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Hemostatic Soluble Plasma Proteins During Acute-Phase Response and Chronic Inflammation

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1. Introduction

Coagulation and inflammation are the interdependent processes attributed to the host defence response to injuries. A synchronized activity of both pathways represents an essential prerequisite for restitution of host homeostasis after ultimate disturbances of the latter. Crosstalk between coagulation and inflammation is considered to inherit from primitive coagulation systems similar to invertebrates. For instance, in Horseshoe "crabs" (*Limulus*) coagulogen, a clotting protein with the bactericidal function, and coagulation-related serine proteases are both located in circulating hemolymph cells (hemocytes) and are capable of simultaneously protecting against injury, as well as to isolate pathogens within cysts (Tanaka et al., 2009). In humans, the complement system as a part of innate immunity remains closely related to the hemostasis system (Markiewski et al., 2007). The organizations of these two systems demonstrate several similarities of both structural and functional features. Both systems are organized into proteolytic cascades; serine proteases of the chymotrypsin family are the components of the latter. Proteases of each system are, in their molecular structure, glycoproteins, which have a highly conservative catalytic site composed of serine, histidine, and aspartic amino acid residues. These proteases exist in the form of inactive zymogens and are subsequently activated by upstream, active proteases. Proteins of the complement and hemostasis systems are mostly synthesized in the liver by hepatocytes, besides being additionally produced during an acute-phase response stimulated by common inflammatory mediators. The biological role of the complement system is mentioned here in order to note that the above-mentioned similarities represent a forcible argument in favour of the common origin of both immunity and hemostasis phenomena. The complement system has been described in detail recently (Castellheim et al., 2009; Markiewski et al., 2007) and will not be further discussed here. Though, it is worthwhile to mention that the blood hemostasis system in humans, hypothetically, has been evolved progressively from the archaic innate immunity organization, diverging to certain narrow-specific pathways, which are responsible for the coagulation control.

Hemostasis in humans is organized as closely interrelated enzyme cascade systems: (i) fibrin clotting system (coagulation); (ii) multilevel system for preventing uncontrolled fibrin formation (anticoagulation); and (iii) system for limiting the amount of cross-linked fibrin (fibrinolysis). Together, coagulation, anticoagulation and fibrinolysis are associated into a

self-regulated and highly organized molecular machine that provides either acceleration or reduction of blood hemostasis process (Spronk et al., 2003). Accurate regulation of the coagulation rate is an effective mechanism, preventing circulatory disorders. This regulatory mechanism is known to be a part of the acute phase of the inflammatory reaction which provides rapid restoration of physiological homeostasis.

Hemostasis is closely linked to innate immunity and inflammation through particular regulatory coupling like protein C system (Fig. 1). Cooperation between hemostasis and innate immunity facilitates injury recognition, vessel wall reparation, and prevention of excessive bleeding without causing thrombosis. These processes are mediated by a balance of cellular surface components, cell-derived factors, and soluble plasma proteins (SPPs). After interruption of the vascular integrity, concentrations of some hemostatic SPPs (HSPPs) change in a manner typical of those of acute-phase proteins (APPs). In agreement with definition of APPs (Kushner & Rzewnicki, 1994; Morley & Kushner, 1982), at least several HSPPs including fibrinogen, plasminogen, and PAI-1 should also be classified as APPs, since: (i) the intensity of synthesis of these HSPPs dramatically changes (by more than 50%) during pathological processes (ii) HSPPs synthesis in hepatocytes during the acute-phase response are regulated by inflammatory-associated cytokines; (iii) due to chronic stimulation by inflammatory mediators, HSPPs may persist in circulation and participate in the formation of a "semantically paradoxical chronic acute-phase response" (Gabay & Kushner, 2001). The above-indicated similarities between inflammation and hemostasis determine their unidirectional changes. These changes involve pro-coagulant activities of inflammatory processes, as well as the pro-inflammatory efficacy of the hemostatic molecular machine.

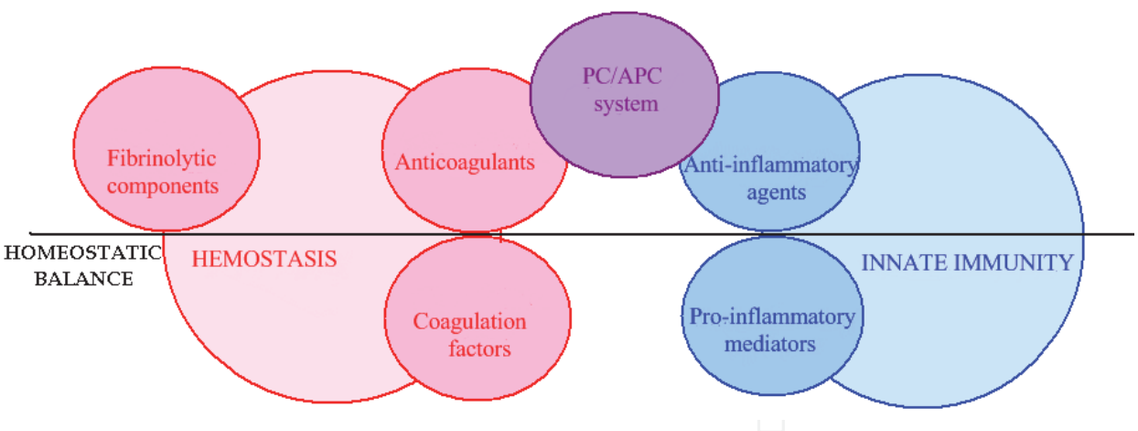

Fig. 1. Schematic representation of the protein C-dependent cross-point between hemostatic and inflammatory pathways. Diagram illustrates a putative regulatory mechanism, maintaining homeostasis within physiological limits. Both pathways are balanced by the PC/APC (protein C/activated protein C) system, directly by attenuation of production of thrombin as a pro-inflammatory and pro-coagulant agent, or indirectly, by controlling NF-kB-dependent anti-inflammatory pathways through EPCRs (endothelial protein C receptors) and PARs (protease-activated receptors) at the surface of the target cells. See the text for discussion in detail.

The capacity of inflammatory mediators to regulate hemostasis, as well as some aspects of the coagulation ability to affect inflammatory events have been extensively reviewed (Butenas et al., 2009; Danese et al., 2010; Esmon, 2005; Jennewein et al., 2011; Levi et al., 2004; Medcalf, 2007). Reciprocal regulation of gene expression is the most important mechanism, by which inflammatory and hemostatic pathways interact with each other. The role of cell surface receptors in providing APP-associated signalling has been recently elucidated (Busso et al., 2008; Guitton et al., 2011). The role of the increased HSPPs in the regulation of hemostasis and inflammation pathways under pathophysiological conditions, however, remains less clear. The molecular mechanisms, responsible for the influence of inflammation upon hemostasis and *vice versa*, remain largely unknown. We would like to review here the most important post-translational events, which might perturb HSPPs structure and functions, as well as those influencing measurable levels of HSPPs during inflammation.

2. Cooperation of inflammatory and hemostatic pathways during acute phase response

In normal conditions, HSPPs are presented by a soluble fibrous protein, fibrinogen, abundant serine protease zymogens (inactive enzymatic precursors), and minute amounts of active proteases, as well as by non-enzymatic cofactors and protease inhibitors (Table 1).

Precursor conversion	HSPP identification	The basic function in hemostasis	Proteolytic activation	Expression during the acute-phase response
Clotting factors				
Fibrinogen (Fg) → fibrin (Fn)	a fibrous (structural) protein	substrate for polymerization to fibrin that is important in tissue repair	by thrombin	increase, 1,5-4,0-fold
Prothrombin (II) → thrombin (IIa)	trypsin-like serine protease (endopeptidase)	the conversion of Fg to Fn leading to the formation of a fibrin clot	by prothrombinase	no change or weak increase
Factor V (V) → activated factor V (Va)	ceruloplasmin-like binding protein	as a cofactor for Xa participates in thrombin activation, not enzymatically active	by thrombin	ND
Factor VII (VII) → activated factor VIIa (VIIa)	serine protease (endopeptidase)	the catalytic component of the extrinsic tenase, activates IX to IXa and X to Xa	by thrombin, IXa, Xa, XIIa	ND
Factor VIII (VIII) → activated factor VIIIa (VIIIa)	ceruloplasmin-like binding protein	cofactor for IXa in conversion of X to Xa, receptor for IXa and X, not enzymatically active	by thrombin	increase
Factor IX (IX) → activated factor IXa (IXa)	serine protease (endopeptidase)	the enzyme component of the intrinsic tenase, activates X to Xa	by XIa or TF-VIIa/PL-Ca^{2+}	ND
Factor X (X) → activated factor Xa	serine protease (endopeptidase)	the enzyme component of the prothrombinase is	by IXa-VIIIa/PL-Ca^{2+},	ND

Precursor conversion	HSPP identification	The basic function in hemostasis	Proteolytic activation	Expression during the acute-phase response
(Xa)		responsible for rapid thrombin activation	TF-VIIa/PL-Ca^{2+}	
Factor XI (X) → activated factor XIa(XIa)	serine protease (endopeptidase)	the conversion of IX to IXa within the intrinsic pathway	by surface bound α-XIIa	ND
Factor XII (XII) → activated factor XII (α-XIIa)	serine protease (endopeptidase)	initiation of the intrinsic coagulation pathway *via* conversion XI to XIa	by kallikrein	decrease
Factor α-XIIa (α-XIIa) → factor β-XIIa (β-XIIa)	serine protease (endopeptidase)	solution phase activation of kallikrein, factor VII and the complement cascade	by kallikrein	decrease
Factor XIII (XIII) → activated factor XIIIa (XIIIa)	transglutami-nase (transpeptidase)	stabilization of the fibrin clot *via* cross linking the α and γ-chains of Fn, α_2-PI, V, vWF	by thrombin	ND
Anticoagulants				
Tissue factor pathway inhibitor (TFPI)	Kunitz-type protease inhibitor	reverse inhibition of Xa and IIa, then TF-VIIa independently from Ca^{2+}	-	decrease
Antithrombin (AT)	serpin	Inhibition of VIIa, IXa, Xa and XIa, kallikrein, plasmin, IIa	-	decrease
Protein C (PC)→ Activated protein C (APC)	trypsin-like serine protease (endopeptidase)	inactivation of Va and VIIIa, that inhibits the prothrombinase and tenase and, finally, IIa	by α-throm-bin/throm-bomodulin, by Xa or IIa without Ca^{2+}	no change or decrease
Protein S (PS)	binding protein	as cofactor for APC	-	increase
Fibrinolytic proteins				
Tissue-type plasminogen activator, single chain form (sc-tPA) → active two-chain form (tc-tPA)	serine protease (endopeptidase)	the main endothelium-derived activator of the fibrinolytic system, converts Pg to Pm	by plasmin	decrease or weak increase
Glu-plasminogen (Glu-Pg) → plasmin (Pm)	serine protease (endopeptidase)	responsible for the fibrin clot digestion	by t-PA, u-PA, elastase, XIIa	increase, 2-3- fold
Urokinase-type plasminogen activator, single chain (sc-uPA)	serine protease (endopeptidase)	activator of the Pg conversion to Pm	-	increase
Proteinase inhibitors				
α_1-Antitrypsin or alpha 1-proteinase	serpin	protects tissues from proteolytic enzymes,	-	increase

Precursor conversion	HSPP identification	The basic function in hemostasis	Proteolytic activation	Expression during the acute-phase response
inhibitor (α_1-PI)		inhibits IIa and APC		
α_2-macroglobulin (α_2-M)	broad-range protease inhibitor	inhibits IIa, APC Pm, kallikrein, a remover of plasma enzymes	-	increase, about 100-fold
α_2-antiplasmin (α_2 – plasmin inhibitor) (α_2-PI)	serpin	inhibitor of Pm, forms covalent complexes interfered with the binding of Pg(Pm) to Fn	-	ND
Thrombin activable fibrinolytic inhibitor (TAFI) → activated form (TAFIa)	carboxypepti-dase	inhibitor of fibrinolysis, removes Pg-binding sites on Fn	by thrombin, plasmin, trypsin	ND
Plasminogen activator inhibitor of type 1 (PAI-1)	serpin	the major inhibitor of tPA that regulates the fibrinolysis by attenuation of Pm production	-	increase
Plasminogen activator inhibitor of type2 (PAI-2)	serpin	inhibitor of urokinase as well as tPA	-	ND
Plasminogen activator inhibitor of type3 (PAI-3 or PCI)	serpin	the major inhibitor of APC as well as tPA and urokinase	-	ND

Table 1. The main characteristics of hemostatic soluble plasma proteins (HSPPs)

Physiological anticoagulants are also available to suppress appropriate clotting factors. Some of the clotting factors (like thrombin or factor V) can promote both coagulation and anticoagulation; thus, these factors are called Janus-faced proteins.

A hemostatic response to the activating signal is manifested by a series of transformations of proenzymes to activated enzymes in a cascade-like manner. The formation of thrombin at the final stage of the coagulation cascade is aimed at conversion of soluble fibrinogen into insoluble fibrin, the non-cellular matrix of blood thrombus. The thrombus formation is considerably accelerated due to accumulation of tissue factor (TF) at the sites of vascular endothelial damage. Tissue factor, a membrane-bound glycoprotein, is considered the common physiologic trigger of both hemostasis and inflammation pathways. Under normal conditions, none cells, which contact with the bloodstream, express TF. An injury, as the initial triggering signal, starts up the TF expression and the externalization at the surface ofinflammatory cells (primarily, monocytes) and vascular wall cells (endothelial or smooth muscle cells). Immediately upon exposure to the bloodstream, TF contacts with activated coagulation factor VIIa (VIIa), whose trace amounts (about 1 % of total inactive enzyme precursor, coagulation factor VII) are conventionally present in circulation. The formation of macromolecular complex TF-VIIa is a crucial event that initiates the first stage of the coagulation process, initiation phase. Alongside with that TF initiates local inflammatory reaction.

2.1 Initiation phase of the coagulation process

Tissue factor-VIIa complexes, newly appeared on the boundary between blood flow and the vessel wall, begin to bind plasma-derived coagulation factor VII to produce additionally factor VIIa. Thus, TF acts as a cofactor in the factor VII/VIIa autoactivation process (Fig. 2). Membrane-bound TF/VIIa complexes also interact with small amounts of coagulation factor X (X) and coagulation factor IX (IX). Activated factors X (Xa) and IX (IXa) start up prothrombin (coagulation factor II) conversion to thrombin (IIa). This first cycle of restricted thrombin production is limited by two plasma-derived inhibitors, tissue factor pathway inhibitor (TFPI) and antithrombin (AT). The former one neutralizes factor Xa when forms a quaternary structure with TF-VIIa-Xa. As well, AT upon binding to heparan sulphate/heparin, rapidly inactivates free factors Xa, IXa, and thrombin, initially accumulated at the site of vascular injury. So, TF-VIIa itself is incapable to generate substantial amounts of thrombin during the initiation phase. However, the TF-dependent cycle of thrombin production can overcome inhibition by TFPI and AT, when TF is maintained at a sufficiently high level (Tanaka et al., 2009). Blood-borne forms of TF (soluble sTF or TF-positive microparticles) shed from disrupted cell membranes of different origin presumably can be an additional driver of the increased TF-initiated thrombin production (Sommeijer et al., 2006). Apparently, cell-exposed and blood-borne TF can promote transduction of inflammatory signals *via* cellular protease-activated receptors (PARs). For example, sTF-mediated inflammation in animal models might develop *via* platelet PAR-4 signalling, while TF-proteases complexes (TF-VIIa and TF-VIIa-Xa) induce the activity of signalling pathways in vascular cells *via* PAR-1 and PAR-2 (Busso et al., 2008; Rao & Pendurthi, 2005; Riewald & Ruf, 2001). Being a mediator of both inflammatory and hemostatic pathways, TF integrates different extra cellular signals and cellular responses, thus participating in the development of a host acute-phase response (Fig. 2). As an extremely potent triggering molecule, TF is capable of translating injury signals into activation of the coagulation cascade, sustaining thrombin initiation, and promoting its propagation.

2.2 Propagation phase of the coagulation process

Trace amounts of thrombin promote formation of a IXa-VIIIa-Ca^{2+}-phospholipid assembly (tenase complex) or a Xa-Va-Ca^{2+}-phospholipid assembly (prothrombinase complex) *via* feedback activation of non-enzymatic cofactors VIII (VIIIa) and V (Va) after their binding to negatively charged phospholipids (phosphatidylinositol and phosphatidylserine) on the surface of activated platelets in the presence of calcium ions. Thrombin also activates factor XI (XIa), which additionally stimulates the tenase complex. Tenase-produced prothrombinase complexes lead to the explosive generation of thrombin, which ultimately leads to generation of a fibrin clot. During an episode of TF-initiated coagulation, tenase and prothrombinase complexes are generated in concentrations that might be sufficient to maintain the TF-independent procoagulant response as long, as the reactants are available. From this moment, normal coagulation may become fully independent of TF (Butenas et al., 2009). The propagation phase can continue and prolong the acute-phase response, where driving of thrombin generation is a requisite for an adequate bleeding prevention *via* more fibrin deposition. By binding to PARs, thrombin activates platelets, endothelial cells, and immune cells. As a result, cytokines and chemokines are additionally expressed, as well as certain HSPP secretion is intensified, leukocyte and platelet recruitment to inflammatory *foci* increases, and fibrin deposition is accumulated. These events considerably enhance local

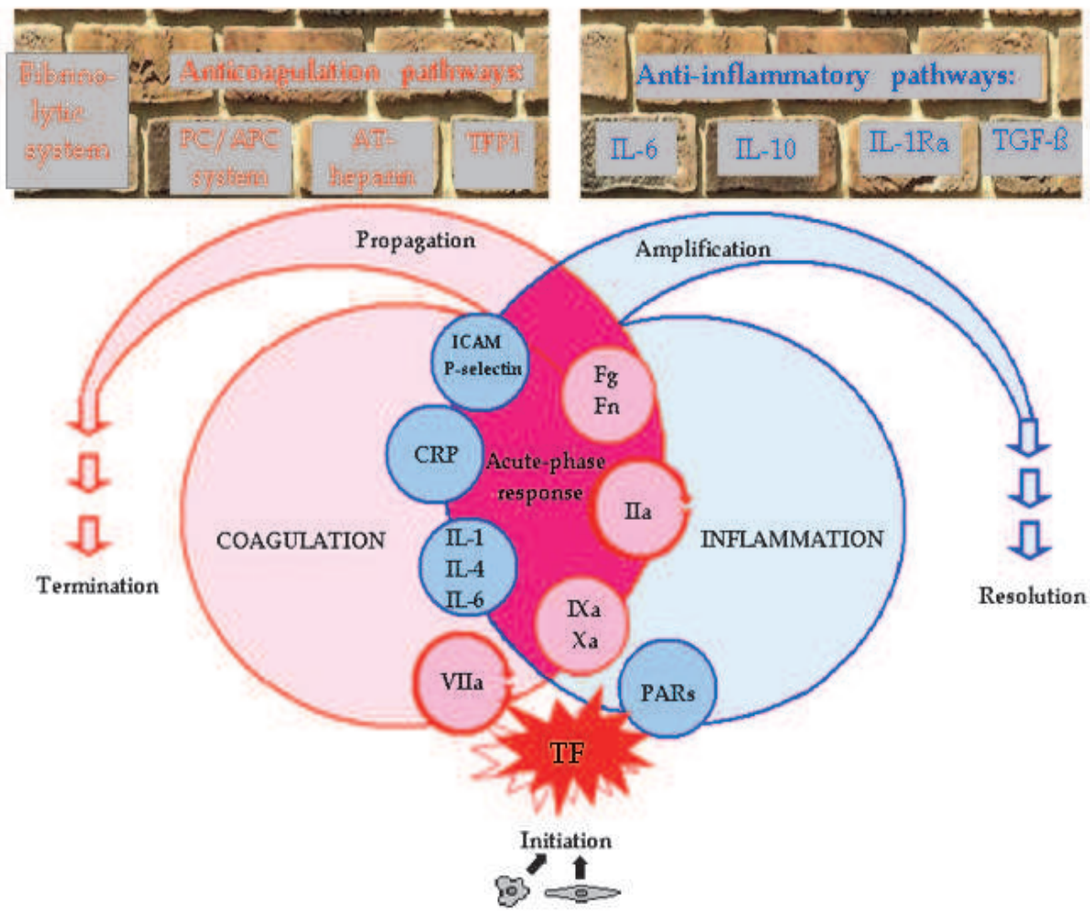

Fig. 2. Schematic representation of the regulatory crosstalk between hemostasis and inflammation in response to exposed TF (coagulation factors and cytokines are all present in circulating plasma whereas TF is the only cell surface glycoprotein shown here). The interconnection of coagulation and inflammation pathways is an essential prerequisite for host homeostasis restitution after injury. Coagulation factors (as shown here with pink circles) and inflammatory mediators (blue circles) promote both coagulation and inflammation through complex and reciprocal interactions, thereby sustaining the acute phase response. At least, two clotting factors, factor VIIa and thrombin, contribute in pro-inflammatory action of coagulation system through positive feed-back autoactivation mechanism (closed-loop arrows). Red curved arrow represents propagation phase of coagulation, blue curved arrow represents amplification of inflammation. In proportion to increasing levels of clotting and inflammatory APP, anticoagulation and anti-inflammation processes are activated. The limiting action of the anticoagulant and fibrinolytic systems on coagulation as well as anti-inflammatory mechanism of inflammation attenuation is depicted by brick-built barrier. Abbreviations: IIa, VIIa, IXa, Xa and XIa indicate activated coagulation factors; Fg, fibrinogen; Fn, fibrin; TF, tissue factor; PARs, protease-activated receptors; IL, interleukin; CRP, C-reactive protein; ICAM, intercellular adhesion molecule-1; PC/APC, protein C/activated protein C; AT, antithrombin; TFPI, tissue factor pathway inhibitor; IL-1Ra, interleukin-1 receptor antagonist; TGF-ß, transforming growth factor beta.

inflammation and coagulation. Furthermore, the formation of thrombi in the microvasculature provides a mechanical barrier that blocks spreading of inflammatory/coagulation mediators into the circulation. Such limited clotting restricts thrombus propagation and prevents from acute local inflammation turning into systemic complications. An anti-clotting molecular process induced by several physiological anticoagulants and fibrinolytic agents is designated for regulation of an adequate clot size and formation of an effective thrombus. Two hemostatic pathways, anticoagulation and fibrinolysis, both are responsible for coagulation termination (Fig. 2).

2.3 Termination phase of the coagulation process

After bleeding is arrested, and the injured vessel is repaired, coagulation attenuation begins to dominate over its propagation due to accumulation of inhibitors of blood clotting. Natural clotting inhibitors are orchestrated through successive induction of three major anticoagulant-dependent pathways: tissue factor pathway inhibitor-, heparin/antithrombin- and the protein C-dependent pathways. A tissue factor pathway inhibitor (TFPI), as was mentioned above, is a most effective inhibitor of coagulation at the initiation phase. This inhibitor specifically blocks the TF-VIIa-Xa complex after trace factor Xa has been formed (Spronk et al., 2003). Antithrombin (AT) is a direct protease inhibitor, which attenuates accumulation of coagulation factors IXa, Xa, and IIa during the propagation phase. Heparin-like glycosaminoglycans accelerate the rate of inactivation of these clotting factors by AT. The protein C system provides multi-directional attenuation of thrombin procoagulant activity and this terminates coagulation. Thrombomodulin (TM), a endothelial cell membrane-associated protein, is capable of to tackling excessive thrombin, thus changing its specific enzyme activity. Within the thrombin-thrombomodulin complex, thrombin looses its affinity to fibrinogen or cellular PARs. Instead this, it possesses an ability to convert precursor protein C (PC) into an anticoagulant enzyme, activated protein C (APC). Activated protein C interrupts thrombin propagation *via* limited proteolysis of cofactors Va and VIIIa. Cofactor protein S and platelet membrane phospholipids provide manifold acceleration of the APC activity. Endothelial protein C receptors (EPCR) enhance the thrombin-thrombomodulin affinity to PC. In such a way the protein C system down-regulates the coagulation cascade to moderate the explosive trend of thrombin production. Its anticoagulant competence enhances, due to the modulation of the thrombin activity through two mechanisms, inhibition of prothrombin converting into thrombin and promotion of thrombin inversion from a procoagulant enzyme into an anticoagulant one. Inhibition of thrombin formation can also reduce thrombin's pro-inflammatory activities (Sarangi et al., 2010).

In a complementary mode with respect to anticoagulation, the surplus clots are dissolved by proteases of the fibrinolytic system. Activation of intravascular fibrinolysis is controlled through enhancing synthesis and secretion of tissue plasminogen activator (tPA) by endothelial cells during fibrin clotting. Tissue plasminogen activator is released into the clot and binds in the clot volume with fibrin(ogen) and plasminogen (Pg). The formation of ternary Fn-tPA-Pg complex extremely effectively accelerates Pg converting into the serine protease plasmin (Pm). Plasmin cleaves fibrin into soluble fragments, the so called fibrin degradation products (FDPs). The rate and extent of local delivery of tPA during the clot formation is important for enhancing the process of fibrinolysis (Schrauwen et al., 1994). To avoid excessive clot digestion, which can affect bleeding, the activity of fibrinolytic system is down-regulated by numerous plasma- and cell-derived inhibitors (Meltzer et al., 2010). These are (i) plasminogen activator inhibitor of type 1 (PAI-1) that highly specifically

inactivates tPA, (ii) α_2-antiplasmin, primary Pm inhibitor that prevents Pm-dependent non-specific proteolysis due to effective neutralization of Pm, and (iii) thrombin activable fibrinolytic inhibitor (TAFI) that down-regulates the cofactor activity of fibrin during activation of plasminogen and, thereby, suppresses fibrinolysis.

The activities of both coagulation and fibrinolytic cascades are normally latent but have the potential to be accelerated in an extremely acute manner during inflammation. The coagulation and fibrinolysis pathways have to follow each other, retaining a delicate physiological balance preventing thrombosis and bleeding. Activation of the coagulation cascade leads to increases in the plasma levels of coagulation factors VIIa, Xa, and thrombin, which are pro-inflammatory factors contributing to the acute-phase response (Fig. 2). In addition, fibrin deposition and fibrin degradation products, FDPs, enhance inflammation. Coagulation factors elicit inflammation *via* affecting a number of blood/vascular cells through protease-activated receptor (PAR)-mediated pathways up-regulating the expression of numerous APPs (tumour necrosis factor-alpha, interleukins, adhesion molecules, and growth factors) (Chua, 2005). At least fibrin, thrombin, and coagulation factor Xa, all are important cell-signalling effector molecules that are responsible for receptor triggering. When PARs are activated constantly (e.g., under the action of repeated stimuli), the acute-phase response can be inverted into a chronic one. Therefore, the inflammatory consequence caused by coagulation should be abolished within a necessary time intervals; otherwise it could be enormous. Resolution of the acute-phase response requires down-regulation of inflammatory/procoagulant APPs expression. In particular, IL-4, IL-10, TGF- ß are anti-inflammatory agents that inhibit the production of numerous inflammatory cytokines, including TNF-α, IL-1ß, IL-6, IL-8, and, finally, IL-10 itself (de Waal et al., 1991; Walley et al., 1996). In fact, human blood monocytes are known to produce both pro- and anti-inflammatory cytokines. During resolution, monocytes and macrophages considerably increase production of the latter above the former, thereby preventing prolongation or escalation of an early inflammatory response (Fig. 2). The concentration of cytokine-induced procoagulants is reciprocally decreased; thus, vascular homeostasis is restored.

A failure of the control of these processes causes incorrect inflammation termination or even its propagation. Such an inconsistency leads to deregulation of hemostasis, which, in turn, might force the further leap of inflammatory responses. Under pathological conditions, cytokines are released by immune regulatory cells in sites of the local inflammatory response. This process may be acute but limited in time (reverting to the normal homeostatic state) or persistent (resulting in chronic activation of coagulation and fibrinolysis). Initially acting within the frame of the adaptive defence system, inflammation and hemostasis might develop from a local response to a systemic host reaction. Escalation of inflammation can induce endothelial dysfunction subsequently activating the coagulation cascade, and *vice versa* — hypercoagulation follows amplification of inflammation (Levi et al., 2004)

Under these conditions mutual activation of coagulation and fibrinolysis might follow to potentially exhausting consumptive coagulopathy and disseminated intravascular coagulation. A detrimental inflammatory response resulting from coupling of procoagulant and pro-inflammatory stimuli might cause thrombophilia, and, furthermore, provoke the thrombotic events. In such a way, inflammation/coagulation interaction drastically increases a risk of thrombus formation implicated in the pathogenesis of several diseases in humans. On the one hand, these are thrombophilias, atherosclerosis, and other cardio-vascular pathologies, as well as intercurrent illnesses (like trauma and cancer) or surgery. On the other hand, these are acute/chronic inflammatory diseases, including sepsis, inflammatory bowel disease, and lung and heart inflammation (Fig. 3).

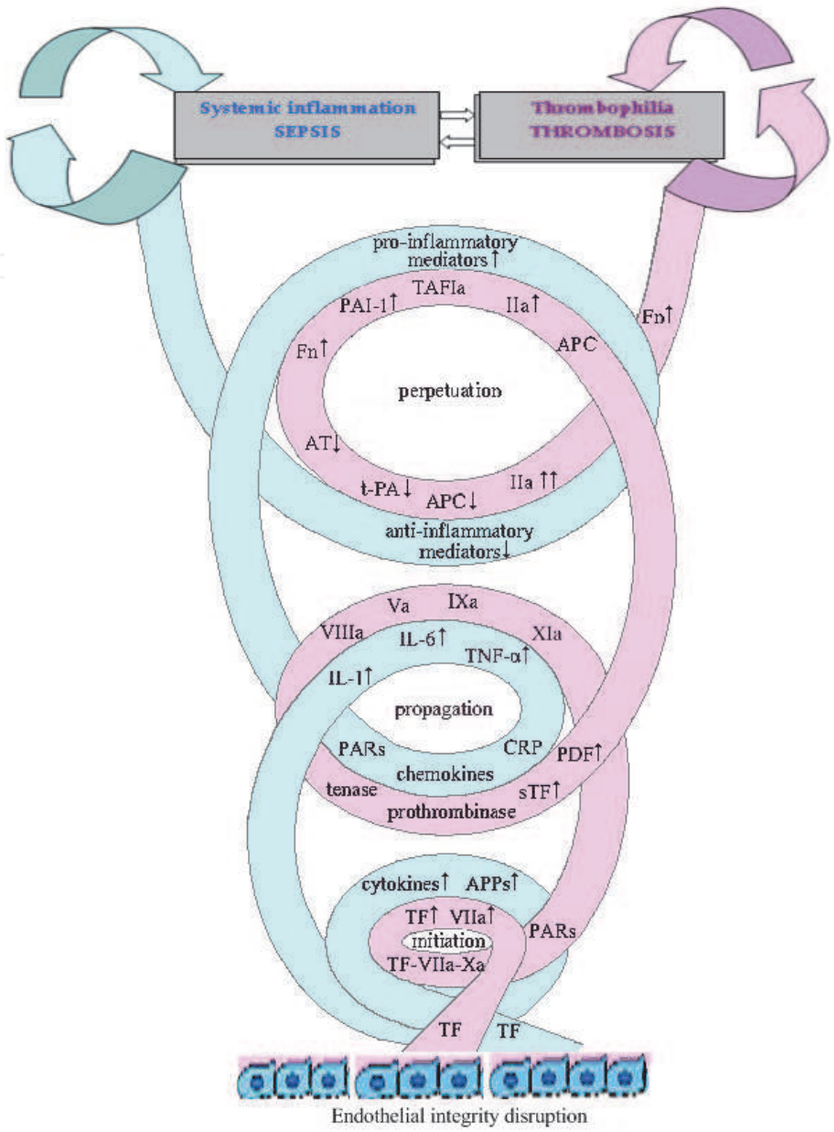

Fig. 3. A simplified hypothetic model of pathophysiological interactions between inflammatory and hemostatic APPs. Each spiral turn represents a potentially vicious cycle driven by excessive concentrations of some components (this is shown as arrows up) and/or insufficient concentrations of other components (as arrows down). Amplification of HSPP activation during an initiation phase is exerted through interaction with the components of the innate immune system, which, in turn, prolongs inflammation during the propagation phase. As a result, both processes, coagulation and inflammation, can come into perpetuation phase. These complex interactions can lead to life-threatening complications, such as thrombosis or sepsis. Refer to the text for discussion in detail.

Abbreviations: IIa, VIIa, IXa, Xa, and XIa indicate activated coagulation factors; Va and VIIIa – non enzymatic cofactors; Fn, fibrin; TF, tissue factor; sTF, blood-borne (soluble) forms of tissue factor; PARs, protease-activated receptors; IL, interleukin; CRP, C-reactive protein; TNF–α, tumour necrosis factor alpha; tPA, tissue activator of plasminogen; APC, activated protein C; AT, antithrombin; PAI-1, plasminogen activator inhibitor of type 1; TAFI, thrombin activable fibrinolytic inhibitor.

3. Pro-inflammatory environment sustains coagulation

Recently, a clear association of high APP levels with a human procoagulant phenotype and impaired fibrinolysis were found in some studies. Indeed, a certain relationship is believed between the plasma levels of C-reactive protein (CRP) and some HSPPs. C-reactive protein is at present used as a sensitive biomarker of acute and chronic inflammation. This is the only APP that correctly displays the severity of vascular pathologies: from an endothelium-derived focal inflammatory response to a hard coronary lesion (Calabrò et al., 2009; Willerson & Ridker, 2004). CRP levels are now detected using a high-sensitivity assay (hsCRP); these indices are found to be an accurate predictor of cardiovascular disease (CVD) (de Ferranti & Rifai, 2007).

The procoagulant function of the C-reactive protein is still debated (MacCallum, 2005), but there is some evidence proving that CRP is associated with the metabolism of HSPP. In *ex vivo* experiments on cell systems, CRP was found to induce expression of inflammatory cytokines or TF in monocytes (Cermak et al., 1993), of thrombomodulin and von Willebrand factor (vWF) in human umbilical vein endothelial cells (HUVECs) (Blann & Lip, 2003), and of PAI-1 in human arterial endothelial cells (HAECs) (Chen et al., 2008; Devaraj et al., 2003). Close correlation of the CRP amount with increasing fibrinogen levels was found in patients with acute ischemic stroke (Tamam et al., 2005). The CRP expression reflects not only to be a predictor but rather an active mediator of atherothrombotic events, as was reported for *in vivo* CRP-dependent induction of TF in blood monocytes (Sardo et al., 2008). Increased levels of hsCRP are associated with such CVD, as severe unstable angina, myocardial infarction, stroke, and peripheral arterial disease (de Ferranti & Rifai, 2007). The causative role of CRP in thrombogenesis is at present believed doubtful, but its active participation is supported by some results described earlier. One of the small group evaluation reports revealed that activation of coagulation and fibrinolysis induced by recombinant CRP infusion provoked increases in the levels of prothrombin F1+2 and D-dimer, as well as in the vWF and PAI-1 concentrations (Bisoendial et al., 2005). CRP also attenuated the fibrinolytic capacity, by inhibiting the tPA activity and stimulating PAI-1. An increased ECLT (euglobulin clot lysis time) and, hence, a decreased fibrinolytic capacity in the blood plasma obtained from volunteers with high CRP levels were found (Zouaoui Boudjeltia et al., 2004). These data confirm a conclusion on down-regulation of fibrinolysis during the enhanced inflammatory response indicated by CRP.

The studies that elucidate the inhibitory role of cytokines in fibrinolysis are not limited to that of CRP. A multifunctional cytokine, IL-1, was shown to stimulate up-regulation of specific mRNA expression of urokinase-type plasminogen activator (u-PA) (Wojta et al., 1994). IL-1 also increased accumulation of PAI-1 in cardiac microvascular endothelial cells (Okada et al., 1998) and also controlled expression of PAI-1 and u-PA in human astrocytes (Kasza et al., 2002). Production of PAI-1 protein in human adult cardiac myocytes was found to be increased up to two times by interleukin-1α and tumour necrosis factor-α (TNF-α) and up to five times by transforming growth factor-ß (TGF- ß) and oncostatin M. However, t-PA production in human cardiac myocytes did not change after cytokine treatment (Macfelda et al., 2002). By contrast, IL-1 and tissue necrosis factor alpha inhibited t-PA in HUVEC (Bevilacqua et al., 1986).

During severe inflammation, the function of fibrinolysis can be impaired. The same is true with respect to anticoagulant pathways. It was recently documented that an increase in serum CRP level in dogs was accompanied by lowering of the AT concentration (Cheng et

al., 2009). Under inflammation conditions, the AT plasma level can decreased due to impaired synthesis (like other negative APPs) or due to protein degradation by elastase produced by activated neutrophils (Viles-Gonzalez et al., 2005). In addition, AT might be consumed proportionally to inhibition of the target proteases or removed from circulation after binding to fluid-phase complement attack complexes within the complement cascade (Esmon, 2005). Another natural anticoagulant, TFPI, seems to be incapable of regulating an enhanced thrombin amount during severe inflammation, since a low endogenous concentration of the anticoagulant does not increase (Bastarache et al., 2008). It would be noted that TFPI concentrations do not follow the development of disseminated intravascular coagulation and cannot prevent hypercoagulation (Wiinberg et al., 2008). TFPI is expressed primarily in the microvessels; thus, it might only nominally participate in hemostasis balancing in the larger vessels. Apparently, this pathway only slightly contributes to the coagulation/inflammation cross-over (DelGiudice & White, 2009). The PC anticoagulant system is more extensively present in the vascular network (Viles-Gonzalez et al., 2005). This system plays a pivotal role in hemostasis, shutting down coagulation and promoting fibrinolysis. These events might fail because of the presence of some vulnerable components. Down-regulation of membrane TM and EPCR by endotoxin, IL-1b, and TNF-α has been noted elsewhere (Esmon, 2005). The disappearance of TM from the endothelial cell surface impairs the process of activation of protein C. Not only the amount of APC but also its anticoagulant activity might be decreased under pathological conditions. Protein S, when forming an inactive complex with complement protein C4b (C4BP), thereby looses its ability to promote APC (Dahlback, 1991). Additionally, soluble forms of TM and EPCR can appear during inflammation in the blood flow. They may bind APC without potentiation of its activity or, moreover, even might inhibit APC anticoagulant function. sEPCRs have been found to block binding of protein C and APC to phospholipids and to alter the active site of APC (Liaw et al., 2000).

Tissue factor, in addition to its procoagulant function, has been recently identified as a key secondary inflammatory mediator that markedly accelerates the feedback intensification of coagulation and inflammation pathways. Its concentration in circulation dramatically increases when the endothelium is disrupted and the blood begins to contact with extra vascular cells. In addition, inflammatory mediators many times increase the tissue factor protein amount and activity through stimulation of expression of this protein and through increasing the number of TF-positive microparticles as a consequence of paracrine and autocrine activity of the inflammatory cells (Esmon, 2005). It should be noted that TNF-α and IL-1ß are produced by lymphocytes and macrophages during vascular inflammation, and these events can also enhance the expression of the TF. The TF expression can be stimulated by several inflammatory mediators, namely TNF-α, IL-1, IL-6, activated complement, and immune complexes (DelGiudice & White, 2009). Activated T cells increase both TF expression and activity *via* paracrine stimulation of endothelial cells (Monaco et al., 2002). LPS-stimulated monocytes enhance intracellular transport of increased amounts of TF to the cell surface as well as the shedding of TF-containing microparticles (Egorina et al., 2005). Subsequently, soluble TF indirectly promotes inflammation by stimulation of thrombin production and by involvement of platelets *via* thrombin-activated PAR-dependent signalling. In PAR-4-deficient mice, recombinant sTF did not induce inflammation but was able to activate thrombin production, demonstrating, in such a way, the necessity of thrombin-sensitive platelets for sTF-mediated inflammation (Busso et al., 2008). Activation of platelets leads to release, from their α-granules, of a cocktail of

chemokines and cytokines including IL-8, platelet factor–4 (PF4), and macrophage inflammatory protein–1a (MIP-1a) and to the expression of platelet surface adhesion molecules including P-selectin and CD40-ligand (CD40L). Platelet-derived CD40L is able to induce TF on the cell surface of endothelial cells and also of monocytes (Lindmark et al., 2000). Apparently, interaction between TF and flowing blood prolongs activation of the coagulation cascade through additional thrombin generation, which, in turn, might potentiate the formation of a platelet-rich thrombus. As was found recently, the inflammatory response involves activation not only of blood-borne cells (leukocytes and platelets), but also of the cells derived from the vascular wall (endothelial and smooth muscle cells, etc.). Binary TF-VIIa and ternary TF-VIIa-Xa complexes can also modulate inflammation *via* protease-activated receptor 2 (PAR 2) cleaving (Ahamed et al., 2006). Some vascular-bed specificities influence the TF-dependent mechanism of modulation of the acute response. In particular, vessel wall- derived TF forces mainly arterial thrombogenesis, since instable or ruptured atherosclerotic plaques are characterized by a high concentration of TF in both cellular and acellular regions. At the same time, soluble TF contributes mainly to venous thrombosis and microvascular thrombosis (Owens & Mackman, 2010). Nevertheless, circulating TF was found to be associated with the increased blood thrombogenicity in patients with unstable angina and chronic coronary artery disease (Corti et al., 2003). TF causes progression of coagulation within initial stages of disseminated intravascular coagulation (DIC) (Wiinberg et al., 2008). In animal models, TF was shown to participate in generalization of deep vein thrombosis (DVT) (Himber et al., 2003). Some reports indicate that myocardial inflammation and cardiomyocytes injuring enhance expression of TF, thereby increasing local formation of thrombin (Erlich et al., 2000; Luther et al., 2000).

Coagulation factor Xa was found to increase induction of endothelial TF and E-selectin by all the pro-inflammatory cytokines (e.g. TNF, IL-1ß, and CD40L). TF, in turn, initiates a new wave of factor Xa production after the formation of the TF-VIIa complex and activation of zymogens of factors IX and X. Binding of TF-VIIa to PAR-2 also results in up-regulation of the inflammatory responses in macrophages and neutrophils (Cunningham et al., 1999). A synergistic pattern of activity of factor Xa and inflammatory cytokines, resulting in both re-activation of coagulation cascade and augmentation of inflammatory mediators, is a good illustration of the apparent positive feedback mechanism, by which enhanced coagulation maintains pro-inflammatory environment and *vice versa* (Hezi-Yamit et al., 2005) .

4. Hypercoagulation and impaired fibrinolysis perpetuate inflammation

The above-mentioned facts proved the capacity of the inflammatory factors to regulate coagulation and fibrinolysis. A converse molecular mechanism, by which hemostasis stimulates inflammation is at present less obvious but undergoes increasing investigations. Fibrinogen, the precursor of fibrin, is considered a rapid and sensitive marker of both coagulation and the acute-phase response, while its synthesis is enhanced during early inflammatory reactions. Fibrinogen contributes to coagulation being a terminal substrate in plasma clotting, which is cleaved specifically by thrombin. By splitting fibrinopeptide A and fibrinopeptide B from fibrinogen thrombin forms fibrin-monomers are spontaneously polymerized producing fibrin. Fibrin, in turn, provides plasma clotting, platelet aggregation and wound healing or thrombus formation. In addition, fibrin(ogen) participates in activation of vascular cells and regulation of the inflammatory response. Pro-inflammatory effects of fibrin(ogen) manifest itself after the abnormal fibrin deposition; this affects the

vascular bed and enhances primarily local and, then, systemic inflammation through expression of the pro-inflammatory mediators. In fact, fibrin(ogen) increases the mRNA levels and induces synthesis of inflammatory cytokines IL-6 and TNF-alpha in human peripheral blood mononuclear cells (PBMCs) (Jensen et al., 2007), of IL-8 in HUVEC (Qi et al., 1997), and of transcription factor NF-kB in mononuclear phagocytes (Sitrin et al., 1998). Fg can manifest a pro-inflammatory action independently of its clotting function due to the existence of a high-affinity integrin binding site or multiple low-affinity binding sites, which interact with inflammatory competent cells. In particular, induction of cytokines IL-1β, IL-6, TNF-α has been found to be associated with fibrin binding to integrin receptors Mac-1 (CD11b/CD18) in monocytes (Trezzini et al., 1991). Cytokine secretion is suggested to be directly triggered by the process of Fg polymerization to Fn. The activity of thrombin that increases in Fg-deficient mice after LPS administration does not correlate with the levels of inflammatory mediators produced by bone marrow-derived macrophages and duration of their action. Both thrombin and Fg acting separately or in combination exert no effect on the cytokine production. It was concluded that up-regulation of secretion results in conformation changes of the Fg molecular structure during its conversion into Fn (Cruz-Topete et al., 2006). Some recently obtained data supported this conclusion. Molecular determinants of fibrin(ogen)-mediated pro-inflammatory activity were found to be localized in a γ-chain. These determinants can enhance the inflammatory cell recruitment and activation *via* interaction with integrin receptors Mac-1. Several specific sequences (all are attributed to the fibrinogen γ-chain) were found to participate in the interaction of fibrinogen with leucocytes. There are γ190-202, γ377-395, and γ383-395 sequences (the latter is localized within the γ-chain of the D nodule), which are capable of affecting leukocyte adhesion, their migration, or cytokine expression (Jennewein et al., 2011). In addition to Mac-1, leukocyte integrin receptors $\alpha_M ß_2$ (but not platelet receptors $\alpha_{IIb} ß_3$) may be involved in the progression of inflammatory disease (Flick et al., 2004; Flick et al., 2007). The core recognition motif, γ-chain residues 383–395, was suggested to determine the affinity of Fg and Fn to $\alpha_M ß_2$. Obviously, soluble Fg has cryptic $\alpha_M ß_2$ binding sites, which are inaccessible for integrin $\alpha_M ß_2$ binding. Structural conformation changes during Fg immobilization or conversion of the latter into Fn permit Fg/Fn binding to integrin and provide local leukocyte activation. Being non-diffusible component fibrin deposition attaches to site of injury, marking spatial and temporal coverage for inflammatory cell targeting. Indeed, one might speculate that fibrin-mediated activation of $\alpha_M ß_2$ in macrophages and neutrophils represents a possible mechanism of the inflammatory response amplification during hypercoagulation. As a result, NF-kB-dependent intracellular signaling, which is triggered by fibrin interaction with $\alpha_M ß_2$, leads to a vicious cycle of cell recruitment, adhesion, degranulation, generalization of oxidative responses and release of inflammatory mediators (Flick et al., 2004; Flick et al., 2007).

The involvement of fibrinogen in coagulation, as well as that of fibrin in the fibrinolytic process, is accompanied by generation of the various fibrin(ogen) degradation products, FDPs. These small and large fragments can exert an independent regulatory effect on the inflammatory process. In particular, fibrinopeptides A and B, the products of Fg conversion into Fn, are suggested to show a pro-inflammatory action on leucocytes functioning as chemoattractants. In contrast, the peptide Bβ15-42 which is generated by plasmin cleavage of fibrin, can mediate powerful anti-inflammatory effects. FDPs, which are formed after Fg(Fn) digestion by plasmin, also seem to modulate inflammation (Jennewein et al., 2011). Fg, Fn and FDPs were shown to induce intensification of CRP production in vascular

smooth muscle cells. Herein, FDPs have the most prominent pro-inflammatory potency, as compared to that of fibrin(ogen) (Guo et al., 2009).

It is interesting that plasmin(ogen) also generates degradation products during activation of fibrinolysis. These are either the first three, or the first four kringle domains (K1-3, K1-4) or only kringle domain 5 (K5). Angiostatin, a proteolytic fragment that contains K1-4, acts as a powerful anti-inflammatory modulator. In particular, angiostatin demonstrated a lowering adhesiveness of leukocytes to extracellular matrix proteins and the endothelium. Interaction of the angiostatin kringle domain K4 with integrin receptor Mac-1 down-regulates transcriptional factor NF-kB, whereby attenuates NF-kB-related expression of neutrophil-derived tissue factor (Chavakis et al., 2005). The kringle domain K5 has been found to restrict the neutrophil chemotactic activity (Perri et al., 2007). Obviously, impaired fibrinolysis looses this anti-inflammatory action.

The formation of fibrin deposition is a direct consequence of increased thrombin production. A pro-inflammatory action of thrombin is realized by two interdependent ways: (i) by direct promotion of hypercoagulation accompanied by the pro-inflammatory effects described above; (ii) by stimulation of vascular and blood-borne cells and their further involvement in the inflammatory response. Being a powerful signal molecule, thrombin interacts specifically with PAR-1, PAR-2, or PAR-3 and activates the signaling pathways in endothelial cells, platelets, mononuclear cells, and fibroblasts. Thrombin-induced intracellular pathways up-regulate the expression of several cytokines and growth factors, as well as the secretion of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Levi & Poll, 2008). Thrombin is a key protease-agonist, which controls the platelet involvement in the formation of thrombi by stimulation of platelet aggregation, granule secretion, and additional recruitment in the inflammatory process. In an *in vitro* endothelial-cell-monolayer model, thrombin was shown to affect PAR-1-mediated signalling in a concentration-dependent manner. Low thrombin concentrations (20–40 pM) results in endothelial barrier protection, whereas high thrombin concentrations (> 80 pM) lead to disruption of this barrier (Feistritzer & Riewald, 2005). When activated protein C occupies PAR-1, thrombin can realise disruptive effects through activation of PAR-4; this effect requires a higher concentration of thrombin (Bae et al., 2007). Upon binding to thrombomodulin, thrombin inverts its coagulant and inflammatory functions into anticoagulant and anti-inflammatory ones. TM competes effectively with procoagulant substrates (fibrinogen, V, VIII, and PARs) for the same exosite-1 of thrombin but inhibits activation of the coagulation cascades. Moreover, a thrombin-TM complexation down-regulates inflammation/coagulation-pathways *via* a feedback inhibition mechanism, while, at the same time, it initializes protein C-dependent anticoagulant pathway *via* PC activation (Bae et al., 2007). APC, in addition to its anticoagulant function, acts as a pleiotropic agent with anti-inflammatory, profibrinolytic, and cytoprotective effects. After its activation, APC dissociates from the thrombin-TM complex and comes into plasma, where it acts as anticoagulant and profibrinolytic agent, or binds to cell membrane EPCR and regulates intracellular inflammatory pathways. APC is now considered as a signaling molecule that possesses an ability to selectively regulate cytokine production during the inflammatory response. On the one hand, APC down-regulates the production of such pro-inflammatory cytokines, as TNF-α, IL-1ß, IL-6, and IL-8 in monocytes (Stephenson et al., 2006). On the other hand, APC up-regulates anti-inflammatory IL-10 that can reduce the protein concentration and activity of TF, as it was found after treatment of LPS-stimulated monocytes with recombinant APC, rAPC (Toltl et al., 2008). NF-kB-mediated signal

transduction events are modulated directly by APC interaction with EPCR on the plasma membrane of endothelial cells and mononuclears. APC-EPCR inhibits NF-kB nuclear translocation, which then results in inhibition of downstream NF-kB-regulated genes, e.g., ICAM-1, VCAM-1, and E-selectin in endothelial cells or TF in mononuclear cells (Joyce et al., 2001; White et al., 2000). APC has recently been reported to impair TNF signaling in vascular endothelial cells by preclusion of phosphorylation of NF-κB p65 and, thereby, by attenuating expression of cell adhesion molecules (including VCAM-1) (Guitton et al., 2011). At the same time, APC does not affect neutrophil respiratory bursts, phagocytic activity, and expression of monocyte adhesion molecules (Stephenson et al., 2006). In fact, APC does not seem to suppress the innate defensive mechanisms. As a consequence, the action of inflammatory cytokines and oxidative agents sharply reduce the efficiency of TM in PC activation and promote pro-inflammatory efficiency of thrombin.

5. Phenotypic variability of hemostasis during acute and chronic inflammation

Abnormal exposure of the procoagulant and pro-inflammatory agents contributes to sustaining of both local and systemic procoagulant/inflammatory potentials. Prolonged activation of inflammatory cells promotes the production of large amounts of inflammatory mediators by downstream-cells affecting not only *via* an autocrine mechanism, but also in a paracrine manner. The duration and amplitude of a cytokine-mediated systemic inflammation signal, upon reaching the liver, determine the probable pattern of HSPPs additionally produced during the acute inflammatory response. The HSPP level is known to be up-regulated by various pro-inflammatory cytokines similarly to other acute phase proteins, i.e., at the transcriptional and post-transcriptional levels. Genetic factors *per se* may contribute in different manners to a total variability of the HSPP systemic levels: cover about 50% variation in the fibrinogen level or 30% in factor VII plasma level, but exert a negligible effect on the plasma level of t-PA (neither that of antigen nor of its activity) (Voetsch & Loscalzo, 2004). Activated protein C, that breaks thrombus generation through regulation of both coagulation and fibrinolysis apparently is not additionally expressed during the acute-host response. There is some evidence that cytokine-dependent down-regulation of protein C synthesis occurs (Yamamoto et al., 1999); this allows one to classify this agent rather as a negative acute-phase protein. In case, lowering of the plasma protein C level is observed in some diseases, which are attended with inflammation (Danese et al., 2010). Another fact, which is a stronger proof, is that cytokines decrease the capacity of the endothelium to activate protein C precursor in activated protein C because they are able to down-regulate the amount of endothelial membrane-bound thrombomodulin (Esmon, 2004). Alternatively, some authors hypothesize that plasma pool of precursor PC can rapidly decline because of enhanced APC consumption after counteracting with plasma inhibitors (Danese et al., 2010; Patalakh et al., 2009). It is obvious, that in pathological states, the relative proportions of HSPPs significantly vary depending on either driving or suppression of their production by inflammation. Changes in the plasma protein ratio can lead to disproportion between procoagulant and anticoagulant patterns under different pathophysiological conditions. Activated proteases are rapidly cleared from circulation and this determines only a crude assessment of their production. That is why their plasma levels do not always respond "in unison" upon systemic inflammation.

5.1 Transcriptional regulation of HSPP production during inflammation

Unlike CRP (type-1 acute phase protein) up-regulated by synergistic action of IL-6 and IL-1beta, most hemostatic proteases (type-2 acute phase proteins) require IL-6 alone for maximal induction of their synthesis (Trautwein et al., 1994). IL-6 is a key effector that effectively promotes the coagulation pathway, not only by up-regulation of expression of some procoagulant factors (such as TF, fibrinogen, and factor VIII) but also by down-regulation of synthesis of some anticoagulants (such as antithrombin and protein S) (Hou et al., 2008). Cytokine IL-6 is suggested to act as a common inductor for several vascular acute-phase proteins (CRP, α- and β- chains of Fg, Pg, α_2-macroglobulin, and PAI-1). Under transcriptional control by the cytokine IL-6, their circulating levels increase *via* cooperative up-regulation of the corresponding gene promoter activity. The congruence of the HSPP gene responses to IL-6 is provided by an IL-6- responsive element (IL6-RE) that is required for maximal stimulation of the promoter activity by IL-6 (Bannach et al., 2004). Interestingly, IL-6-REs were identified in human CRP and α_2-macroglobulin genes, as well as in two genes responsible for synthesis of fibrinogen α- and ß-chains. The same IL-6-REs is located in the region identified as a cytokine-response region of murine Pg and human PAI-1(Bannach et al., 2004; Loppnow & Libby, 1990). More than one IL-6-RE can exist in the promoter region required for the full responsiveness to IL- 6. Two macroglobulin promoters, e.g., have two functionally cooperated REs, which provide the full IL-6 response of the gene (Trautwein et al., 1994). It was assumed that any small differences in the amount or sequence homology of IL-6-REs in the HSPP genes can vary their inducibility by IL-6 (Hattory et al., 1990). Likewise, distinct transcription factors help to transduce the inflammation signal from cytokine to IL-6-RE in a cell- and/or tissue-specific manner. Such mechanisms might allow differential regulation of HSPPs gene expression induced by IL-6. IL-6-dependent regulatory machine is a good example for demonstration how the overall expression of a single plasma protease gene can be controlled by the inflammatory signal that begins in the extracellular milieu and terminates at separate sites on the promoter region of the gene.

Not only IL-6, but a number of cytokines, alone or in a combination, may also influence HSPP synthesis. TNF-α and other inflammatory agents are known to markedly suppress fibrinolysis, mainly *via* stimulation of PAI-1 and down-regulation of t-PA expression. The transcriptional and post-transcriptional regulation of the fibrinolytic system by inflammatory signals was recently reviewed in detail (Medcalf, 2007). Simultaneously acting cytokines can exert additive, inhibitory, or synergistic effects on the HSPP production. TNF-α and IL-1 provide mutual down-regulation of the mRNA for murine protein C. These cytokines are able to control gene expression independently or in combination with IL-6 (Yamamoto et al., 1999), whereas IL-6-mediated induction of Fg synthesis was partially inhibited by IL-1 or TNF-α (Mackiewicz et al., 1991). Various environmental factors and individual features of the patient (including age, body mass index, levels of plasma triglycerides and atherosclerotic transformation of the vessel wall) influence the cytokine-regulated levels of HSPP. For instance, shear stress can up-regulate cytokine-induced expression of t-PA, TGF-ß1, and ICAM-I genes at the transcriptional level (Kawai et al., 1996). These additional influences modify local or systemic inflammatory responses depending on the host phenotype (Lowry, 2009).

5.2 Alterations of the HSPP plasma levels caused by post-transcriptional and post-translational events

Marked alterations in the plasma HSPP levels following an acute-phase stimulus are determined not only by transcriptional regulation but also by post-transcriptional and post-

translational mechanisms. The latter were found for such classic APPs, as serum amyloid A (SAA), complement factors B, and C3 (Jiang et al., 1995). It was reported that the α_2-macroglobulin gene transcription rate might increase (up to nine-fold) during the acute inflammatory response, while its protein plasma concentrations can rise much more significantly (to 100-fold) (Hattory et al., 1990). The other study demonstrated that aortic endothelial cells decreased production and secretion of t-PA after incubation with CRP without any alteration of the tPA mRNA level, thus underlying a suggestion that CRP-mediated tPA inhibition is a posttranscriptional event (Singh et al., 2005). In contrast, post-transcriptional regulation should not play a substantial role in monocyte-derived production of fibrinogen α-chain or α-1 protease inhibitor (α -PI) proteins (Jiang et al., 1995).
Despite the HSPPs are largely regulated by transcriptional control, they still strongly require the post-transcriptional regulation (including co-translational and post-translational modification) to confer their optimal functionality. They should form the disulfide bonds to get native conformation as well as should be carboxylated, hydroxylated, phosphorylated, sulfated or glycosylated to achieve a specific function. In particular, the main coagulation factors II, VII, IX, X and protein C (all are the vitamin K-dependent proteins) are processed through further post-translational modification to become biologically active. Prior to secretion into the blood they should be modified by a vitamin K-dependent gamma-glutamyl carboxylase, getting in such a way, an amount of negatively charged γ-carboxyglutamic acid (Gla) residues. Gla-residues have a chelating activity oriented to Ca^{2+}-cations (Table 2). They are orchestrated in the "Gla domain" to participate in the Ca^{2+}-dependent binding of parent protein to cell membrane or macromolecular complexes.
Similarly to most secretory proteins, HSPPs are enriched by disulfide bonds (Table 2). Before secretion, they undergo oxidative maturation that leads to binding of the appropriate pairs of cystein residues. The disulfide bonds are formed in the rough endoplasmic reticulum, since this process requires an oxidative environment. These functional groups are well-known as playing an important role in protein folding (by stabilizing the tertiary and quaternary structure). Furthermore, disulfide bonds can be responsible for intra- and intermolecular reorganization or even proteins aggregation. In the few last years, a number of studies on functional disulfides have highlighted their two important functions, namely catalytic and allosteric (Chen & Hogg, 2006; Manukyan et al., 2008; Popescu et al., 2010).
In addition to carboxylation and formation of disulfide bonds, a series of post-translational modifications occurs to attach N- or O-linked glycans to secreted proteins (Table 2). Several N-linked glycosylation sites are well-known to be an attributive feature of HSPPs, which are glycoproteins. N-glycosylation has been recently discovered to be a crucial event in the regulation of the glycoprotein structure and function. *Via* promotion or inhibition of intra- and intermolecular binding, glycans can regulate protein folding, cell adhesion and aggregation. Glycosylation can also modulate the activity of plasma membrane receptors at the surface of the vascular endothelial cells, platelets, and leukocytes influencing in such a way intracellular signal transduction systems, which are responsible for homeostasis in circulation (Skropeta, 2009). Probably, a degree of initial core glycosylation might affect the efficiency of protein's γ–carboxylation in endoplasmic reticulum before secretion (McClure et al., 1992).
There are available data, suggesting that glycosylation is higher in proteins synthesized during the acute-phase responses. *In vitro* studies with isolated hepatocytes and hepatoma cell lines proved that inflammatory cytokines regulate changes in glycosylation independently of the rate of synthesis of the APP (Van Dijk & Mackiewicz, 1995). Variations

Protein	Percent carbo-hydrate (w/w)	number of modified residue			
		Glycosylation	Disulfide bond	Hydroxylation, phosphorylation or sulfation	Carboxylation
fII		3 N-linked	10 (+2 predicted)	none	10 Gla residues
fV	~25%	5 N-linked (+21 predicted)	6 (+1 predicted)	1 phosphothreonine 1 phosphoserine 7 sulfotyrosine	none
fVII	13%	2 O-linked 2 N-linked	12	one β-hydroxyaspartate	10 Gla residues
fVIII		1 N-linked (+21predicted)	7 (+1 predicted)	6 sulfotyrosine	none
fIX	17%	4 O-linked 2 N-linked	11	one β-hydroxyaspartate 2 phosphoserine 1 sulfotyrosine	12 Gla residues
fX	15 %	2 O-linked 2 N-linked	12	one β-hydroxyaspartate	11 Gla residues
fXI	5%	5 N-linked	18	2 phosphorilated	none
fXII	17%	7 O-linked 2 N-linked	20	none	none
Glu-Pg	~2%	2 O-linked 1 N-linked	24	1 phosphoserine	none
tPA		1 O-linked 3 N-linked	17	none	none
TFPI		2 N-linked 3 O-linked	9	none	none
AT	9%	4 N-linked	3	1 phosphoserine	none
PC	23 %	4 N-linked	12	one β-hydroxyaspartate	9 Gla residues
α_2 -PI	14%	4 N-linked	1	1 sulfotyrosine	none
PAI-1		2 N-linked (+1 predicted)	none	none	none
TAFI		5 N-linked	3	none	none

(Data based on Protein Knowledgebase UniProtKB)

Table 2. Post-translational modifications of the major hemostasis soluble plasma proteins

in different glycoforms of APP in circulation most likely result from alterations in oligosaccharide branching, increased sialylation, and decreased galactosylation (Gabay & Kushner, 1999). The replacement of individual N-glycans by other ones exerts very specific and diverse effects on the protein structure and/or function. Human hemostatic proteins, coagulation factor IX and protein C, which both are the vitamin K-dependent proteins

synthesized and secreted by hepatocytes, vary extensively in their glycosylation levels. Coagulant factor IX has two N-glycosylation sites and is characterised by significantly more heterogeneity of N-glycan structures than anticoagulant protein C. PC has four N-glycosylation sites, Q97, Q248, Q313, and Q329; the latter has an unusual consensus sequence, Asn-X-Cys. Desialylation of PC and factor IX was shown to result in a two-threefold increase in the anticoagulant activity of protein C and in a loss of the coagulant activity of factor IX (Gil et al., 2009; Grinnell et al., 1991). Alterations in the glycosylation pattern have been suggested to be specific in certain diseases (An et al., 2009; Ohtsubo & Marth, 2006). Nevertheless, it remains unclear whether inflammation signals control processing of coagulation proenzymes or not. Well-documented inflammation impact on glycosylation of classic APPs allows researchers to suggest such a control. The most important mechanism, through which the inflammatory environment is able to alter the enzyme activity and/or substrate specificity in local environments or in a systemic disease are modifications of the glycan moiety or heterogeneity. Experiments with glycoprotein deglycosylation showed that the removal of distinct glycan or total deglycosylation usually leads to remarkable reduction of the protein binding and enzymatic activity. However, at least two examples have been recently elucidated (Skropeta, 2009), where the enzyme activity increased upon deglycosylation of HSPPs. In particular, removal of the two of four existing glycosylation sites in the human protein C molecule resulted in a two–threefold increase in the anticoagulant activity of APC due to an enhanced affinity of thrombin, the natural activator of PC. Interestingly, two fibrinolytic proteins, tPA and its specific substrate, Pg, interact more or less effectively depending on the peculiarities of attached glycans. Indeed, tPA can exist as two glycoforms, type I with three N-glycosylated sites and type II with two N-glycans. Plasminogen also exists in two glycoforms; type 1 has both N- and O-linked glycans, while type 2 has only an O-linked glycan. The combination of type II tPA with type 2 plasminogen induced a twofold more intense conversion of plasminogen to plasmin compared to interaction of more heavily glycosylated type I tPA with type 1 Pg. Changes in the microheterogenity and unique structure of glycans are now known to be ensued from folding of the glycoprotein early form during post-translation processing in the secretory pathway. Glycosylation is an enzymatic process regulated by distinct glycosyltransferases in the endoplasmic reticulum, which modulate unfolded glycoproteins prior to trafficking to the Golgi apparatus. Unexpectedly, one experiment demonstrated that an alterated O-glycosylation pathway affects the N-glycosylated coagulation proteins in NAcT-1-deficient mice. In particular, the deficiency of a polypeptide GalNAc transferase (ppGalNAcT) contributed to shifting of O-glycan repertoire by other glycosyltransferases, as well as affected blood coagulation resulting in prolongation of the activated partial thromboplastin time, APTT, and bleeding time. These abnormalities were accompanied by mild or moderate decreases in the circulating levels of factors V, VII, VIII, IX, X, and XII, whereas the level of von Willebrand factor tended to raise (Tenno et al., 2007). The reported results might be interpreted as a consequence of pleuotropic effects of O-glycosylation that contribute to regulation of HSPP expression and/or turnover (primarily secretion and clearance). Additionally, alterations in the degree of branching and of levels of sialylation, fucosylation, and mannosylation can dramatically change the glycoprotein turnover. Although our information about glycan-mediated pathophysiological mechanisms is still very limited, their impact on the enzyme secretion, stability, and activity and on molecular trafficking and clearance allows researchers to suggest that glycosylation plays a special role

in the phenotypical variability of hemostatic and inflammatory proteins in circulation. Apparently, the acute-phase response generates a characteristic protein profile by alteration of synthesis, secretion, and clearance of protein reflected in their final concentrations. The actual level of plasma proteins under pathological conditions is also determined by changes in their stability, post-secretion proteolysis, functional activity, and accessibility for interaction.

Marked alterations in the plasma protein levels are probably paralleled by modifications of their disulphide bonds. The role of disulfides in regulation of the functional activity of HSPPs was subjected to intense research. A direct influence of inflammatory conditions on the structure or functions of plasma proteins is an intriguing question. Recently, we demonstrated that the concentration of DTNBA-active polypeptides produced in the course of the reaction of plasma and serum proteins with 5,5'-dithiobis(2-nitrobenzoic acid), was noticeable increased in patients with stable *angina pectoris* compared to healthy subjects. *In vitro* blood coagulation was accompanied by a six-fold drop of the SS-containing components and 2,5-fold elevation of SH-containing polypeptides in patients, whereas mild changes were documented in control subjects. In addition, positive correlation of the plasma level of SH-containing polypeptides with concentrations of CRP and low-density lipoproteins was observed. Based on our findings, we can speculate that hypercoagulation in sclerotized vessels can enhance inflammation by promoting the development of oxidative stress. Activated, and thereby, partially degraded HSPPs, after their more open conformation has been obtained, can exhibit earlier buried disulphide bridges, which can serve as pro-oxidant derivates during thiol-disulfide exchange (Patalakh et al., 2008). Earlier, in the study of Procyk and colleagues (1992), it was found that thrombin looses its ability to cleave Fg in a calcium-free medium under non-denaturing conditions after reduction of several disulfide bonds in α- and γ-chains of fibrinogen. The loss of thrombin clottability was suggested to result from perturbation of carboxy-terminal polymerization sites in the fibrinogen γ-chain. It is interesting that tPA converted Pg into Pm more effectively on the surface of non-clottable (partially reduced) Fg rather than on untreated Fg (Procyk et al., 1992). These data confirm the statement on the ability of disrupted disulfide bonds to modulate the functional activity of major HSPPs *via* conformational changes. Newly obtained data suggest that particular SS-bonds are involved in regulation of HSPP functions *via* reduction or oxidation. Most hemostasis-related proteins probably contain these functionally active allosteric disulfide bonds; among them, there are TF, Fg, Pg(Pm), tPA, uPA, an uPA receptor, vitronectin, glycoprotein 1bα, ß$_3$ subunit of $\alpha_{IIb}ß_3$ integrin, and thrombomodulin (Chen & Hogg, 2006). We hypothesized that at least one common sensitive element in the protein structures of the plasma pattern might facilitate the adequate integrated response of the hemostasis system to an inflammatory impact. Redox-mediated signals, which are generated in plasma during inflammation, might control hemostasis pathways *via* such a sensitive element in protein structures. And *vice versa*, exposed disulfide bonds through one-electron reduction can generate active intermediates transmitting pro-inflammatory or pro-oxidant extracellular signals to cell receptors and, thus, can induce production of more APPs and HSPPs, especially *via* the MAPK-mediated pathway (Forman et al., 2004; Rees et al., 2008).

Although HSPPs are synthesised and secreted principally in hepatocytes (Ruminy et al., 2001) other cell types can be additionally involved. For example, vascular endothelial cells represent an almost exclusive source of such a fibrinolytic component, as tPA produced by

the endothelium in both physiological and pathophysiological states. Another fibrinolytic component, PAI-1, has additional sites of synthesis, such as vascular endothelial cells, leukocytes, adipocytes, and platelets, but this occurs predominantly after their activation at inflammatory *foci*. Synthesis of protein C, which mainly occurs in the liver, was also identified in the kidneys, lungs, brain, and male reproductive tissue. Therefore, a systemic or local inflammatory signal is able to recruit more than one cells source of HSPP. Aggregated platelets, activated leukocytes, and cells presented in the vascular wall release cytokines thereby altering local HSPP secretion. Impairment of total HSPP production because of disorders of the liver functions during systemic inflammation can be accompanied by increased protein consumption or by a decrease in the hepatic clearance for individual proteins. Perpetuation of inflammation in patients suffering from sepsis is known to depress the activity of Pg or α_2-plasmin inhibitor (α_2-PI) rather because of a low synthetic function of the liver but not consumption coagulopathy (Asakura et al., 2001). In contrast, increased consumption is the main reason for suppression of the plasma level of such enzymatically active proteases, as APC, thrombin, Pm, and tPA. In turn, depletion of the pool of proteases results in ineffective consumption and clearance of their substrates. Additionally to the protein expression, this mechanism can participate in elevation of such hemostatic APPs as factors VIIIa and Va, Fg, Fn, and Pg (Baklaja & Pešic, 2008). Finally, the rate of secretion and/or clearance processes of plasma proteins should be markedly distinct from the rate of their synthesis. Respectively, the half-life time of involved factors is shortened or prolonged.

It is obvious that the plasma levels of naturally active (e.g., tPA) or *in situ* activated hemostatic proteases (e.g., thrombin or APC) fluctuate during inflammation rather due to stimulation of secretion, reactivity, and clearance than due to the respective gene expression in the cells. The above-mentioned regulatory mechanisms can affect significantly the HSPP kinetic profile with either a rise or a decline of their plasma levels. According to the study of Jern and colleagues (Jern et al., 1999), there is no correlation between the net release rate of total t-PA and plasma levels of either total or active tPA. These authors also suggested that the local endothelial release rate, rather than the systemic plasma level of t-PA, determines the plasma fibrinolytic potential destined to clot digestion *in situ*. The assay-measured plasma concentration of tPA insufficiently displays this local discrete increment. Moreover, while cytokine-induced PAI-I secretion increases, tPA secretion alternatively decreases (as after CRP-regulated secretion) or remains unchanged. Platelets have a large PAI-1 storage pool within secretory α-granules (about 90 % of the plasma level). After platelet activation, PAI-1 is released from α-granules along with other coagulation proteins, adhesion molecules, integrins, growth factors, and inflammatory modulators. Such a pro-inflammatory milieu facilitates the recruitment of additional platelet and inflammatory cells encouraging generate and amplify inflammation signals. Tissue plasminogen activator is secreted from the intracellular storage compartment after stimulation of PARs on the surface of endothelial cells. There are two pathways involved in tPA secretion from endothelial cells, constitutive and regulated secretion. Rates of the constitutive tPA release is differentiated markedly by the genotype; however, genetic variation most likely is not reflected in the circulating plasma t-PA levels. It was reported that CRP impaired the release of tPA *via* Fc-γ receptors but did not affect tPA mRNA (Devaraj et al., 2005). Stimulation of endothelial cells with IL-1ß or TNF-α did not change their ability to produce tPA (Jern et al., 1999). Shear stress can modulate the cytokine effects by enhancing t-PA secretion and attenuating the PAI-1 release (Kawai et al., 1996).

Probably, the recovery of the tPA plasma pool in proportion to excessive consumption by PAI-1 is rapid but transient, since the augmented local tPA secretion is limited by the rate of its synthesis. Because of the fact that the tPA-PAI-1 complex is usually cleared at a lower rate than free tPA, this can lead to the appearance of a disproportion between the antigen and activity values. Notably, activated protein C is suggested to compete with tPA for PAI-1 complexation. The importance of APC-PAI-1 *in vivo* association is still disputable because the PAI-1 reactivity with respect to APC is very low in a purified system. Nevertheless, it was demonstrated that vitronectin, a pro-inflammatory protein, enhances the reactivity of PAI-1 with APC about 300 times (Rezaie, 2001).

In a study with patients suffering from chronic cardiac failure (CCF) and stable *angina pectoris* (SAP) we found an abnormality of the ratios between the plasma levels of t-PA, PC, and PAI-1 (Patalakh et al, 2009; Patalakh et al, 2007). An insufficiency of PC and t-PA proteins was accompanied by increase in the PAI-1 concentration and activity in the blood plasma of patients with high intravascular inflammation (hs-CRP levels were 12,95±1,81 and 6,83±1,48 mg/ml for SAP and CCF, respectively). We believe that these changes are a manifestation of reduction of the blood fibrinolytic potential. Using a regression analytical procedure, we simulated a potential profibrinolytic effect of endogenous PC as association of its plasma level with PAI-1 attenuation. The effect became apparent within a close-cut range of the PAI-1 concentrations and descended at low (<0,8 nM) or high (>3 nM) PAI concentrations. It was also predicted that the profibrinolytic function of APC during CCF duration might be realized under conditions where the precursor PC concentration did not decrease below 50-60 nM.

Some evidence do exist that the plasma levels of PC are associated with the systemic inflammatory response to trauma, infection, resuscitated cardiac arrest, non-stable *angina pectoris*, etc. It seems that most cardio-vascular diseases during their severe inflammation stage are complicated by a transient PC deficiency. The nature of this failure is not completely clear. We suppose that the PAI-1 inhibitory activity is involved into PC plasma pool depletion during acute inflammation. It seems that phenotypic PC alterations reflect different aspects of the APC turnover, up-regulated by inflammation stimuli. It seems that conversion of PC into APC, forced by the increasing thrombin production, can lead to rapid consumption of PC since APC undergoes action of the abundant amount of serine protease inhibitors, accumulated in the blood during the acute-phase response. PAI-1 is the most up-regulated inhibitor of APC during acute inflammation. Activated platelets additionally produce PAI-1 during coagulation and thrombus formation. Particularly due to vitronectin activation PAI-1 should contribute significantly to the acquired protein C deficiency. Only when present in physiological concentrations, APC can deplete PAI-1 and, thus, promote the involvement of t-PA in fibrinolysis. Due to severe or prolonged conversion of PC into APC, the plasma pool of PC may be exhausted. As a result, further generation of activated protein C will be disturbed. The retarded turnover of protein C ($t_{1/2}$~ 8 hours) and an extremely short clotting time (about 2-3 min) might cause depression of the protein C pathway and, consequently, uncontrolled promotion of the thrombin pathway. As a result APC loses its crucial role in the regulation of hemostasis and inflammation. While coagulation and inflammation are escalated, anticoagulant and fibrinolytic blood potentials are dropped. The described progression of events might provoke inflammatory and thrombogenic diseases in a manner we illustrate in figure 3.

6. Conclusion

Recent advances in our understanding of the nature of critical factors, linking hypercoagulation with both acute and chronic inflammation are rather promising. Nevertheless, we only can propose some speculations predicting the balance disruption between procoagulant and anticoagulant components under conditions of abnormal hemostasis, as well as consequences of their ratio abnormality on inflammation duration. The problem is complicated by the existence of poorly predictable mechanisms of most urgent thrombotic events that are happened rather "now" and "here". A transient deficiency or acute inactivation of common hemostatic soluble plasma proteins, affecting hemostasis and inflammation by a mutual regulatory mechanism, was suggested as a key pathogenic factor of such life-threatening complications. Post-translational HSPPs modifications reviewed here could be considered as crucial phenomenon impacted by the inflammatory process. Apparently, inflammation-associated variations in the structure and function of hemostatic proteins can influence their catalytic efficiency and measurable plasma levels. These changes should be taken into account in indication of pathological hemostasis. The recent knowledge on regulatory crosstalk between hemostatic system components and the inflammatory system allows discovering new therapeutic targets to be developed. This new approach could not only change the traditional paradigm of clotting factor substitution therapy, but also anti-inflammatory therapies. Activated protein C is expected to be an attractive therapeutic target with prominent anticoagulant, profibrinolytic, and anti-inflammatory properties, which can simultaneously regulate both inflammation and coagulation. Nevertheless, the results of several clinical trials with recombinant APC or modified rAPC were found to be rather disappointing. Indeed, the peculiarities of the protein structure, attributed to regulatory components with pleiotropic action such as APC, may play a pivotal role in providing clinical benefit of designed protein variants. Hemostasis is a thorough "molecular machine", which can not readily be improved. To understand and to reconstruct perturbed functions of this machinery should be a prominent goal for both basic and clinical research studies.

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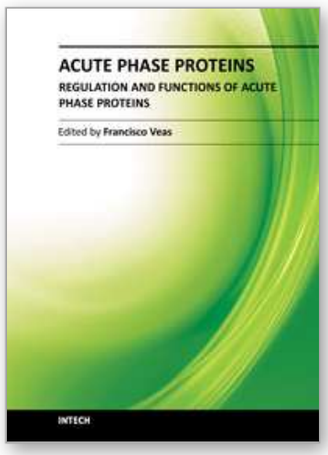

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The two volumes of Acute Phase Proteins book consist of chapters that give a large panel of fundamental and applied knowledge on one of the major elements of the inflammatory process during the acute phase response, i.e., the acute phase proteins expression and functions that regulate homeostasis. We have organized this book in two volumes - the first volume, mainly containing chapters on structure, biology and functions of APP, the second volume discussing different uses of APP as diagnostic tools in human and veterinary medicine.

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