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# Changes in the coagulation-fibrinolysis balance of endothelial cells and mononuclear phagocytes: role in disseminated intravascular coagulation associated with infectious diseases

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**Summary.** Over the last few years, evidence has accumulated that the pathogenetic mechanism of disseminated intravascular coagulation encountered in patients with infectious diseases is extraordinarily complex and involves multiple interactions between the microorganism itself and/or a number of mediators, both microorganism derived and host manufactured, and multifunctional cellular systems, namely endothelial cells and mononuclear phagocytes. In particular, infectious agents and mediators shift the coagulation-fibrinolysis equilibrium of these cells towards fibrin formation and accumulation, via enhancement of procoagulant properties and reduction of both anticoagulant and fibrinolytic capacities. New insights into the pathogenetic mechanism may have important implications for the management of infected patients with disseminated intravascular coagulation.

**Key words:** Disseminated intravascular coagulation – Infection – Coagulation-fibrinolysis – Endothelial cells – Mononuclear phagocytes

## Introduction

Infectious diseases are known to be associated with hemostatic disorders. Although isolated thrombocytopenia is the usual abnormality, the most dramatic complication is acute disseminated intravascular coagulation (DIC) [38]. It is a complex, dynamic, pathological process triggered by the continuous exposure of blood to pathogenic clot-promoting factors. The induced intravascular blood clotting activation results in widespread thrombus formation in the microcirculation, responsible for tissue ischemia and organ failure, followed by secondary fibrinolysis and consumption of hemostatic components (platelets, coagulation factors, inhibitors) eventually leading to bleeding. DIC is relatively uncommon and classically associated with gram-negative sepsis, although it can also be observed in gram-positive sepsis and occasionally in certain viral, rickettsial, or even parasitic infections [38]. The Waterhouse-Friderichsen syndrome is considered the extreme manifestation, being the condition with the highest mortality risk and the most fulminant clinical course. Variations in the acuteness of onset and in the degree of consumption, possibly related to the type of infectious agent as well as to the age and general health of the patient, account for the wide spectrum of clinical manifestations, including patients with only laboratory abnormalities.

Although the pathogenetic mechanism of DIC has been the subject of controversy, as has practically every other aspect of the condition, it is generally agreed that the manifestations of the disease can in large part be regarded as a consequence of thrombin formation. Therefore, many efforts have been made to understand the mechanisms leading to the activation of the clotting cascade and fibrin formation. There is quite a lot of evidence that, in naturally occurring and in experimentally induced infections (particularly in gram-negative sepsis), activation of the contact system takes place [13]. However, its role in precipitating DIC has been questioned, since impairment of the intrinsic coagulation pathway, caused by depletion of factor VIII, does not prevent endotoxin-induced coagulative changes in rabbits [58]. Garner and Evensen [27] showed that endotoxin-induced thrombus formation in the microcirculation of tissues from normal dogs is much greater than in factor-VII-deficient dogs, clearly pointing to an involvement of tissue factor. Recently, direct experimental evidence has been provided that exposure of blood to tissue factor is, indeed, the major mechanism triggering DIC after endotoxin. Anti-tissue factor monoclonal antibodies given to rabbits before endotoxin administration substantially reduce the consumption of platelets and clotting factors and prevent fibrin deposition [66]. In baboons anti-tissue factor antibodies have been shown, not only to attenuate the coagulopathy, but also to protect the animals from the lethal effects of *Escherichia coli* [62].

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Accumulation of fibrin deposits in the microcirculation may be facilitated by a simultaneous impairment of the fibrinolytic system. Infusion of des-A-fibrin or thrombin, at doses unable to induce fibrin accumulation in normal animals, causes diffuse renal microthrombosis in animals pretreated with antifibrinolytic agents [31, 39]. Interestingly, a single endotoxin injection is sufficient to render the animals sensitive to thrombogenic stimuli [31, 34], probably due to inhibition of fibrinolysis.

During the last few years, there has been an increasing awareness that vascular endothelial cells and mononuclear phagocytes, under appropriate conditions, may express clot-promoting properties [procoagulant activities (PCAs)], whereby blood coagulation is initiated and propagated. In addition, both cell types are major participants in the fibrinolytic pathways, producing both plasminogen activators and their inhibitors and thus influencing fibrin deposition. The present survey provides a concise description, based mainly on in vitro studies, of the vast repertoire of procoagulant-fibrinolytic properties acquired by endothelial cells and mononuclear phagocytes on exposure to infection-related stimulating agents. Available evidence indicating the involvement of these cellular properties in experimental or human DIC associated with infectious diseases will also be outlined.

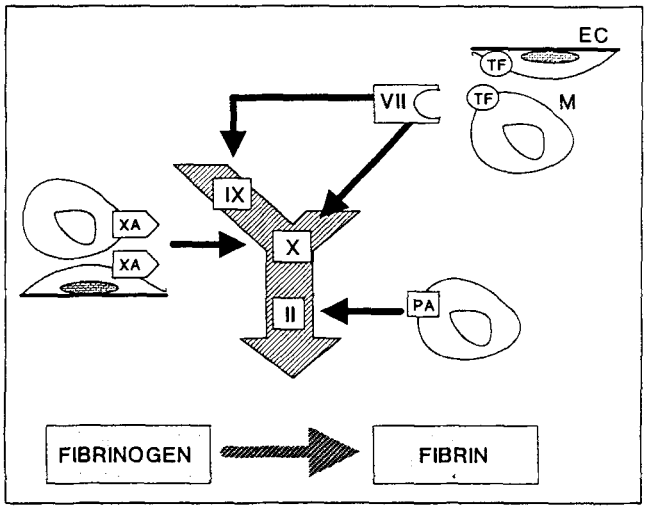


**Fig. 1.** Procoagulant properties of endothelial cells (*EC*) and mononuclear phagocytes (*M*): site of action in the clotting cascade. *TF*, tissue factor; *XA*, factor X activator; *PA*, prothrombin activator. *Black arrows* indicate activation

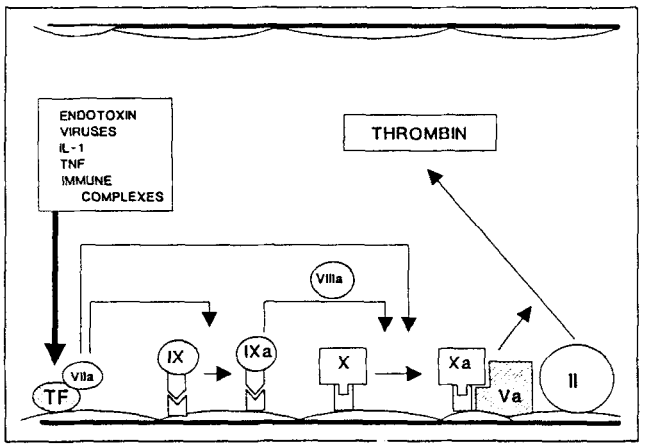


**Fig. 2.** Coagulation cascade on perturbed EC. *IL-1*, Interleukin-1; *TNF*, tumor necrosis factor

## Endothelial cells

As endothelial cells form the luminal vascular surface, endothelium is strategically located to regulate the coagulation-fibrinolysis events. The basal state of the endothelial cell surface is thought to be essentially anticoagulant or non-thrombogenic. Indeed, endothelial cells provide two major active anticoagulant mechanisms [48]: (1) by expressing heparin-like substances, mainly heparan sulfate, they catalyze the antithrombin pathway; (2) by expressing thrombomodulin and secreting protein S, they catalyze the activation of the protein C pathway. Moreover, unlike other vascular cells, namely fibroblasts and smooth muscle cells, that express significant amounts of tissue factor, endothelial cells are unable to activate the coagulation pathways, as shown by functional and immunohistochemical studies and by in situ hybridization [21, 67]. However, cultured human or animal endothelial cells, on exposure to a number of stimulating agents, synthesize and express a PCA that has been identified as tissue factor (Fig.1) [6, 14, 20, 60, 65]. This procoagulant is induced at the level of gene transcription and possibly also by inhibiting mRNA degradation [51]. Agents that induce PCA production in endothelial cells include: bacterial endotoxin; some viruses; interleukin-1 (IL-1); tumor necrosis factor (TNF); immune complexes, whose pathogenetic relevance in infectious disease is well established. Recently, it has been shown that the exposure of cultured or native endothelium to a hypoxic environment induces the generation of a new membrane-associated procoagulant that directly activates coagulation factor X (Fig. 1) [43]. This observation provides a basis for understanding the initiation of vascular pathology in hypoxemic states.

Other procoagulant properties of endothelium include the production of coagulation factor V and the expression of specific membrane receptors for factors IX/IXa and X/Xa. TNF has been shown to enhance the number of factor IX/IXa binding sites, thus potentially enhancing factor Xa formation [60]. In addition, a number of stimulating agents, including some viruses and complement proteins C5b-9, are able to efficiently promote the assembly of the prothrombinase complex on the endothelial cell surface, thereby increasing the rate of thrombin formation [28, 65].

Due to this complex array of procoagulant properties, "perturbed" endothelial cells may represent a surface onto which clotting pathways are initiated and propagated leading to fibrin formation (Fig. 2). The expression of tissue factor on the cell surface allows the binding of factor VII/VIIa and results in the formation of a molecular complex capable of converting the proenzymes factor X and factor IX to their active forms; factor IXa, in turn, in the presence of factor VIII, also activates factor

X. Factor Xa, in the presence of endothelial factor V/Va, then converts prothrombin to thrombin. Of particular interest is the observation that some stimulating agents induce the shedding from the endothelial cell plasma membrane of microparticles that retain their procoagulant properties [28], thus potentially contributing to generalized intravascular coagulation.

It should be emphasized that most of the agents that induce PCA generation also cause a progressive reduction of thrombomodulin and, in the case of TNF and IL-1, of heparin-like substances expressed by endothelial cells [33, 40, 60]. The appearance of clot-promoting properties and the simultaneous loss of physiological anticoagulant mechanisms will eventually favor uncontrolled fibrin formation on the endothelial surface. Perturbed endothelial cells may further promote fibrin accumulation by influencing the fibrinolytic system. Under normal conditions, endothelial cells produce tissue-plasminogen activator (t-PA) and an excess of plasminogen activator inhibitor-1 (PAI-1). However, on demand the endothelium rapidly releases large amounts of t-PA which overcomes the inhibitor capacity [3, 29]. When stimulated with some microorganisms (viruses, rickettsiae), endotoxin, IL-1, TNF, interferon-$\gamma$, and other agents, endothelial cells produce larger amounts of PAI-1 which greatly reduce the cellular fibrinolytic potential [15, 22, 30, 37]. This has been shown to be due to enhanced transcription of the PAI-1 gene [50]. Moreover, cytokines cause a concomitant reduction in t-PA production, further depressing the fibrinolytic potential of the endothelium [37].

Until quite recently, disruption of endothelial cell continuity was considered the only event leading to thrombus formation. Nowadays it is apparent that subtle changes in the endothelial cell function ("biochemical lesions" or "endothelial dysfunction"), resulting in an inbalance of the coagulation-fibrinolysis equilibrium, may well contribute to fibrin deposition (Fig. 3).

## Mononuclear phagocytes

Peripheral blood monocytes, like other intravascular cells, express little, if any, PCA under normal conditions. However, on exposure to a wide variety of stimulating agents, they synthesize and express clot-promoting substances on their surface [23, 24, 42, 55]. This property is also shared by macrophages from different anatomical sites (peritoneal cavity, spleen, pulmonary alveoli, marrow) [54]. Among the agonists that induce a procoagulant response, many are of pathogenetic importance in infectious diseases, including whole bacteria, endotoxin, some viruses, immune complexes, anaphylotoxin C5a, antigens, and TNF [19, 36, 55]. Although the majority of these stimuli can directly induce the synthesis of procoagulants, more complex cellular pathways have been described for some of them [23, 36]. In some instances, especially in immune induction of monocyte PCA, the collaboration of T-lymphocytes (via cell-cell interaction or mediated by lymphokines) is an absolute requirement. Tissue factor is the procoagulant most commonly produced by mononuclear phagocytes, especially by human cells (Fig. 1). Its expression appears to be primarily regulated at the level of gene transcription, which is rapidly and transiently induced by the agonist. Other reported procoagulants are: a direct prothrombin activator (prothrombinase) and a factor X activator [23, 36]. Monocytes-macrophages are also able to synthesize a number of clotting factors (vitamin K-dependent proteins, factor V, factor XIII) and to assemble the prothrombinase complex on their surface, thus promoting thrombin formation. As with endothelial cells (Fig. 2), the expression of multiple procoagulants enables mononuclear phagocytes to initiate and propagate the coagulation pathways leading to fibrin formation.

Mononuclear phagocytes are also endowed with fibrinolytic properties. They are known to produce, at least in culture, two major components of the fibrinolytic system, the urokinase-type plasminogen activator (prou-PA) and its inhibitor (PAI-2) [7, 10, 68]. When exposed to endotoxin, muramyl dipeptide, some viruses, TNF, and other agents, monocytes/macrophages exhibit a marked increase in the release of PAI-2 [2, 25, 35]. Secretion of such an inhibitor would be expected to favor the persistence of fibrin, especially if it occurs concomitantly with fibrin formation caused by increased PCA [52].

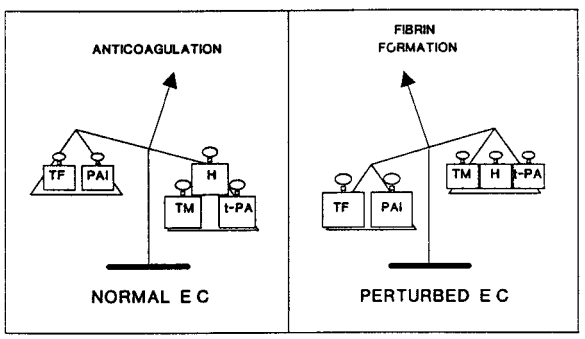


**Fig. 3.** The coagulation-fibrinolysis balance in normal and perturbed EC. *PAI*, Plasminogen activator inhibitor-1; *TM*, thrombomodulin; *H*, heparin-like substances; *t-PA*, tissue plasminogen activator

## Pathophysiological role

Although the exact pathophysiological significance of the observations reviewed above remains to be established, there are some indications that they may reflect mechanisms and reactions operating in vivo. Our knowledge of the relationship between changes in the coagulation-fibrinolysis equilibrium of vascular cells and mononuclear phagocytes and fibrin formation/deposition in infectious disease stems from numerous animal studies and a limited number of human studies.

### *Animal studies*

The pathological condition in which the essential role of these phenomena has been perhaps best established is DIC induced by endotoxin in rabbits. Mononuclear phagocytes recovered from blood and other anatomical sites (peritoneum, spleen, marrow) of rabbits treated with endotoxin express strong tissue factor activity, clearly suggesting that endotoxin stimulates tissue factor production in vivo [49, 53]. We have recently shown that

aortic endothelium, obtained from rabbits after a single endotoxin injection, expresses significantly higher levels of tissue factor than control vessels [57]. Moreover, aortic vascular segments from endotoxin-treated animals, when incubated in culture medium, release much more PAI-1 activity than control samples (Colucci et al., unpublished work). These changes at the cellular level are associated with laboratory signs of blood clotting activation and a marked increase in plasma PAI-1 [15]. A significant enhancement of PCA, associated with fibrin deposition has been reported in glomeruli from rabbits with bilateral cortical necrosis induced by two endotoxin injections (Sanarelli-Shwartzman reaction) [9]. Although in these studies the precise source of PCA was not established, infiltrating mononuclear phagocytes and/or intrinsic glomerular cells (particularly endothelial cells) could well be responsible for the augmented glomerular PCA. Experiments in our laboratory have shown that glomeruli from rabbits given a continuous infusion of endotoxin or TNF have a markedly decreased fibrinolytic activity compared with glomeruli from normal animals (Colucci et al., unpublished work). This phenomenon appears to be due to a reduced production of plasminogen activators (t-PA and u-PA) rather than to an increase in PAI. Altogether these data clearly suggest that fibrin deposition in tissue microcirculation (for instance at glomerular level) results from the simultaneous activation of blood coagulation and inhibition of fibrinolysis by cells.

Many years ago Thomas and Good [63] first stressed the importance of leukocytes in the pathogenesis of endotoxin-induced DIC, as they showed that endotoxin does not initiate DIC in animals rendered leukopenic by cytotoxic drugs. These data were confirmed by other investigators [41]. It was also shown that the infusion of normal leukocytes in leukopenic animals restores the susceptibility to endotoxin-induced coagulative changes. Moreover, passive transfer of "activated" leukocytes (expressing PCA) in normal animals causes DIC. Although it was originally thought that polymorphonuclear cells were involved in the activation of coagulation, these fundamental experiments, if reinterpreted in the light of present knowledge, strongly support the concept that monocytes/macrophages play a key role in endotoxin-induced DIC. Indeed, these cells not only synthesize and express clot-promoting substances, but are the main source of mediators (TNF, IL-1) that shift the hemostatic balance of endothelial cells towards promotion of fibrin deposition. Infusion of IL-1 in rabbits results in a time- and dose-dependent induction of tissue factor with a concomitant decrease in endothelial thrombomodulin activity [60].

The rat is considered much more resistant than the rabbit to endotoxin-induced DIC. We observed that rat mononuclear phagocytes generate little, if any, PCA in response to even large amounts of endotoxin, both in vitro and in vivo [53]. Moreover, endotoxin has been shown to increase the expression of PCA and to decrease the plasminogen activator activity (due to PAI secretion) in macrophages from endotoxin-responsive mouse strains, but not in cells of unresponsive animals [11]. These findings further support a major role for cellular procoagulant-fibrinolytic properties in endotoxin-induced DIC.

There is evidence that mouse blood mononuclear cells, stimulated in vitro by murine hepatitis virus strain 3 (MHV-3), respond with an increase in PCA (prothrombinase), and that this response directly correlates with susceptibility of animals to hepatic injury [36]. In animals infected with MHV-3, a dramatic increase in spontaneous PCA of blood and spleen mononuclear cells was observed in disease-sensitive, but not in disease-resistant, mouse strains. This occurred before histological abnormalities and persisted during the development of the disease. These data, while clearly suggesting that monocyte/macrophage PCA may play a role in the pathogenesis of murine virus hepatitis, point out the need for similar studies in other viral infections.

*Human studies*

The pathophysiological role of the multiple activities of mononuclear phagocytes and endothelial cells is for obvious reasons much more difficult to study in man. Nevertheless, there is some evidence that mononuclear phagocytes may undergo in vivo functional changes, similar to those observed in vitro, on exposure to stimulating agents. Conkling et al. [18], in a phase 1 trial to evaluate recombinant human TNF in the treatment of patients with refractory cancer, found that TNF infusion induced a trend toward higher tissue factor in blood monocytes. In some severe infectious diseases (meningococcal infections, bacterial peritonitis), as well as in other conditions associated with endotoxemia (severe obstructive jaundice), peripheral blood monocytes have been shown to exhibit a significant increase in tissue factor activity [1, 44, 56]. Interestingly, the highest cellular tissue factor levels have been found in patients with a lethal outcome, suggesting that this parameter may have prognostic significance. Some investigators have exploited the accessibility of alveolar and peritoneal macrophages to assess the procoagulant and fibrinolytic properties of these cells in relation to infectious diseases. Macrophages obtained from the peritoneal fluid of patients with peritonitis showed a substantial increase in tissue factor compared with control cells [1]. In patients with adult respiratory distress syndrome (ARDS) secondary to sepsis, the PCA of bronchoalveolar lavage fluid was found to be markedly higher than in patients with interstitial lung disease or normal subjects and was associated with fibrin deposition in the lung, whereas fibrinolytic activity (urokinase-type) was virtually absent, due to the appearance of PAIs [12, 32]. PCA was identified as tissue factor/factor VII and most probably originated from "activated" alveolar macrophages, although other cellular sources could not be excluded (lung fibroblasts, parenchymal cells). Interestingly, in situ hybridization studies of acute-phase ARDS lung biopsies showed evidence of macrophage PAI-1 mRNA rather than the expected PAI-2 mRNA, suggesting a differential expression of the two inhibitors in vivo.

Even more difficult to assess is the precise role of the multiple activities of endothelial cells in man. Perhaps the best marker of endothelial function is the plasma level of t-PA [29]. In healthy volunteers receiving a bolus injec-

tion of endotoxin (2–4 ng/kg), a clear increase in plasma t-PA levels, indicative of endothelial cell activation, was observed about 2 h after endotoxin administration [61, 64]. The release of t-PA coincided with activation of the fibrinolytic pathway, as measured by plasmin-$\alpha_2$ antiplasmin complexes. However, the fibrinolytic activity of t-PA was rapidly offset by the subsequent and long-lasting increase in the plasma levels of PAI-1.

In patients with different types of bacterial infections, a significant increase in plasma PAI-1 has been consistently reported by several investigators and, in some studies, PAI-1 has been proposed as a prognostic marker in patients with septic shock [8, 15, 17, 45, 47]. Assuming that circulating PAI-1 is mostly of vascular origin, its increase could represent an additional marker of endothelial stimulation in vivo.

Using highly sensitive and specific assays, some investigators have shown that a bolus injection of endotoxin or TNF in healthy volunteers induces an early and sustained subclinical activation of the coagulation system (increase in plasma levels of the activation peptide of factor X, prothrombin fragment F1+2, thrombin-antithrombin III complexes) [46, 64]. Interestingly, neither treatment caused activation of the contact system of blood coagulation, further suggesting the involvement of the tissue factor pathway. Bauer et al. [4] studied the in vivo effect on the hemostatic system of recombinant TNF administered as a continuous 24-h infusion to cancer patients. There were clear signs of blood clotting activation during TNF infusion but little or no changes in plasma D-dimer and fragment B$\beta$1-42, two sensitive parameters of fibrinolysis activation. These phenomena have been interpreted as reflecting the changes induced by TNF in endothelial cells in vitro (expression of tissue factor, reduced production of t-PA and/or increase in PAI-1 production).

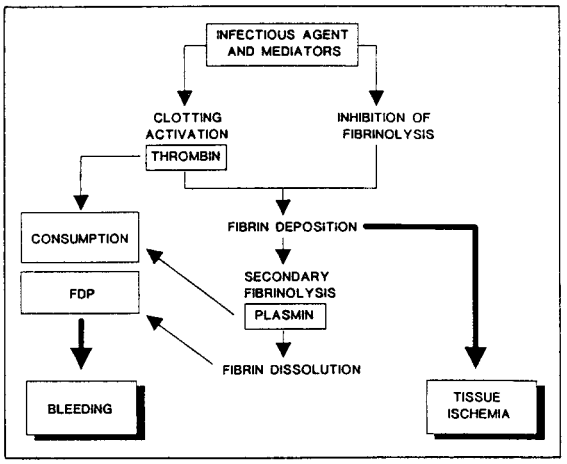


**Fig. 4.** Pathogenetic sequence leading to thrombotic and hemorrhagic manifestations in infection-associated disseminated intravascular coagulation. *FDP*, Fibrin degradation products

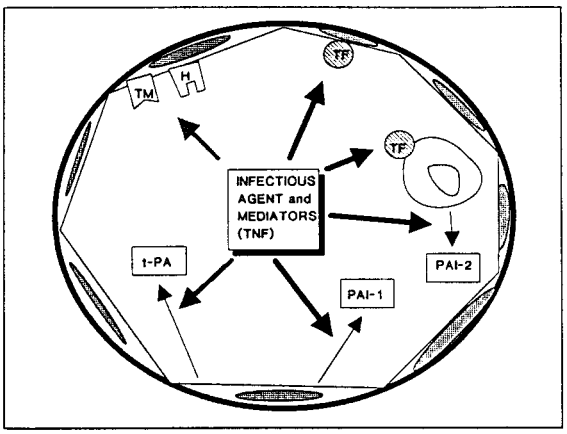


**Fig. 5.** Schematic representation of the changes in the procoagulant, anticoagulant and fibrinolytic properties of EC and M. *Black arrows* indicate induction; *grey arrows* indicate inhibition

## Concluding remarks

Figure 4 schematically shows how the complex processes reviewed above may lead to clinical appearance of thrombotic and hemorrhagic phenomena during DIC. Infectious agents and/or their products will trigger intravascular coagulation via the expression of tissue factor (and/or other procoagulants) on the surface of monocytes/macrophages and endothelial cells, and the simultaneous suppression of endothelial anticoagulant mechanisms (Fig. 5), thus leading to thrombin generation and fibrinogen to fibrin conversion. Infectious agents and/or their products are also able to down-regulate the fibrinolytic balance of mononuclear phagocytes and endothelial cells via the induction of synthesis and release of PAIs (PAI-2 and PAI-1, respectively) and the decrease in the production of plasminogen activators (Fig. 5). This will favor the persistence of fibrin in the circulation. Infection-related vasomotor reactions will then localize fibrin deposition to defined microvascular areas. Clearly, vessel obstruction will entail tissue ischemia and organ failure, which are prominent features of DIC, and, when red cells manage to traverse the fibrin mesh, microangiopathic features can be observed. Hemorrhage will eventually occur, due to the consumption of platelets and clotting factors that follows the continuous activation of coagulation pathways. Moreover, the formation of fibrin-rich microthrombi will result in induction of t-PA release from the vessel wall (due to stasis, hypoxia, acidosis, and perhaps other factors) and initiation of secondary fibrinolysis, despite the presence of high plasma levels of PAI-1 [16]. As a consequence, fibrin degradation products having anticoagulant effects will be generated and, if an excess of plasmin is formed, further consumption of coagulation factors will ensue, thus aggravating the bleeding.

Considering that endotoxin represents perhaps the most powerful stimulus for both mononuclear phagocytes and endothelial cells and is also a potent inducer of virtually all the endogenous mediators (especially cyto kines) that affect the function of these cells, the pathogenetic sequence outlined above provides a likely explanation for DIC observed in the course of gram-negative sepsis. Recently, it has become apparent that effects similar to those induced by endotoxin may be caused by gram-positive bacteria or their cell wall components

(muramyl dipeptide, and perhaps others), some bacterial exotoxins, rickettsiae, and viruses. More importantly, cytokines, particularly TNF, can be released in vivo in infections other than gram-negative sepsis and could also represent the pivotal endogenous mediators in these conditions [26]. Based on these considerations, it is not surprising that virtually every infectious agent may alter the coagulation-fibrinolysis balance of endothelial cells and mononuclear phagocytes and thus cause DIC. Our present knowledge may have important implications in the management of infected patients with DIC. There is no doubt that the most important aspects of therapy are those directed towards elimination of the infectious agent and general support measures. However, antibiotics appear to have limited immediate effect on the outcome of acute DIC, although they often rapidly eliminate the causative bacteria and sterilize the bloodstream. Release of endotoxin and/or other bacterial products caused by the action of antibiotics may even result in clinical deterioration. Moreover, in most viral diseases elimination of the infectious agent is not yet possible. On the other hand, treatments aimed at reversing coagulation abnormalities, particularly heparin, are difficult and often unsuccessful, although new approaches for inhibiting blood clotting are being developed and look promising (antithrombin III concentrates, recombinant hirudin). These concerns have focussed interest on developing therapeutic strategies that inhibit the key toxins (e.g., anti-endotoxin monoclonal antibodies) or mediators (e.g., anti-TNF monoclonal antibodies). The validity of this approach is underscored by the encouraging results obtained in experimental models and in a few human studies [5, 59].

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