destroys proteolytically F Va and F VIIIa. These are the two cofactors of the tenase and prothrombinase complexes[35]. In this was the amounts of thrombin and factor Xa generated from their respective proenzymes are regulated. PS serves as the cofactor for APC. This regulatory system thus functions fundamentally different from AT.

Congenital defects of both, APC and PS, are associated with a thrombophilic state characterized by venous and arterial forms of thromboembolism. Also acquired deficiencies lead to hypercoagulability[36]. In DIC patients, PC levels decrease to variable degrees, in part due to increased consumption, in part due to impaired synthesis[2,6]. As with AT, increased clotting leads to increased consumption which, in turn, leads to more clotting. During sepsis PC levels in plasma decrease and clinical as well as experimental evidence suggests that this may contribute to clinical outcome[20,37].

The possible relationship between the APC/PS/thrombomodulin system and inflammation is less well known at this time. In vitro and in vivo data revealed, however, that cytokines can modulate this system. TNF and IL-1 were shown to reduce TM activity and TNF down-regulates the protein C receptor on endothelial cell surfaces[38,39]. This would impair the anticoagulant activity of this system. Components of the APC/PS/TM system were also found to influence inflammation. APC was noted to inhibit endotoxin-induced production of TNF, IL-1, IL-6 and IL-8 from monocytes and macrophages[12,40].

As with AT, these findings prompted experiments in which animal models of sepsis were treated with recombinant PC. PC improved outcome in septic baboons and in rabbits with DIC, suggesting a beneficial effect in humans as well[41,42]. Following reports of successful treatment of septic patients with PC, a major, multicenter, double-blind and placebo-controlled trial was conducted involving 1690 patients[31,43]. Recombinant APC was administered to patients with severe sepsis. This treatment significantly reduced all-cause mortality, although an increased bleeding tendency was observed. It should be noted that APC also activates the fibrinolytic system by destroying PAI-1 which could be

an extra benefit for the developing MODS.

### Perspective

Sepsis is still one of the major causes of mortality, and about 50% of patients still die despite of improved diagnostic capabilities and care[44]. Optimal treatment is still elusive. This is very likely due to the complexity of the metabolic derailments that occur during sepsis which Bone termed "metabolic anarchy"[45,46]. Many attempts have been made to intercept therapeutically key metabolic steps or key triggers of sepsis[27]. All were based on animal models with favorable outcome, but almost all failed to demonstrate in humans significant improvements[47]. There are, of course, many reasons why it is difficult to extrapolate results from animal experiments to human trials, yet valuable information can be gained from such investigations. The data obtained with recombinant APC in patients with severe sepsis are, in view of the previous experiences, encouraging and appear to suggest that approaches, where antiinflammatory and anticoagulantly active therapeutics are used to treat sepsis, are viable and need to be explored further[42].

**Footnote:** This article is dedicated to Professor Orhan N. Ulutin, MD, for his early contributions to the development of protein C, then known as "autoprothrombin II-A."

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**Address for Correspondence:**

Eberhard F. MAMMEN, MD

Route 1, 1553-H-2 Michibay Drive
49854, Manistique MI, USA

e-mail: *efmammen@up.net*