Coagulopathy of Acute Sepsis

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Abstract

Coagulopathy is common in acute sepsis and may range from subclinical activation of blood coagulation (hypercoagulability), which may contribute to venous thromboembolism, to acute disseminated intravascular coagulation, characterized by widespread microvascular thrombosis and consumption of platelets and coagulation proteins, eventually causing bleeding. The key event underlying this life-threatening complication is the overwhelming inflammatory host response to the pathogen leading to the overexpression of inflammatory mediators. The latter, along with the microorganism and its derivatives drive the major changes responsible for massive thrombin formation and fibrin deposition: (1) aberrant expression of tissue factor mainly by monocytes-macrophages, (2) impairment of anticoagulant pathways, orchestrated by dysfunctional endothelial cells (ECs), and (3) suppression of fibrinolysis because of the overproduction of plasminogen activator inhibitor-1 by ECs and thrombin-mediated activation of thrombin-activatable fibrinolysis inhibitor. Neutrophils and other cells, upon activation or death, release nuclear materials (neutrophil extracellular traps and/or their components such as histones, DNA, lysosomal enzymes, and High Mobility Group Box-1), which have toxic, proinflammatory and prothrombotic properties thus contributing to clotting dysregulation. The ensuing microvascular thrombosis–ischemia significantly contributes to tissue injury and multiple organ dysfunction syndromes. These insights into the pathogenesis of sepsis-associated coagulopathy may have implications for the development of new diagnostic and therapeutic tools.

Keywords

- infection
- coagulation
- fibrinolysis
- neutrophil extracellular traps
- microvascular thrombosis

Coagulopathy is a common feature of acute sepsis and comprises a wide spectrum of hemostatic changes ranging from thrombocytopenia and/or subclinical activation of blood coagulation (hypercoagulability) to uncontrolled, systemic clotting activation with massive thrombin formation and fibrin deposition in the microcirculation, eventually leading to consumption of platelets and proteins of the hemostatic system (acute disseminated intravascular coagulation, DIC).[1,2] From a clinical standpoint, septic patients may present with localized thrombotic manifestations, as indicated by the observation that they are at increased risk for venous thromboembolism.[3,4] However, the most common and dramatic clinical feature is widespread thrombosis in the microcirculation of different tissues which causes hypoxic-ischemic tissue injury and contributes to the altered function of one or more organs.[1,2] The development of multiple organ dysfunction syndrome (MODS), the hallmark of severe sepsis and septic shock, is a major determinant of the high morbidity and mortality in these conditions. Although several closely interlinked mechanisms have been proposed to explain this dramatic event,[5] an important role of DIC is supported by several lines of evidence[2,6]: (1) thrombosis in small and mid-size vessels of multiple organs and its relationship with organ ischemia and dysfunction has been documented by

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numerous histological studies in septic patients and in animals with sepsis or endotoxemia; (2) in experimental models of sepsis, amelioration of DIC by various interventions improves organ failure and, in some cases, mortality; (3) DIC is an independent predictor of organ dysfunction and mortality in septic patients. In severe (fulminant) DIC, the progressive consumption of platelets and coagulation proteins will result in simultaneous or delayed bleeding of different severity, ranging from oozing at arterial or venous puncture sites to profuse hemorrhage from various sites. DIC is classically associated with Gram-negative bacterial infections but it can also occur in Gram-positive sepsis (with a similar incidence) and in systemic fungal, viral, and parasitic infections.[1,2,7]

The pathophysiology of sepsis-associated DIC is extremely complex and still under extensive investigation. The key event is the systemic overwhelming host inflammatory response to the infectious agent (SIRS, systemic inflammatory response syndrome).[8] After sensing danger-associated molecular patterns (DAMPs), including both unique constituents expressed by the causative microorganism (PAMPs, pathogen-associated molecular patterns) and factors derived from damaged host cells (alarmins), through specific receptors (PRRs, pattern recognition receptors, primarily the TLRs, Toll-like receptors), innate immune and other host cells (monocytes-macrophages, neutrophils, platelets, and endothelial cells) synthesize and release large amounts of proinflammatory mediators, mainly cytokines and chemokines (►**Fig. 1**). The latter, together with other mediators generated by the inflammatory cascade, including complement activation products,[8] act in concert with the microorganisms and/or their derivatives to trigger inflammation and coagulation pathways, DIC and organ dysfunction.[1,2,6] Enzymes generated during the clotting cascade (thrombin, factor Xa, and factor VIIa), in turn, interact with specific cellular receptors (PARs, protease-activated receptors) and elicit cell responses that amplify the inflammatory reactions,[9,10] creating a vicious cycle. In addition, evidence accumulated during the last years indicate that nuclear products released by activated innate immune cells (mainly neutrophils) and/or by dead cells are endowed with inflammatory, cytotoxic, and prothrombotic properties and thus they may significantly contribute to the initiation and propagation of inflammation and coagulation pathways, and to tissue injury and organ failure occurring in acute sepsis.[11,12] This review will briefly outline current knowledge on the pathogenesis of sepsis-associated DIC and the ensuing development of new potential diagnostic and therapeutic tools.

Pathogenesis of Sepsis-Associated Coagulopathy and Thrombus Formation

It is widely recognized that the causative agent and especially the mediators generated by the inflammatory response drive thrombus formation by at least three simultaneously acting mechanisms: (1) upregulation of procoagulant pathways, (2) downregulation of physiological anticoagulants, and (3) suppression of fibrinolysis.[1,2,7] Virtually all cells participating in acute systemic inflammation, that is, endothelial cells (ECs), monocytes-macrophages, neutrophils, and platelets, variably cooperate to each of these mechanisms.

Upregulation of Procoagulant Pathways: The Central Role of Tissue Factor

Currently, the aberrant in vivo expression of tissue factor (TF) is thought to play a pivotal role in sepsis-associated blood clotting activation. This view is strongly supported by the following observations. (1) The impairment of the TF pathway by various means prevents coagulation abnormalities (including fibrin deposition in target tissues) and lethality

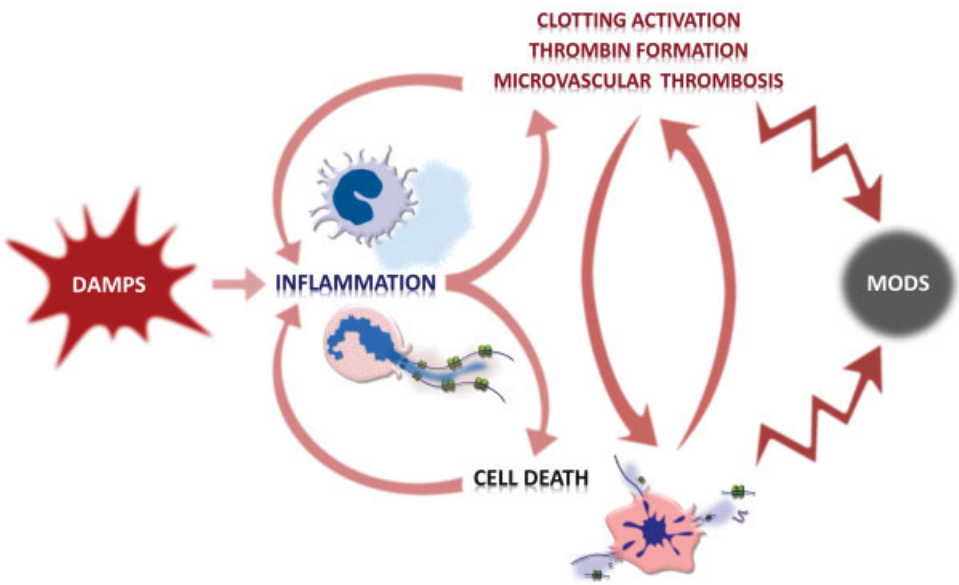

Fig. 1 Crosstalk between inflammation, coagulation, and tissue damage in the development of sepsis-associated multiorgan failure (see text for details).

in numerous animal models of sepsis or endotoxemia.[2,7,13] (2) The plasma levels of TF are increased in septic patients and generally associated with raised concentrations of markers of clotting activation.[2,7,14]

As to the cellular source of TF in sepsis there is still some debate. In vitro, ECs and monocytes-macrophages have long been known to synthesize TF in response to a wide variety of stimulating agents or conditions that are of pathophysiological importance in sepsis,[2,15] and, more recently, TF expression has been detected also in human polymorphonuclear leukocytes (especially neutrophils) upon stimulation by inflammatory agents[2,16] and in platelets activated by various agonists.[2,17,18] It should be noted that, according to some investigators, neutrophils and platelets do not synthesize TF but rather they acquire it by binding TF-expressing microparticles (MPs).[2,13,19] MPs are small-membrane vesicles released from activated or apoptotic cells that can be transferred to the surface of other cells via specific receptors (for instance, PSGL-1 on leukocyte-derived MPs and P-selectin on activated platelets or ECs) making the recipient cell capable of triggering and propagating coagulation.[2,15]

Although all the aforementioned cells, being actively involved in the systemic inflammatory response, might contribute to the aberrant in vivo expression of TF, available studies point to activated monocytes-macrophages as the main triggers of blood coagulation during sepsis. In animal models of endotoxemia or sepsis, TF is increased in target organs where fibrin is often deposited during DIC (i.e., lung, kidney, liver, brain, and spleen) and, at cellular level, it is detected mainly in monocytes present in the microcirculation and macrophages infiltrating the involved tissues; in these animals, also blood monocytes and macrophages of different origin express strong TF activity.[2,13,15,20] In addition, a selective genetic deficiency of TF expression by hematopoietic cells as well as the deletion of TF gene in myeloid cells reduced lipopolysaccharide-induced coagulation, inflammation, and mortality in mice.[13,21] Increased expression of monocyte-macrophage TF has been also documented in healthy volunteers after the administration of low-dose endotoxin,[22] in septic or endotoxemic patients, in whom TF was associated with clotting activation, MODS and lethal outcome, and in patients with peritonitis or acute respiratory distress syndrome.[2,15,20] Moreover, increased numbers of circulating TF-positive MPs of monocyte origin have been detected in patients with meningococcal sepsis and in human low-dose endotoxemia,[2,23] and levels of MP TF activity were correlated with coagulation activation in endotoxemic mice.[24] Surprisingly, and in contrast with the abundant in vitro evidence, ECs were negative for TF in most animal studies, with very few exceptions.[2,13,19,20] Notably, the deletion of the TF gene in ECs had no significant effect on clotting activation in endotoxemic mice,[13,21] ruling out a major involvement of EC-derived TF. ECs, however, may contribute to clotting activation and thrombus formation by other mechanisms. These cells play a critical role in orchestrating the host response to sepsis and are the target of DAMPs and inflammatory mediators.[25] As a consequence, ECs become activated and adopt a proinflammatory phenotype that initiates the recruitment and activation of innate immune cells (mainly monocytes and neutrophils) through the expression of adhesion molecules. P-selectin is of particular importance in this context because, as mentioned above, it binds TF-positive MPs via PSGL-1.[2,13] Interestingly, MPs taken up by ECs are internalized and the TF moiety is recycled to the cell surface thus inducing a substantial increase in the cell procoagulant potential.[26] In addition, activated ECs are known to secrete von Willebrand factor (VWF) in its highly platelet-agglutinating form (i.e., ultralarge VWF multimers) from Weibel–Palade bodies, eventually resulting in platelet activation and platelet-mediated clotting stimulation.[25] As a matter of fact, increased plasma levels of VWF have been reported in systemic inflammation including sepsis.[27] In parallel, decreased plasma levels of the VWF-cleaving protease ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motif) are seen, likely due to downregulation at transcriptional level, proteolytic degradation, and consumption.[27] In some studies the levels of ADAMTS13 and VWF correlated with disease severity, organ dysfunction, and/or outcome[27,28] suggesting that these parameters might be useful for the diagnosis and the therapeutic monitoring of septic patients.

Similar to ECs, the role of neutrophils and platelets as a direct source of TF in vivo during sepsis remains controversial.[2,13,21,29] These cells, however, may participate in the activation of coagulation and thrombus formation by other mechanisms, besides the binding of TF-positive MPs.[2,13] During sepsis platelets are activated by DAMPs and other inflammatory mediators (e.g., platelet-activating factor, PAF), by adhesion to damaged endothelium and VWF (see above) or by thrombin. The expression of P-selectin mediates the binding of platelets to monocytes and enhances the production of TF by these cells.[6] Platelet activation also provides a suitable phospholipid surface (anionic phospholipids, mainly phosphatidylserine) that catalyzes the coagulation reactions several folds and renders clotting enzymes less susceptible to fluid phase protease inhibitors. Moreover, activated platelets and platelet-derived MPs may induce thrombin generation independently of TF via activation of factor XII (FXII). There is now ample evidence that the platelet-derived surface activating FXII is provided by soluble polyphosphates (poly-P) that are composed of 60–100 linear linked phosphate subunits and are released from platelet dense granules upon activation.[30] The mechanism whereby neutrophils contribute to dysregulation of coagulation in sepsis is discussed below.

Pawlinski et al[13,21] have shown that the selective inhibition of TF expressed by nonhematopoietic cells reduces the clotting activation in endotoxemic mice suggesting other unknown cellular sources of TF. In endotoxemic and septic animals, TF expression is increased not only in monocytes-macrophages but also in tissue cells, for example, lung and kidney epithelial cells, and brain astrocytes.[2,13,20] Therefore, considering also that the role of ECs and vascular smooth muscle cells[21] remains uncertain, it is likely that TF upregulation in parenchymal cells of target organs may contribute to clotting coagulation during sepsis. Moreover, the obvious increase in vascular permeability and vascular damage occurring during severe

inflammation will allow the exposure of extravascular (e.g., fibroblast-associated) TF to blood.

Concerning the relative role of the main endogenous proinflammatory mediators in the in vivo induction of TF-induced clotting activation associated with sepsis or endotoxemia, the neutralization studies with specific antibodies against individual cytokines and with inhibitors of complement activation would suggest a major role of interleukin-6 (IL-6), IL-1β (albeit to a lesser extent), and complement-derived mediators.[31,32]

Downregulation of Physiological Anticoagulant Mechanisms

Among the various components of the anticoagulant pathways physiologically expressed by ECs, namely, thrombomodulin (TM), endothelial protein C receptor (EPCR), protein S (PS), tissue factor pathway inhibitor (TFPI) and the heparin-like proteoglycan heparan sulfate, those involved in the protein C (PC) pathway have been most extensively investigated in sepsis. In cultured ECs, inflammatory mediators consistently reduced the expression of TM and EPCR, and the PS secretion.[2,7,33,34] Although animal studies on the expression of TM and EPCR by ECs produced rather controversial results,[2,7,33,34] the rise in plasma levels of soluble TM and EPCR observed in endotoxemic animals suggests that endothelial activation/damage by DAMPs and inflammatory mediators does occur in vivo.[2,33,34] The central role of the PC pathway in sepsis-associated DIC is definitely demonstrated by the observation that compromising the PC system resulted in a marked worsening of DIC and in increased morbidity and mortality in different animal models, whereas restoring an adequate activated protein C (APC) function (e.g., treatment with APC) prevented the coagulopathy and improved organ failure and survival.[6,33] Interestingly, mice with heterozygous PC deficiency had more severe DIC and a higher mortality than the wild-type controls and mice homozygous for a point mutation of the TM gene that deletes the anticoagulant activity of the protein exhibited 10- to 30-fold greater amounts of fibrin in the microcirculation of several organs than the wild-type mice.[1,2]

Studies in human sepsis have in general confirmed the dysfunction of the PC pathway. The plasma levels of soluble TM and EPCR were increased and TM levels were often correlated with disease severity and poor outcome.[2,33,35] Moreover, septic patients have low levels of PC and PS, due to impaired liver synthesis and/or consumption, and low levels of free PS, due to increased C4b-binding protein.[7,33] Acquired severe PC deficiency is associated with early death.[36] Notably, the expression of TM and EPCR on morphologically intact ECs of dermal vessels was reduced in biopsy specimens of purpuric lesions from children with meningococcal sepsis, as compared with control skin-biopsy specimens.[35] Plasma levels of APC remained low in some of these patients even after treatment with PC concentrates, confirming downregulation of TM in vivo and impaired PC activation. APC plasma levels were found to vary markedly among patients with severe sepsis and were significantly higher in survivors than in nonsurvivors (28-day mortality), suggesting that endogenous APC serves as protective functions.[37] As a matter of fact, apart from its anticoagulant and profibrinolytic activities, APC is endowed with several anti-inflammatory effects, including downregulation of cytokines and TF in activated leukocytes, antioxidant and antiapoptotic activities, and preservation of endothelial barrier function.[38]

During sepsis, an impairment of the heparan-sulfate-antithrombin (AT) axis has also been reported. Indeed, inflammatory stimuli are able to downregulate the expression of heparan sulfate in cultured ECs,[7,33] and plasma levels of AT are generally decreased in septic patients because of consumption, the lowest levels being associated with increased mortality.[7]

With respect to TFPI, a decreased expression was found in ECs of several organs in animal models of endotoxemia or sepsis.[33,39] In addition, anti-TFPI antibodies increased fibrin accumulation in the lungs of septic baboon,[39] suggesting that TFPI underexpression, coupled with TF upregulation, might augment the local procoagulant potential, thus promoting fibrin formation in tissues. Despite these findings, the role of TFPI in the regulation of sepsis-associated coagulation activation still remains incompletely understood, particularly in humans.

Downregulation of the anticoagulant pathways in vivo has been attributed mainly to tumor necrosis factor-α (TNF-α), IL-1 and complement-derived mediators, as evidenced by neutralization studies in animal models of sepsis or endotoxemia.[31,32]

Suppression of Fibrinolysis

One of the main mechanisms responsible for sepsis-associated hypofibrinolysis is an increased production of plasminogen activator inhibitor-1 (PAI-1) by ECs, as consistently demonstrated by several in vitro studies on cultured ECs challenged with endotoxin or inflammatory mediators and in vivo experiments in animal models of endotoxemia or sepsis.[6,33,40] In general, a simultaneous increase in tissue-type plasminogen activator (t-PA) does occur, but the net result is almost invariably a fibrinolytic shutdown because of the large amounts of PAI-1.[1,2,40] It should be noted that, in some models of endotoxemia or cytokinemia, hypofibrinolysis and fibrin deposition in adrenals and/or kidneys are most dependent on a decrease in PAs.[1,41] Therefore, the impairment of fibrinolysis mediated by PAI-1 increase and other tissue- and species-specific alterations, such as decreased PAs in some models, appears to be essential for fibrin deposition in tissue vasculature. This view is supported by the observation that, when challenged with endotoxin, mice deficient in PAs have more extensive fibrin deposition in tissues, whereas PAI-1 knockout mice, in contrast to wild-type controls, have no microvascular thrombosis.[1,41] The increase in endothelium-derived PAI-1 in animal models of sepsis or endotoxemia appears to be due primarily to TNF-α, IL-1, and complement-derived mediators.[31,32]

In human sepsis, a sustained increase in plasma PAI-1 has been consistently reported by numerous studies and, in some of them, PAI-1 appears to have a prognostic value.[7,41] Again, plasma t-PA is also elevated,[7,41] but the net effect is definitely

antifibrinolytic. The role of PAI-1 is supported by the finding that a 4G/5G polymorphism in the PAI-1 promoter influencing PAI-1 expression is associated with the clinical outcome of severe sepsis.[7,41] Moreover, in a multicenter clinical trial, the fibrinolytic shutdown in septic patients was confirmed by a plasma clot lysis assay, which showed that fibrinolytic resistance increased with the severity of sepsis and predicted shock and kidney failure (Colucci et al, in preparation).

More recent evidence indicates that other thrombin-dependent mechanisms might contribute to hypofibrinolysis during sepsis. Thrombin is known to cause resistance to fibrinolysis by forming more compact and less permeable clots[42] and by activating thrombin-activatable fibrinolysis inhibitor (TAFI), a plasma procarboxypeptidase that, once activated (TAFIa), removes the C-terminal lysines from partially degraded fibrin, thereby reducing plasmin generation.[43] Enhanced thrombin generation, the hallmark of sepsis, might influence the fibrin structure as suggested by the following observations. ECs stimulated by inflammatory cytokines to express TF cause the production of abnormally dense, fibrinolysis-resistant fibrin networks.[44] In addition, activated platelets, commonly found in sepsis, increase fibrinolytic resistance either by altering the fibrin structure via the direct interaction between fibrin and αIIbβ3 integrin[44] and via the release of inorganic poly-P,[45] or by promoting TAFI activation.[46] Finally, activated human monocytes were shown to inhibit fibrinolysis through a TF-mediated enhancement of TAFI activation.[47]

In animal models of endotoxemia or sepsis, TAFI levels are usually reduced, likely because of activation and consumption.[41] In addition, blocking TAFIa with synthetic inhibitors or inhibiting thrombin-TM-dependent TAFI activation enhances the rate of fibrin degradation and reduces fibrin deposition in target tissues.[41] In human studies, TAFI levels are consistently decreased in septic patients and in healthy volunteers with low-grade endotoxemia.[41] Of note, in severe meningococcal infection,[48] the levels of TAFI activation markers are increased in patients with DIC as compared with those without, are significantly higher in nonsurvivors than in survivors and strongly correlated with severity scores of the disease. Therefore, TAFI activation seems to occur in severe sepsis and the measurement of TAFI activation markers may be clinically useful. The role of TAFI is further supported by the fact that a single nucleotide polymorphism in the TAFI gene that causes the substitution of Thr325Ile and produces increased TAFIa stability/activity is associated with a poor outcome in meningococcal sepsis.[41]

The Role of Nuclear Products in Sepsis-Associated Thrombus Formation

Over the last few years, new players have been found to importantly contribute to the pathological derangement of coagulation and thrombus formation during sepsis. These novel thrombogenic agents are represented by nuclear products, exposed to the extracellular space either in isolated form or arranged in complex structures called neutrophil extracellular traps (NETs).[49] NETs are networks of chromatin filaments made up of histones and DNA strands, decorated with proteins and lysosomal enzymes (myeloperoxidase, elastase, and cathepsin G among others) and are released by neutrophils upon exposure to a variety of stimuli such as major types of microorganisms (bacteria, fungi, protozoa, viruses) and their products, inflammatory mediators and reactive oxygen species (ROS). Noteworthily, activated platelets are potent inducers of NET formation as a consequence of their interaction with neutrophils.[50] Extracellular traps (ETs) can be actively extruded also by other innate immune cells, such as mast cells, eosinophils, and mononuclear phagocytes upon activation.[49] Individual components (histones and DNA, mainly as nucleosomes) can be passively released by dying cells.[51] NET formation involves the unwinding of nuclear DNA fibers and the breakdown of the nuclear membrane before the final active discharge in the extracellular milieu. This process is mediated by nuclear factor kappa B (NF-kB) signaling,[52] peptidylarginine deiminase 4 (PAD4), and neutrophil elastase (NE), which cooperate to modify histones and enable DNA decondensation, and ROS via NADPH oxidase, although ROS may not be needed in the presence of some neutrophil stimuli.[12,49]

Since the characterization of NETs as a major innate immunity mechanism to trap, restrain, and eventually neutralize invading microorganisms,[49] numerous studies underscore the role of NETs as a new interface between inflammation and the hemostatic system. As shown for the first time by Fuchs et al,[53] NETs per se are able to promote thrombosis as they may provide a three-dimensional scaffold for recruitment of platelets and red blood cells (RBCs), and adsorb several proteins involved in thrombus formation such as VWF, fibronectin, fibrinogen, and even cell-derived TF.[54] NETs co-localize with fibrin and likely they interact closely with fibrin strands in the thrombus, thus potentially influencing thrombus organization and stability.[53]

Mechanistically, NET's constituents are primarily responsible for thrombus formation as they display a variety of prothrombotic activities (►**Fig. 2**). Histones, the most abundant proteins in NETs, induce platelet activation (adhesion and spreading, fibrinogen binding, platelet aggregation, VWF release, P-selectin expression, and the formation of platelet–leukocyte aggregates) either directly[55] or indirectly (via the binding of VWF or fibrinogen). Histones promote thrombin formation through different pathways: (1) they make red blood cells procoagulant through the exposure of anionic phospholipids[56]; (2) via a TLR4- and TLR2-dependent platelet activation pathway, they induce the release of poly-P from platelets, which trigger coagulation independently of FXII[57]; (3) enhance the expression of platelet procoagulant properties (anionic phospholipids and factor V/Va)[55]; and (4) impair TM-mediated protein C activation.[58] Histones are also endowed with general cytotoxic effects; of particular relevance, in the context of this discussion, is the histone-induced injury of ECs which will result in the exposure of the thrombogenic subendothelial surface.[11] Most of these effects are attributable to histone H3 and, especially, to H4. The importance of histones is supported by in vivo experiments showing that, when administered to animals at low doses,

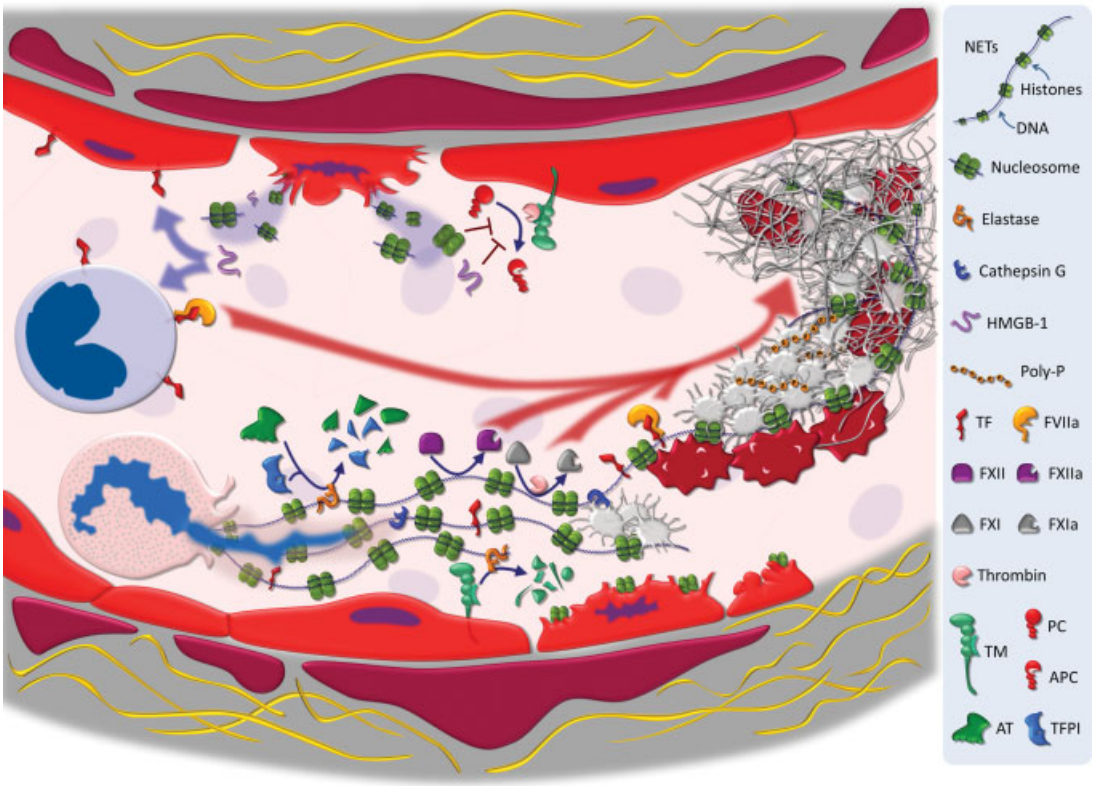

Fig. 2 Schematic representation of the prothrombotic mechanisms of NETs and nuclear products. The components of NETs trigger thrombin generation and fibrin deposition by several mechanisms: (1) activation of the intrinsic and extrinsic coagulation pathways; (2) degradation of anticoagulant factors; and (3) binding and activation of platelets and erythrocytes. Histone-induced toxicity and extensive cell damage further enhance the prothrombotic tendency by stimulating TF exposure, impairing anticoagulant pathways and exposing subendothelial structures. See text for details.

they cause thrombocytopenia and stimulate thrombin generation,[12] whereas at high doses they are lethal and mimic the manifestations of sepsis, including microvascular thrombosis, organ failure, and death.[11] Interestingly, neutralization of histones by non-anticoagulant heparin-derived compounds[59] or by antibodies specifically targeting histone H4[11] protects mice in different models of endotoxemia and sepsis. It should also be noted that recombinant APC degrades histones thus lowering their toxicity toward ECs in vitro, abolishing their ability to activate platelets and RBCs[57,58] and preventing lethality in histone-treated animals.[11] Similar to the inorganic poly-P released by activated platelets, double-stranded DNA serves as a suitable negatively charged surface that initiates the intrinsic pathway of coagulation by favoring the auto-activation of FXII and potentiating FXI activation by thrombin.[60] Among proteins and enzymes hosted in NETs, elastase cleaves the major physiological anticoagulants TFPI, AT, and TM, and thus allows the coagulation reactions to proceed uncontrolled[6,12,41]; myeloperoxidase oxidizes and inactivates TM, and cathepsin G further augments platelet activation on the NET surface.[61] Finally, NETs can harbor neutrophil or blood-derived TF which initiates the extrinsic coagulation pathway.[54]

It is worth mentioning that, in infections, the microvascular thrombosis triggered by the innate immune cells activated by contact with blood-borne microorganisms, through the release of NETs (neutrophils) and through the expression of TF (monocytes), has been proposed to act as an antimicrobial mechanism that protects the host against pathogens. This process, called immunothrombosis, also involves activated platelets and ECs that promote both local accumulation of innate immune cells and thrombus formation.[62] Of course, in acute sepsis, the situation is completely different. The widespread dissemination of the pathogen and its derivatives into the circulation and the ensuing SIRS with its plethora of inflammatory mediators will cause massive recruitment and activation of innate immune cells eventually leading to excessive NET release and TF expression, and DIC. The latter, therefore, may be considered a form of uncontrolled immunothrombosis.[62] Excessive activation of inflammation and unrestricted formation of thrombi in microvasculature are further worsened by the ability of both processes to potentiate each other. NETs are abundant in venous and arterial thrombi from animals and patients[12] as well as in microvascular thrombi[63] and, in some models, inhibiting NET formation prevents thrombosis,[12] indicating their importance for thrombus formation.

Similar to DNA and histones, another nuclear product, namely HMGB-1, can be actively secreted by stimulated immune cells or passively released by necrotic cells; upon translocation to the extracellular milieu it acts as a lethal mediator of systemic inflammation.[64] In both animal models

and human sepsis, HMGB-1 levels rise into the circulation, and targeting HMGB-1 with antibodies confers protection against lethal endotoxemia and sepsis.[64] HMGB-1 is significantly involved in sepsis-associated microvascular thrombosis by stimulating TF expression in monocytes and ECs and by reducing the activity of thrombin-TM complex with consequent reduction in protein C activation.[65,66] Interestingly, platelet-derived HMGB-1 promotes the extrusion of NETs in a process that involves the HMGB-1 receptor RAGE (receptor for advanced glycation end products).[67]

Acute sepsis is considered to be the most relevant clinical disorder in which necrosis and apoptosis occur, and, as a matter of fact, a marked increase in apoptosis has been observed in septic patients compared with nonseptic, critically ill patients and healthy controls.[68] Because of a massive apoptosis-necrosis overwhelming the clearance mechanisms, extracellular nucleosomes, DNA, histones, and HMGB-1 released during the late stages of sepsis can amplify inflammation, coagulation, and cell death and thus importantly contribute to MODS. This is supported by the increase in the levels of free histones, DNA, nucleosomes, and HMGB-1 in septic patients and, more importantly, by their direct correlation with disease severity and mortality,[69–71] which supports the potential use of these markers as useful prognostic factors.

Conclusion and Perspectives

As briefly summarized earlier, considerable progress has been made over the last few years in regard to our understanding of the complex events involved in the pathological derangement of the hemostatic process that leads to DIC and contributes to MODS in acute sepsis. Whether the current knowledge will prove useful for the development of new diagnostic and therapeutic tools remains to be established. Although numerous laboratory tests are available, including global assays and markers of endothelial activation,[72,73] the diagnosis of DIC is still based on the combination of a typical underlying disease, such as sepsis, with simple laboratory markers, including platelet count, prothrombin time, activated partial thromboplastin time, fibrinogen concentration, and a fibrin-related marker, reflecting intravascular fibrin formation, such as D-dimer, all of which are used in the DIC scores.[72] Some new parameters that could be of clinical utility are currently being investigated. For instance, elevated plasma levels of nucleosomes, and/or cell-free DNA have been reported in septic patients that paralleled the disease severity,[69,71,74] and single histones H3 and H4 were increased in patients with severe sepsis.[70] Moreover, circulating HMGB-1 is consistently augmented in septic patients, being significantly higher in nonsurvivors than in survivors, and its plasma concentration has been proposed as a possible prognostic marker of DIC and organ failure.[75] Nuclear proteins, therefore, might be new sensitive biomarkers of disease progression and useful predictors of outcome in sepsis.

As regard the supportive treatment for sepsis-associated hemostatic abnormalities, different strategies have been developed based on the insights into the pathogenetic mechanisms responsible for microthrombosis in sepsis. TF inhibitors would be the most logical treatment considering the pivotal role of TF in clotting activation during sepsis. However, a phase III clinical trial with recombinant TFPI did not show an overall survival benefit in septic patients.[6] Likewise, treatment with AT concentrates, despite several reported beneficial effects (improvement of laboratory parameters, shortening of DIC duration, and amelioration of organ function), failed to significantly reduce the mortality of septic patients in a large-scale clinical trial.[6] Based on the notion that the depression of the PC system significantly contributes to the pathophysiology of DIC, supplementation of APC might be of benefit, also considering that this drug, besides restoring the PC anticoagulant pathway, has well-known anti-inflammatory action[34,38] and is able to degrade histones.[11] In fact, recombinant human (rh)-APC was found to reduce mortality of patients with severe sepsis at high risk of death[7] and, until recently, it has been approved for use in these patients. However, after the failure of the most recent randomized controlled trial, PROWESS-SHOCK,[76] rhAPC was withdrawn from the world market and its role in the treatment of severe sepsis appears to have subsided, although controversies remain.[77,78] TM would represent another therapeutic option. Besides favoring PC activation, TM can also neutralize circulating histones[79] and aid thrombin in the cleavage of HMGB-1.[80] Administration of rhTM has been associated with reduced in-hospital mortality in adult patients with sepsis-induced DIC[78] and a phase III study is being conducted in subjects with severe sepsis and coagulopathy.

Considering the emerging role of NETs and/or their constituents in dysregulation of coagulation and formation of microthrombi associated with acute sepsis, active regulation or neutralization of these compounds could be a novel therapeutic strategy. Inhibitors of the enzymes PAD4 and NADPH-oxidase, and of the transcription factor NF-kB, all of which are involved in NETs formation, are possible candidates for active regulation of NETs. Actually, an NADPH oxidase inhibitor ameliorated the influenza A virus-induced lung inflammation in which excessive NETs were involved.[81] Dismantling of NETs by deoxyribonuclease (DNase) 1 could be another potential strategy for the treatment of sepsis-associated coagulopathy, as suggested from animal studies of deep venous thrombosis that DNase1 administration suppresses thrombosis through reduced NET formation.[12] Of note, impaired DNase1-mediated degradation of NETs has recently been shown to be associated with acute thrombotic microangiopathies.[82] Since histones, the most toxic NET components, along with HMGB-1, seem to be critical mediators of organ dysfunction and death in septic patients, an attractive approach to treat MODS and prevent death could be the development of effective histone and HMGB-1 antagonists,[11] which might prove therapeutic without the bleeding complications that can result from APC therapy.

Conflict of Interest
The authors state that they have no conflict of interest.

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